3

Algae as Bioindicators

Biological indicators (bioindicators) may be defined as particular species or communities, which, by their presence, provide information on the surrounding physical and/or chemical environment at a particular site. In this book, freshwater algae are considered as bioindicators in relation to water chemistry – otherwise referred to as 'water quality'.

The basis of individual species as bioindicators lies in their preference for (or tolerance of) particular habitats, plus their ability to grow and out-compete other algae under particular conditions of water quality. Ecological preferences and bioindicator potential of particular algal phyla are discussed in Chapter 1. This chapter considers water quality monitoring and algal bioindicators from an environmental perspective, dealing initially with general aspects of algae as bioindicators and then specifically with algae in the four main freshwater systems – lakes, wetlands, rivers and estuaries.

3.1 Bioindicators and water quality

Freshwater algae provide two main types of information about water quality.

- *Long-term information*, *the status quo*. In the case of a temperate lake, for example, routine annual detection of an intense summer bloom of the colonial blue-green alga *Microcystis* is indicative of pre-existing high nutrient (eutrophic) status.
- *Short-term information*, *environmental change*. In a separate lake situation, detection of a change in subsequent years from low to high blue-green dominance (with increased algal biomass) may indicate a change to eutrophic status. This may be an adverse transition (possibly caused by human activity) that requires changes in management practice and lake restoration.

In the context of change, bioindicators can thus serve as early-warning signals that reflect the 'health' status of an aquatic system.

3.1.1 Biomarkers and bioindicators

In the above example, environmental change (to a eutrophic state) is caused by an environmental stress factor – in this case the influx of inorganic nutrients into a previously low-nutrient system. The resulting loss or dominance of particular bioindicator species is preceded by biochemical and physiological changes in the algal community referred to as 'biomarkers.' These may be defined (Adams, 2005) as short-term indicators of exposure to environmental stress, usually expressed at suborganismal levels – including biomolecular, biochemical and physiological responses. Examples of algal biomarkers include DNA damage (caused by high UV irradiation, exposure to heavy metals), osmotic shock (increased salinity), stimulation of nitrate and nitrite reductase (increased aquatic nitrate concentration)

Freshwater Algae: Identification and Use as Bioindicators Edward G. Bellinger and David C. Sigee

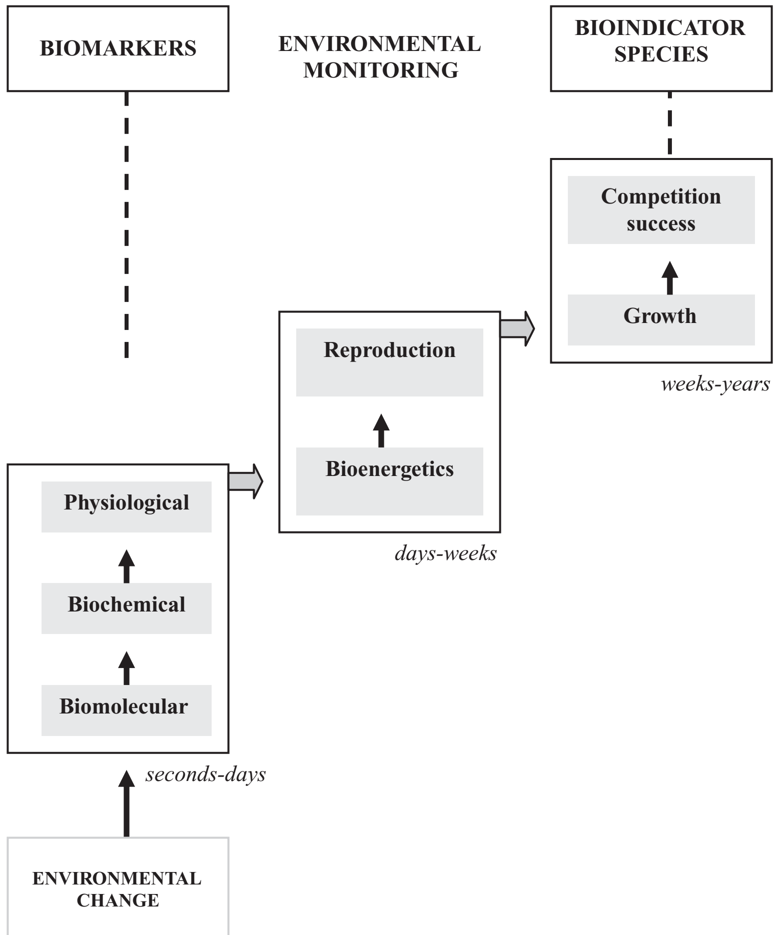

Figure 3.1 Hierarchical responses of algae to environmental change, such as alterations in water quality. The time-response changes relate to sub-organismal (left), individual (middle) and population (right) aspects of the algal community. Environmental monitoring of the algal response can be carried out at the biomarker or bioindicator species level. Adapted from Adams (2005).

and stimulation of phosphate transporters/reduction in alkaline phosphatase secretion (increased aquatic inorganic phosphate concentration).

The time scale of perturbations in the algal community that results from environmental change (stress) can be expressed as a flow diagram (Fig. 3.1), with monitoring of algal response being carried out either at the biomarker or bioindicator species level. Although the rapid response of biomarkers potentially provides an early warning system for monitoring environmental change (e.g. in water quality), the use of bioindicators has a number of advantages (Table 3.1) including high ecological relevance and the ability to analyse environmental samples (chemically-fixed) at any time after collection.

3.1.2 Characteristics of bioindicators

The potential for freshwater organisms to reflect changes in environmental conditions was first noted by Kolenati (1848) and Cohn (1853), who observed that biota in polluted waters were different from those in non-polluted situations (quoted in Liebmann, 1962).

Since that time much detailed information has accumulated about the restrictions of different organisms (e.g. benthic macroinvertebrates, planktonic algae, fishes, macrophytes) to particular types of aquatic environment, and their potential to act as environmental monitors or bioindicators. Knowledge of freshwater algae that respond rapidly and predictably

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
Primary indicator of	Exposure	Effects
Sensitivity to stressors	High	Low
Relationship to cause	High	Low
Response variability	High	Low
Specificity to stressors	Moderate-high	Low-moderate
Timescale of response	Short	Long
Ecological relevance	Low	High
Analysis requirement	Immediate, on site	Any time after collection (fixed sample)

Adapted from Adams, 2005.

to environmental change has been particularly useful, with the identification of particular indicator species or combinations of species being widely used in assessing water quality.

Single species

In general, a good indicator species should have the following characteristics:

- a narrow ecological range
- rapid response to environmental change
- well defined taxonomy
- reliable identification, using routine laboratory equipment
- wide geographic distribution.

Combinations of species

In almost all ecological situations it is the combination of different indicator species or groups that is used to characterize water quality. Analysis of all or part of the algal community is the basis for multivariate analysis (Section 3.4.3), application of bioindices (Sections 3.2.2 and 3.4.4) and use of phytopigments as diagnostic markers (Section 3.5.2)

3.1.3 Biological monitoring versus chemical measurements

In terms of chemistry, water quality includes inorganic nutrients (particularly phosphates and nitrates), organic pollutants (e.g. pesticides), inorganic pollutants (e.g. heavy metals), acidity and salinity. In an ideal world, these would be measured routinely in all water bodies being monitored, but constraints of cost and time have led to the widespread application of biological monitoring.

The advantages of biological monitoring over separate physicochemical measurements to assess water quality are that it:

- reflects overall water quality, integrating the effects of different stress factors over time; physicochemical measurements provide information on one point in time.
- gives a direct measure of the ecological impact of environmental parameters on the aquatic organisms.
- provides a rapid, reliable and relatively inexpensive way to record environmental conditions across a number of sites.

Biological monitoring has been particularly useful, for example, in implementing the European

Table 3.2 Trophic Classification of Temperate Freshwater Lakes, Based on a Fixed Boundary System

	Trophic Category				
	Ultraoligotrophic	Oligotrophic	Mesotrophic	Eutrophic	Hypertrophic
Nutrient concentration ($\mu g\, l^{-1}$)					
Total phosphorus (mean annual value)	<4	4–10	10–35	35–100	>100
Orthophosphate[a]		<2	2–5	5–100	>100
DIN[a]		<10	10–30	30–100	>100
Chlorophyll a concentration ($\mu g\, l^{-1}$)					
Mean concentration in surface waters	<1	1–2.5	2.5–8	8–25	>25
Maximum concentration in surface waters	<2.5	2.5–8	8–25	25–75	>75
Total volume of planktonic algae[b]	0.12	0.4	0.6–1.5	2.5–5	>5
Secchi depth (m)					
Mean annual value	>12	12–6	6–3	3–1.5	<1.5
Minimum annual value	>6	>3.0	3–1.5	1.5–0.7	<0.7

Table adapted from Sigee (2004). Lakes are classified according to mean nutrient concentrations and phytoplankton productivity (shaded area). Boundary values are mainly from the OECD classification system (OECD, 1982), with the exception of orthophosphate and dissolved inorganic nitrogen (DIN), which are from Technical Standard Publication (1982).

[a]Orthophosphate and DIN are measured as the mean surface water concentrations during the summer stagnation period.

[b]Total volumes (% water) of planktonic algae are for Norwegian lakes, growth season mean values (Brettum, 1989).

Union Directive relating to surface water quality (94C 222/06, 10 August 1994), where Member States were obliged to establish freshwater monitoring networks by the end of 1998.

3.1.4 Monitoring water quality: objectives

Environmental monitoring of aquatic systems, particularly in relation to water quality, provides information on:

- *Seasonal dynamics*. In temperate lakes these include hydrological measurements (water flows, residence time), thermal and chemical stratification and changes in nutrient availability at the lake surface. Epilimnion concentrations of nitrates and phosphates can fall to very low levels towards the end of stratification (late summer), and the lake could then be dominated by algae such as dinoflagellates (e.g. *Ceratium*) and colonial blue-greens (e.g. *Microcystis*) which are able to carry out diurnal migrations into the nutrient-rich hypolimnion.

- *Classification of ecosystems in relation to water quality, productivity and constituent organisms*. The most widely used classification (for both lotic and lentic systems) is based on inorganic nutrient concentrations, with division into oligotrophic, mesotrophic and eutrophic systems (Table 3.2). Detection and analysis of indicator algae (Table 3.3) provides a quick assessment of trophic status and possible human contamination of freshwater bodies.

- *Dynamics of nutrient and pollutant entry into the aquatic system via point or diffuse loading*. Localized or diffuse entry of contaminants can be studied by analysis of benthic algal communities. The potential use of these littoral algae in lakes, where water quality at the lake edge directly relates to different types of inflow from the catchment area, is shown in Fig. 3.2.

- *Human impacts*. Long-term monitoring of anthropogenic effects within the ecosystem – including changes (Section 3.2.3) such as eutrophication, increase in organic pollutants, acidification and heavy metal contamination. Analysis of indicator diatoms within sediment cores has been particularly useful in monitoring long-term changes in water quality and general ecology (Section 3.2.2).

Table 3.3 Lake Trophic Status: Phytoplankton Succession and Algal Bioindicators

Lake Type	Spring / Summer / Autumn	Mid-Summer Algal Bioindicators	Example
Oligotrophic	**DIATOMS** *Cyclotella* → **DINO** *Ceratium* **BG** *Gomphosphaeria*	**DIA** *Cyclotella comensis* *Rhizosolenia* spp. **G** *Staurodesmus spp.*	Carinthian Lakes[1] Wastwater[2] Ennerdale
Mesotrophic	**DIATOMS** Asterionella → **CHRYSO** Mallomonas → **DINO** Ceratium **BG** *Gomphosphaeria* **GREEN** Sphaerocystis → **DIATOMS** *Asterionella*	**DIA** *Tabellaria flocculosa* **CHR** *Dinobryon divergens, Mallomonas caudata* **G** *Sphaerocystis schroeteri, Dictyosphaerium elegans, Cosmarium spp, Staurastrum spp* **DINO** *Ceratium hirundinella* **BG** *Gomphosphaeria spp.*	Lunzer Untersee[1] Bodensee[3] Erken[4] Windermere[2] Grasmere
Eutrophic	**DIATOMS** Asterionella → **GREEN** Eudorina **CRYPT** *Cryptomonas* → **BG** Anabaena Aphan. → **DINO** Ceratium **BG** *Microcystis* → **DIATOMS** Steph.	**DIA** *Aulacoseira spp., Stephanodiscus rotula* **G** *Eudorina spp., Pandorina morum, Volvox spp.* **BG** *Anabaena spp., Aphanizomenon flos-aquae, Microcystis aeruginosa*	Prairie Lakes[5] Norfolk Broad[2] Rostherne Mere[2]
Hypertrophic	**SMALL DIATOMS** Steph. → **GREEN** Scenedesmus → **GREEN** Pediaastrum → **BG** Aphanocapsa	**DIA** *Stephanodiscus* hantzschii **G** *Scenedesmus spp., Ankistrodesmus spp., Pediastrum spp.* **BG** *Aphanocapsa spp., Aphanothece spp, Synechococcus spp.*	Fertilised waters e.g. Třeboň fishponds[6]

Abbreviations: Main phytoplankton groups: BG, blue-green algae; Chryso, chrysophytes; Crypt, cryptomonads; Dino, dinoflagellates.
Genera: *Steph.*, *Stephanodiscus*; *Aphan.*, *Aphanizomenon.*
Location of lakes: [1]Austria, [2]UK, [3]Germany, [4]Sweden, [5]USA, [6]Czech Republic.
Table adapted from Sigee (2004), originally from Reynolds (1990).

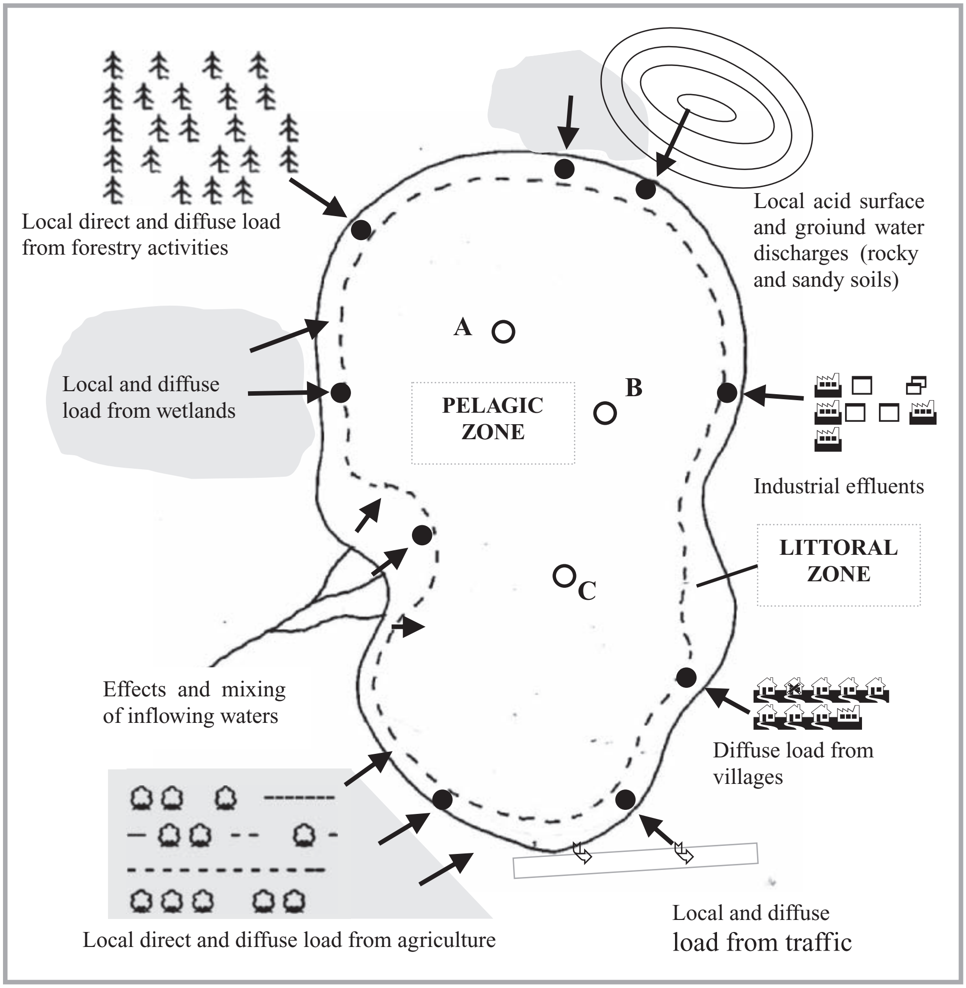

Figure 3.2 Lake water quality: phytoplankton and periphyton as bioindicators. General lake water quality: phytoplankton in pelagic zone (○ sites A, B, C). Local water quality at edge of lake (● periphyton in littoral zone), with inputs (→) from a range of surrounding terrestrial sources. Figure adapted and redrawn from Eloranta, 2000.

- *Suitability of water for human use*. This includes compliance of water quality with regulations for human consumption and recreation. Build-up of colonial blue-green algal populations, with increased concentrations of algal toxins, can lead to closure of lakes for production of drinking water and recreation. The use of a reactive monitoring programme for blue-green algal development over the summer months is now an essential part of water management for in many aquatic systems (e.g. Hollingworth Lake, United Kingdom – Sigee, 2004).

- *Conservation assessment*. Analysis of freshwater algae has become an important part of the survey and data collection programme used in the evaluation of lakes for their nature conservation value (Duker and Palmer, 2009). Evaluation of water quality is also important for existing conservation sites. In the United Kingdom, a large number of freshwater Sites of Special Scientific Interest (SSSIs) are believed to be affected by eutrophication (Nature Conservancy Council, 1991; Carvalho and Moss, 1995). In the case of lakes, the role of algal bioindicators in this assessment is based both on contemporary organisms (Section 3.2.1) and fossil diatoms within sediments (Section 3.2.2).

3.2 Lakes

The use of algae as bioindicators of water quality is influenced by the long-term retention of water in lake systems and also by the age of the lake. Retention of water can be quantified as 'water retention time' (WRT), which is the average time that would be taken to refill a lake basin with water if it were emptied. WRT for most lakes and reservoirs is about 1 to 10 years, but some of the world's largest lakes have values far in excess of this – including extreme values of 6000 and 1225 years respectively for Lakes Tanganyika (Africa) and Malawi (Africa).

Characteristics of lake hydrology and age result in:

- *Phytoplankton dominance*. In moderate to high nutrient deep lakes, phytoplankton populations are able to grow and are retained by the system (not flushed out). Their dominance over non-planktonic algae and macrophytes means they are routinely used as bioindicators.

- *Long-term exposure*. In many lakes, planktonic and benthic algae have relatively long-term exposure to particular conditions of water quality and relate to specific chemical and physical characteristics over extended periods of time (weeks to years).

- *Endemic species*. Some of the world's largest lakes have existed over a long period of time – including Lakes Tanganyika (Africa: 2–3 million years [My]), Malawi (Africa: 4–9 My) and Baikal (Russia: 25–30 My). Long-term evolution within these independent and enclosed aquatic systems has led to the generation of high proportions of unique species (endemism), with over 50% endemic fauna and flora in Lakes Tanganyika and Baikal (Martens, 1997). The presence of substantial levels of endemism in these large water bodies, together with the fact that even non-endemic species may have unique adaptations, means that conventional bioindices will need to be adjusted to suit particular situations.

3.2.1 Contemporary planktonic and attached algae as bioindicators

Both planktonic algae (present in the main water body of the lake – pelagic zone), and attached algae (occurring around the edge of the lake – littoral zone) have been used to monitor water quality (Fig. 3.2).

Phytoplankton: general water quality

Most studies on lakes (see Section 3.2.3) have used the phytoplankton (rather than benthic) community for contemporary environmental assessment, since it is the main algal biomass, is readily sampled at sites across the lake and many planktonic species have defined ecological preferences. Analysis of the phytoplankton community from a number of sites across the lake also provides information about aquatic conditions in general, and is the basis of broad categorization of lakes in relation to water quality, particularly trophic state – see later.

Attached algae: ecological status and localized water quality

Although there have been relatively few studies using attached (benthic and epiphytic) algae to assess water quality, analysis of non-planktonic (mainly littoral) algae can provide useful information on general ecology and local water quality.

General ecological status As well planktonic algae, attached algae are also important in providing a measure of the general ecology of the lake. This is recognized in the European Union Framework

Directive (WFD: European Union, 2000), which requires Member States to monitor the ecology of water bodies to achieve 'good ecological status'. Macrophytes and attached algae together form one 'biological element' that needs to be assessed under this environmental programme (see also 'multiproxy approach' – Section 3.2.2).

Local water quality Various authors have analysed benthic or epiphytic algal populations in relation to water quality, including the extensive periphyton growths that occur in the littoral region of many lakes. These algae are particularly useful in relation to local water conditions (e.g. localized accumulations of metal toxins, point source and diffuse loading at the edge of the lake), since their permanent location at particular sites gives a high degree of spatial resolution within the water body.

Localized metal accumulations Cattaneo *et al.* (1995) studied periphyton growing epiphytically in macrophyte beds of a fluvial lake in the St Lawrence River (Canada), to see if they could resolve periphyton communities in relation to water quality (toxic and non-toxic levels of mercury) under differing ecological conditions (e.g. fine versus coarse sediment). The periphyton, composed of green algae (40%), blue-greens (25%) diatoms (25%) and other phyla, was collected from various sites and analysed in terms of taxonomic composition and size profile. Multivariate (cluster and biotic index) analysis of periphyton communities gave greatest separation in relation to physical ecological (particularly substrate) conditions rather than water quality. The authors recommended that the use of benthic algae as aquatic bioindicators should involve sampling from similar substrate sites to eliminate ecological variation other than water quality.

Point source and diffuse loading at the edge of the lake Water quality in the littoral zone may differ considerably from that in the main part of the lake. This is partly due to the proximity of the terrestrial ecosystem (with inflow from the surrounding catchment area) and partly due to the distinctive zone of littoral macrophyte vegetation, making an important buffer zone between the shore and open water (Eloranta, 2000). Analysis of littoral algae, either by multivariate analysis or determination of bioindices, has the potential to provide information on water quality at particular sites along the edge of the lake in relation to point discharges (stream inflows, industrial and sewage discharges) and diffuse loadings. The latter include input from surrounding agricultural land, discharges from domestic areas, traffic pollutants and loading from local ecosystems such as forests and peat bogs (Eloranta, 2000) – Fig. 3.2.

Sampling and analysis of littoral algae Although attached algal communities (as with phytoplankton) can theoretically be related to water quality in terms of total biomass, this does not correlate well with nutrient loading (King *et al.*, 2006) – chiefly due to grazing and (in eutrophic waters) competition with phytoplankton. Also, nutrients in the water can be supplemented by nutrients arising from the substratum.

Species counts, on the other hand, can provide a useful measure of water quality. Recent recommendations for littoral sampling (King *et al.*, 2006: see also Section 2.10) concentrate particularly on diatoms – collecting specimens from stones and macrophytes (Fig. 2.29), since these substrata are particularly common at the edge of lakes. Epipelic diatoms (present on mud and silt) are probably less useful as bioindicators since they are particularly liable to respond to substrate 'pore-water' chemistry rather than general water quality. The epipelic diatom community of many lowland lakes also tends to be dominated by *Fragilaria* species, which take advantage of favourable light conditions in the shallow waters, but are poor indicators of water quality – having wide tolerance to nutrient concentrations. Having obtained samples and carried out species counts of diatoms from habitats within the defined littoral sampling area, weighted-average indices can be calculated as with river diatoms (Section 3.4.5/6) and related to water quality.

3.2.2 Fossil algae as bioindicators: lake sediment analysis

Recent water legislation, including the US Clean Water Act (Barbour *et al.*, 2000) and the European Council Water Framework Directive (WFD: European Union, 2000) have required the need to assess current water status in relation to some baseline state in the past. This baseline state (referred to as 'reference conditions') defines an earlier situation when there was no significant anthropogenic influence on the water body. In the United Kingdom, this reference baseline is generally set at about 1850, prior to the modern era of industrialization and agricultural intensification. Having defined the reference conditions, contemporary analyses can then be used to make a comparative assessment of human influences on lake biology, hydromorphology and water chemistry. For a particular water body, the absence of long-term contemporary data means that reference conditions have to be assessed on a retrospective basis, including the use of palaeolimnology – the study of the lake sediment record. The use of lake sediments to generate a historical record only gives reliable results under conditions of optimal algal preservation (see below) and if the sediments are undisturbed by wind, bottom-feeding fish and invertebrates.

Lake sediments – algal accumulation and preservation

Continuous sedimentation of phytoplankton from the surface waters (euphotic zone) of lakes leads to the build-up of sediment, with the accumulation of both planktonic and benthic algal remains at the bottom of the water column. In a highly productive lake such as Rostherne Mere, United Kingdom (Fig. 3.5), the wet sedimentation rate in the deepest parts of the water body has been estimated at 20 mm year^{-1} (Prartano and Wolff, 1998), with subsequent compression as further sedimentation and decomposition occurs. Decomposition of algal remains leads to the loss of most organic biomass, and algal identification is largely based on the relatively resistant inorganic (siliceous) components of diatoms and chrysophytes (Section 1.9). Optimal preservation of this cell wall material requires anaerobic conditions, and sediment samples are best taken from central deep parts of the lake rather than from shallow regions such as the littoral zone (Livingstone, 1984).

Diatom bioindicators within sediments

Within lake sediments, diatoms have been particularly useful as bioindicators (Section 1.10) of past lake acidification (Battarbee *et al.*, 1999), point sources of eutrophication (Anderson *et al.*, 1990) and total phosphorus concentration (Hall and Smol, 1999). The widespread use of lake sediment diatoms for reconstruction of past water quality is supported by the European Diatom Database Initiative (EDDI). This web-based information system is designed to enhance the application of diatom analysis to problems of surface water acidification, eutrophication and climate change. The EDDI has been produced by combining and harmonizing data from a series of smaller datasets from around Europe and includes a diatom slide archive, electronic images of diatoms, new training sets for environmental reconstruction (see below) and applications software for manipulating data. In addition to the EDDI, other databases are also available – including a large-scale database for benthic diatoms (Gosselain *et al.*, 2005).

Diatoms within sediments are chemically cleaned to reveal frustule structure (Section 2.5.4), identified and species counts expressed as percentage total. Numerous examples of cleaned diatom images from lake sediments are shown in Chapter 4. As with fossil chrysophytes (Section 1.9), subsequent analysis of diatom species counts to provide information on water quality can involve the use of transfer functions, species assemblages and may be part of a multiproxy approach. The data obtained, coupled with radiometric dating of sediment cores, provide information on times and rates of change and help in setting targets for specific restoration procedures to be carried out.

Transfer functions Transfer functions are mathematical models that allow contemporary data to be applied to fossil diatom assemblages for the quantitative reconstruction of (otherwise unknown) past water quality. Various authors (Bennion *et al.*, 2004; Taylor *et al.*, 2006; Bennion and Battarbee, 2007) have described the use of this approach, which is as follows.

- Generation of a predictive equation (transfer function) from a large number of lakes, in each case relating the dataset of modern surface-sediment diatoms samples to lake water quality data (Bennion *et al.*, 1996). The 'training set' of lakes should match the lake under study in terms of geographic region and lake morphology, and should have a range of water quality characteristics extending beyond the investigation site. In the study by Bennion *et al.* (2004), for example, a north-west European training set of 152 relatively small shallow lakes was used for the smaller productive Scottish lochs, and a training set of 56 relatively large, deep lakes was used for the larger, deeper, less productive test sites.

- Application of the training set transfer functions to fossil diatom assemblages in sediment cores to derive past water chemistry. Reconstruction of past environmental conditions involves weighted averaging (WA) regression and calibration, with WA partial least squares (WA-PLS) analysis (Bennion *et al.*, 2004).

Species assemblages Changes in water quality from historic times to the present day can be inferred by comparison of species associations at the top (current time) and bottom (reference state) of the core. In the study by Bennion *et al.* (2004) on Scottish freshwater lochs, diatom species counts were expressed as percentage relative abundances, and a detrended correspondence analysis (DCA) carried out of the top and bottom of the cores to assess the direction and magnitude of the floristic (algal) change. DCA results reveal two clear axes of variation (accounting for 62% and 49% of sample variance) in the species data. The DCA biplot (Fig. 3.3) shows clear separation of species into distinctive groups (diatom assemblages), and clear floristic changes for some

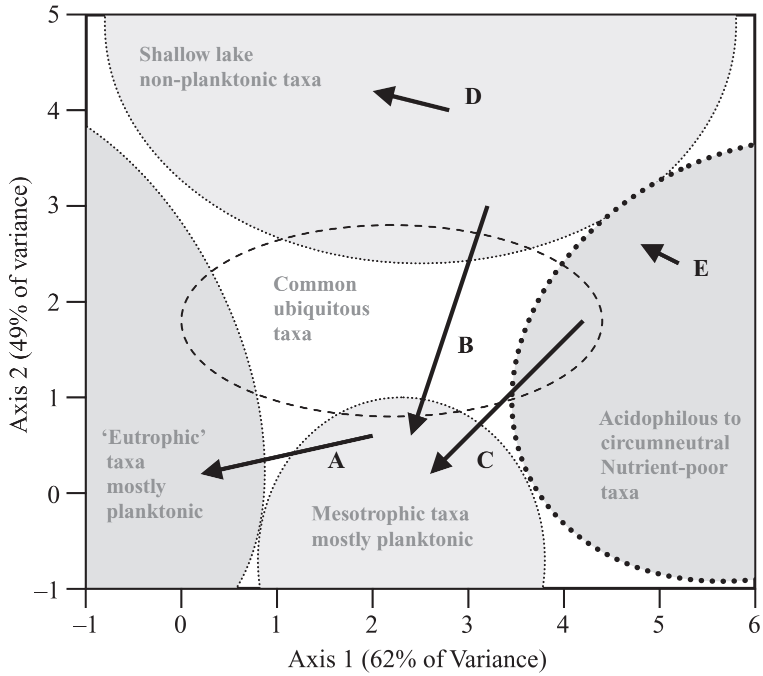

Figure 3.3 Diatom assemblages in sediment cores taken from 24 fresh-water Scottish lakes. (Figure adapted and redrawn from Bennion *et al.*, 2004). Diagrammatic view of a detrended correspondence analysis (DCA) biplot showing: (a) Individual sites (arrows). Each arrow denotes the transition from the bottom of the sediment core (reference sample) to the top (current state). Five sites are shown: A, mesotrophic to eutrophic plankton. B, mainly benthic to planktonic diatoms. C, oligotrophic to mesotrophic state. D. Shallow lake, little change. E. Deep oligotrophic lake, little change. (b) Diatom assemblages, in different regions of the plot. Shallow lake, non-planktonic (*Achnanthes*, *Cocconeis*, *Cymbella*, *Fragilaria*, *Navicula*); acidophilous nutrient-poor (*Achnanthes minutissima*, *Brachysira vitrea*, *Cyclotella comensis*, *Cyclotella kuetzingiana*, *Tabellaria flocculosa*); mesotrophic, planktonic (*Asterionella formosa*, *Aulacoseira subarctica*, *Fragilaria crotonensis*); eutrophic planktonic (*Aulacoseira granulata*, *Cyclostephanos*, *Stephanodiscus*); common ubiquitous taxa.

sites (Fig. 3.3 A, B, C), which indicated clear alterations in water quality. In a number of deep lochs (C), limited eutrophication has occurred, with transition from a *Cyclotella*/*Achnanthes* assemblage to a species combination (*Asterionella*/*Aulacoseira*) typical of mesotrophic waters. Some shallow lochs (B) also showed nutrient increase, indicated by transition from a non-planktonic (largely benthic) to a plankton-dominated diatom population. In other cases, deep oligotrophic (E) and shallow (D) lochs showed little change in diatom assemblage, indicating minimal alteration in water quality.

Multiproxy approach In a multiproxy approach, diatoms are just one of a number of groups of organisms that are counted and analysed within the lake sediments (Bennion and Battarbee, 2007). For European limnologists, the stimulus for a multiproxy approach has come with the most recent Water Framework Directive (WFD; European Union, 2000). This focuses on ecological integrity rather than simply chemical water quality, for which the use of hydro-chemical transfer functions and diatom species assemblage analysis are not sufficient.

Multiproxy analysis uses as broad a range of organisms within the food web (e.g. pelagic food web) as possible, commensurate with those biota with remains that persist in the sediment in an identifiable form. In addition to micro-algae (diatoms, chrysophytes), fossil indicators also include macroalgae (Charophyta), protozoa (thecamoebae), higher plants (pollen and macro-remains), invertebrates (chironomids, ostracods, cladocerans) and vertebrates (fish scales).

This approach is illustrated by the study of Davidson *et al.* (2005) on Groby Pool, United Kingdom, a shallow lake that has undergone nutrient enrichment in the past 200 years. Comparison of 20-year slices from the sediment surface (recent: 1980–2000) and base (reference: 1700–1720) indicate major changes in lake ecology (Fig. 3.4), driven primarily by alterations in water quality (Bennion and Battarbee, 2007). The ecological reference state is one of dominance by benthic diatoms, colonization by low nutrient-adapted macro-algae and higher plants, with detectable invertebrates restricted to plant-associated Chydoridae. In contrast to this, the current-day ecosystem is much more productive – dominated by planktonic algae, high

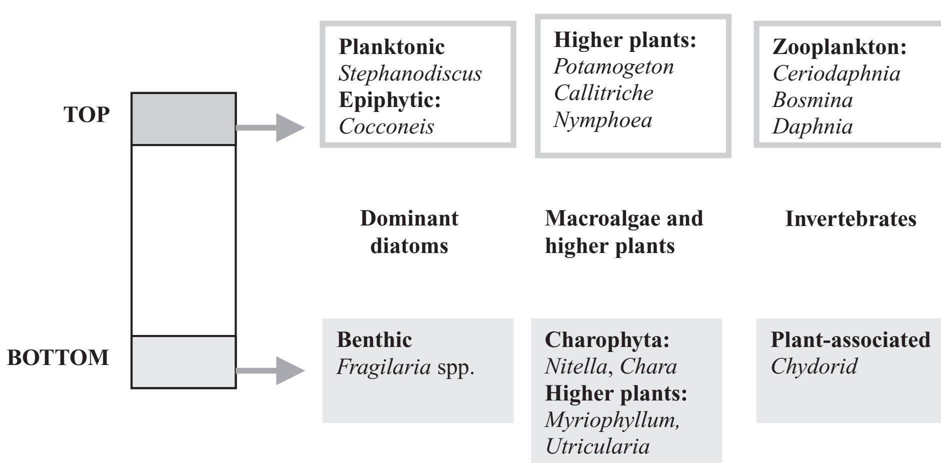

Figure 3.4 Multi-proxy palaeoecological analysis of a sediment core: Groby Pool, United Kingdom. Analysis of 20-year slices from the top (recent ecology, ∼1980–2000) and bottom (reference ecology, ∼1700–1720) samples of the core. Figure adapted and redrawn from Bennion and Battarbee (2007), original data from Davidson *et al.* (2005).

nutrient-adapted macrophytes and abundant zooplankton populations.

Other examples of a multiproxy approach to lake sediment analysis include the studies of Cattaneo *et al.* (1998) on heavy metal pollution in Italian Lake d'Orta (Section 3.2.2) and studies on eutrophication in six Irish lakes (Taylor *et al.*, 2006). The latter study involved sediment analysis of cladocerans, diatoms and pollen from mesotrophic-hypertrophic lakes, to reconstruct past variations in water quality and catchment conditions over the past 200 years. The results showed that five of the lakes were in a far more productive state compared to the beginning of the sediment record, with accelerated enrichment since 1980.

3.2.3 Water quality parameters: inorganic and organic nutrients, acidity and heavy metals

A wide range of chemical parameters can be considered in relation to general lake water quality – including total salt content (conductivity), inorganic nutrients (nitrogen and phosphorus), soluble organic nutrients, acidity, heavy metal contamination and presence of coloured matter (caused particularly by humic materials). The diversity and inter-relationships of different lakes in relation to these characteristics was emphasized by the study of Rosen (1981), evaluating lake types and related planktonic algae from August sampling of 1250 Swedish standing waters. A summary from this study of algae characteristic of Swedish acidified lakes, oligotrophic forest lakes (varying in phosphorus content and conductivity), humic lakes, mesotrophic and eutrophic lakes is given by Willen (2000).

In this section, the role of algae as bioindicators is considered in reference to four main aspects of lake water quality – inorganic nutrients, soluble organic nutrients, acidity and heavy metal contamination.

Inorganic nutrient status: oligotrophic to eutrophic lakes

The trophic classification of lakes, based primarily on inorganic nutrient status, has become the major descriptor of different lake types. Its importance reflects:

- the key role that nutrients have on the productivity, diversity and identity of algae and other lake organisms
- a major distinction between deep mountain lakes (typically oligotrophic) and shallow lowland lakes (typically eutrophic)
- the major impact that humans have on changing the ecology of lakes, typically from oligotrophic to eutrophic
- ecological problems that may arise as lakes change from eutrophic to hypertrophic, leading to degenerative changes that can only be reversed by human intervention.

The diverse ecological effects that increasing nutrient concentrations have on lake ecology have been widely reported (e.g. Kalff, 2002; Sigee, 2004), including the ecological destabilization that occurs at very high nutrient concentrations.

Definition of terms Lake classification, from oligotrophic (low nutrient) to mesotrophic and eutrophic (high nutrient), is based on the twin criteria of productivity and inorganic nutrient concentration – particularly nitrates and phosphates. Various schemes have been proposed to define these terms, including one developed by the Organization for Economic Cooperation and Development (OECD, 1982). This scheme (Table 3.2) uses fixed boundary values for nutrient concentration (mean annual concentration of total phosphorus), and productivity (chlorophyll-*a* concentration, Secchi depth). In this scheme, for example, the mean annual concentration of total phosphorus ranges from 4–10 $\mu g\, l^{-1}$ for oligotrophic lakes, and 35–100 $\mu g\, l^{-1}$ for eutrophic waters. In addition to total phosphorus, boundaries for the main soluble inorganic nutrients – orthophosphate and dissolved inorganic nitrogen (nitrate, nitrite, ammonia) have also been designated (Technical Standard Publication, 1982).

Phytoplankton net production (biomass) is determined either as chlorophyll-*a* concentration (mean/maximum annual concentration in surface waters) or as Secchi depth (mean/maximum annual value). Examples of total volumes of planktonic algae at different trophic levels (Norwegian lakes) are also given in Table 3.2, together with characteristic bioindicator algae.

Algae as bioindicators of inorganic trophic status

Planktonic algae within lake surface (epilimnion) samples can be used to define lake trophic status in terms of their overall productivity (Table 3.2) and species composition (Table 3.3). Species composition can be related to trophic status in four main ways – seasonal succession, biodiversity, bioindicator species and determination of bioindices.

1. **Seasonal succession**. In temperate lakes, the development of algal biomass and the sequence of phytoplankton populations (seasonal succession) directly relate to nutrient availability. In all cases the season commences with a diatom bloom, but subsequent progression (Reynolds, 1990) can be separated into four main categories (Table 3.3).

- Oligotrophic lakes. In low nutrient lakes the spring diatom bloom is prolonged, and diatoms may dominate for the whole growth period. Chrysophytes (*Uroglena*) and desmids (*Staurastrum*) may also be present, and in some lakes *Ceratium* and *Gomphosphaeria* may be able to grow in the nutrient-depleted waters by migrating down the water column to higher nutrient conditions.

- Mesotrophic lakes. These have a shorter diatom bloom (dominated by *Asterionella*), often followed by a chrysophyte phase then mid-summer dinoflagellate, blue-green and green algal blooms.

- Eutrophic lakes. In high nutrient lakes, the spring diatom bloom is further limited, leading to a clear-water phase (dominated by unicellular algae), followed by a mid-summer bloom in which large unicellular (*Ceratium*), colonial filamentous (*Anabaena*) and globular (*Microcystis*) blue-greens predominate.

- Hypertrophic lakes. These include artificially fertilized fish ponds (Pechar *et al.*, 2002) and lakes with sewage discharges, and are dominated throughout the season by small unicellular algae with short life cycles. The algae form a succession of dense populations, out-competing larger colonial organisms which are unable to establish themselves.

2. **Species diversity**. Bioindices of species diversity can be derived from species counts and fall into three main categories (Sigee, 2004) – species richness (Margalef index), species evenness/dominance (Pielou index, Simpson index) and a combination of richness and dominance (Shannon–Wiener index).

One of the most commonly-used indices (d), developed by Margalef (1958), combines data on the total number of species identified (S) and total number of individuals (N), where:

$$d = (S - 1)/\log_e N \qquad (3.1)$$

During the summer growth phase, species diversity is typically low in oligotrophic lakes, rising progressively in mesotrophic and eutrophic lakes, but falling again in some eutrophic/hypertrophic lakes where small numbers of species may out-compete other algae. The effects of increasing nutrient levels on algal diversity (d) are illustrated by Reynolds (1990), with summer-growth values of 3–6 for the nutrient-deficient North Basin of Lake Windermere (United Kingdom) – falling to levels of 2–4 in a nutrient-rich lake (Crose Mere, United Kingdom) and 0.2–2 for a hypertrophic water body (fertilized enclosure, Blelham Tarn, United Kingdom).

3. **Bioindicator species**. Some algal species and taxonomic groups show clear preferences for particular lake conditions, and can this act as potential bioindicators. In a broad comparison of oligotrophic versus eutrophic waters, desmids (green algae) tend to occur mainly in low nutrient waters while colonial

blue-green algae are more typical of eutrophic waters. Such generalizations are not absolute, however, since some desmids (e.g. *Cosmarium meneghinii*, *Staurastrum* spp.) are typical of meso- and eutrophic lakes, while colonial blue-green algae such as *Gomphosphaeria* are also found in oligotrophic waters.

Although it is not possible to pin-point individual algal species in relation to particular trophic states, it is possible to list organisms that are typical of summer growths in different standing waters (Table 3.3). Identification of such indicator species, particularly at high population levels, gives a good qualitative indication of nutrient state. As an example of this the high-nutrient lake illustrated in Fig. 3.5 is characteristic of temperate eutrophic water bodies,with high productivity, characteristic seasonal progression (Fig. 2.8) and with the eutrophic bioindicator algae listed in Table 3.3. In addition to phytoplankton bioindicators, the trophic status of the lake is also reflected in extensive growths of attached algae such as *Cladophora* (Fig. 2.28) and in the dense periphyton communities (Fig. 2.29) that occur in the littoral reed beds. Analysis of lake sediments (Capstick, unpublished observations) indicates increased eutrophication in recent historical times, with higher proportions of the diatoms *Asterionella formosa* plus *Aulacoseira granulata* var. *angustissima* and marked decreases in *Cyclotella ocellata* and *Tabellaria flocculosa* (more typical of low-nutrient waters) over the last 50 years.

Figure 3.5 Eutrophic lake (Rostherne Mere, United Kingdom). The high nutrient status of the lake is indicated by water analyses (mean annual total phosphorus $>50\ \mu g\ l^{-1}$), high productivity (maximum chl-*a* concentration typically $>60\ \mu g\ l^{-1}$) and characteristic bioindicator algae. These include planktonic blooms of *Anabaena*, *Aphanizomenon*, *Microcystis* (colonial blue-greens) plus various eutrophic algae (see text). Attached macroalgae (*Cladophora*) and periphyton communities (present on the fringing reed beds Fig. 2.29) are also well-developed.

Although individual algal species can be rated primarily in terms of trophic preferences, they are also frequently adapted to other related ecological factors.

- *Acidity*: oligotrophic waters are frequently slightly acid with low Ca concentrations, and vice versa for eutrophic conditions.

- *Nutrient balance*: mesotrophic waters may be nitrogen-limiting (high P/N ratio), promoting the growth of nitrogen-fixing (e.g. *Anabaena*) but not non-fixing (e.g *Oscillatoria*) colonial blue-green algae.

- *Long-term stability*: In hypertrophic waters, domination by particular algal groups may vary with the long-term stability of the water body. High-nutrient lakes, with established populations of blue-greens and dinoflagellates, often have these as dominant algae during the summer months. Small newly-formed ponds, however, are often dominated by rapidly-growing chlorococcales (green algae) and euglenoids. The latter are particularly prominent at high levels of soluble organics (e.g. sewage ponds), using ammonium as a nitrogen source. Some of the most hypertrophic and ecologically-unstable waters are represented by artificially fertilized fish ponds, such as those of the Třeboň wetlands, Czech Republic (Pokorny *et al.*, 2002a,b).

In addition to considering individual algal species, taxonomic grouping (assemblages) may also be useful environmental indicators. Reynolds (1980) considered species assemblages in relation to seasonal changes and trophic status, with some groupings (e.g. *Cyclotella comensis*/*Rhizosolenia*) typical of oligotrophic waters and others typical of eutrophic (e.g. *Anabaena*/*Aphanizomeno*/*Gloeotrichia*) and hypertrophic (*Pediastrum*/*Coelastrum*/*Oocystis*) states. Consideration of algae as groups rather than individual species leads on to quantitative analysis and determination of trophic indices.

4. **Phytoplankton trophic indices**. In mixed phytoplankton samples, algal counts can be quantitatively expressed as biotic indices to characterize lake trophic status (Willen, 2000). These indices occur at three levels of complexity (Table 3.4).

1. Indices based on major taxonomic groups Early phytoplankton indices, developed by Thunmark (1945), Nygaard (1949) and Stockner (1972) used major taxonomic groups that were considered typical of oligotrophic (particularly desmids) or eutrophic (chlorococcales, blue-greens, euglenoids) conditions. The proportions of eutrophic/oligotrophic species generated a simple ratio which could be used to designate trophic status (Table 3.4a). Using the chlorophycean index of Thunmark (1945), for example, counts of chlorococcalean and desmid species can be expressed as a ratio, which indicates trophic status over the range oligotrophy (<1) to eutrophy (>1).

Although such indices provided useful information (see below), they tended to lack environmental resolution since many algal classes turn out to be heterogeneous – containing species typical of oligo- and eutrophic lakes. Problems were also encountered in some of the early studies with sampling procedures,

Table 3.4 Lake Trophic Indices

Index	Calculation	Result	Reference
(a) Major taxonomic groups: numbers of species			
Chlorophycean index	Chlorococcales spp./Desmidiales spp.	<1 = oligotrophy >1 = eutrophy	Thunmark (1945)
Myxophycean index	Cyanophyta spp./Desmidiales	<1 = oligotrophy >1 = eutrophy	Nygaard (1949)
Diatom index	Centrales spp./Pennales		
Euglenophycean index	Euglenophyta/ Cyanophyta + Chlorophyta		
A/C diatom index	Araphid pennate/centric diatom spp.	<1 = oligotrophy >2 = eutrophy	Stockner (1972)
Compound index	Cyanophyta + Chlorococcales + Centrales + Euglenophyta spp./ Desmidiales spp.	<1 = oligotrophy 1–3 = mesotrophy	Nygaard (1949)
(b) Indicator algae: species counts or biovolumes			
Species counts	Eutrophic spp./ Oligotrophic spp.	<8 = oligotrophy	Heinonen (1980)
Species biovolumes	Eutrophic spp./oligotrophic spp.	<35 = oligotrophy	
(c) Indicator algae: species given weighted scores			
Trophic index	$I_L = \sum(fI_s)/\sum f$	10–100, high values indicate higher pollution	Hörnström (1981)
Trophic index – biovolumes	$I_T = \sum(vI_s)/\sum v$	Index values for each trophic level	Brettum (1989)

Hörnström's index: I_L = trophic index of lake, f = species frequency on a 5-degree scale, I_s = trophic index of species.
Brettum's index: I_T = index of trophic level T, v = volume of species per litre, I_s = trophic index of species.

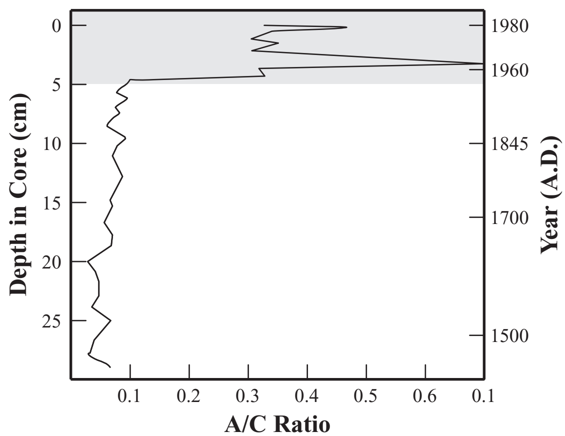

Figure 3.6 Changes in the ratio of araphid pennate to centric diatoms (A/C ratio) as an indicator of gradual eutrophication in Lake Tahoe (United States). The dramatic increase in the A/C ratio during the late 1950s is associated with increasing human population around the lake. Taken from Sigee (2004), adapted and redrawn from Byron and Eloranta, 1984.

where net collection of algae resulted in loss of small-sized (single cells or small colonies) species. Such algae are often dominating elements in the plankton community, and their loss from the sample meant that the index was not representative.

Example: The A/C diatom index of Stockner (1972). The ratio of araphid pennate/centric diatoms (A/C ratio) provides a good example of the successful use of a broad taxonomic index in a particular lake situation. Studies by Byron and Eloranta (1984) on the sediments of Lake Tahoe (United States) showed a clear change in the diatom community during the late 1950s (Fig. 3.6), consistent with eutrophication. The increase in A/C ratio (<0.7 to 0.7) at this time was related to increases in the indicator species *Fragilaria crotonensis* and *Synedra* spp. (with decreases in *Cyclotella ocellata* and *Melosira* spp.), and occurred at a time of increasing human population around the lake. Changes in the A/C ratio (fossil record) parallel contemporary indicators of trophic transition (Carney *et al.*, 1994) – including an increase in primary productivity (annual rise of 5.6% from 1959 to 1990), decrease in Secchi depth and an increase in available nitrogen (as NO_3).

2. Indices based on indicator species Improvements in sampling procedure, recording the full spectrum of species sizes, led to quantitative information being obtained on all planktonic algae and the development of indices based on separate indicator species rather than taxonomic groups.

The most direct indices are based simply on the recorded presence of eutrophic and oligotrophic indicator species (Table 3.4b). Heinonen (1980), for example, studied the occurrence of algae in a range of Finnish lakes, listing about 100 species indicating eutrophy and 25 indicating oligotrophy. Species were classified as eutrophic indicators when their occurrence in eutrophic/oligotrophic waters was >2. The corresponding ratio for oligotrophic indicators was 0.7. The trophic state of lakes was calculated as the ratio of eutrophic/oligotrophic species counts or as biovolumes. Index values <8 indicated oligotrophic conditions in the case of the species count, and <35 for the biovolume ratio (Table 3.4).

3. More complex indices These are based on quantitative rating of individual indicator species, and been devised by Hörnström (1981) and Brettum (1989). Brettum has developed a system in which 150 algal species are scored on their proportion of total biomass for various classes of environmental variable (pH, total P content, total N content, N/P ratio, etc.). A final trophic index could then be constructed for individual trophic levels from ultra-oligotrophy to hypertrophy.

Organic pollution

According to Palmer (1969), organic pollution tends to influence the algal flora more than many other factors in the aquatic environment such as water hardness, trophic status, light intensity, pH, DO (dissolved oxygen), rate of flow, size of water body and other types of pollutants. Organic pollution resembles trophic status (previous section) in relating to nutrient availability, but differs in being soluble organic rather than inorganic nutrients. The terms oligo-, meso- and eutrophic are used specifically for inorganic nutrients and not soluble organics (Table 3.2). The use of algae (and other organisms) for monitoring organic

Table 3.5 Organic Pollution: Most Tolerant Algal Genera and Species (Palmer, 1969)

No.	Taxon	Class	Pollution Index	Freshwater Habitat
	Genus			
1	*Euglena*	Eu	5	Planktonic
2	*Oscillatoria*	Cy	5	Planktonic or benthic
3	*Chlamydomonas*	Ch	4	Planktonic
4	*Scenedesmus*	Ch	4	Planktonic
5	*Chlorella*	Ch	3	Planktonic
6	*Nitzschia*	Ba	3	Benthic or planktonic
7	*Navicula*	Ba	3	Benthic
8	*Stigeoclonium*	Ch	2	Attached
9	*Synedra*	Ba	2	Planktonic and epiphytic species
10	*Ankistrodesmus*	Ch	2	Planktonic
	Species			
1	*Euglena viridis*	Eu	6	Ponds and shallow lakes
2	*Nitzschia palea*	Ba	5	Lakes and rivers
3	*Oscillatoria limosa*	Cy	4	Stagnant or standing waters
4	*Scenedesmus quadricauda*	Ch	4	Lake phytoplankton
5	*Oscillatoria tenuis*	Cy	4	Ponds and shallow pools
6	*Stigeoclonium tenue*	Ch	3	Epiphyte, shallow waters
7	*Synedra ulna*	Ba	3	Lake phytoplankton
8	*Ankistrodesmus falcatus*	Ch	3	Lake phytoplankton
9	*Pandorina morum*	Ch	3	Lake phytoplankton
10	*Oscillatoria chlorina*	Cy	2	Stagnant or standing waters

Ten most tolerant algal genera and species. listed (Palmer, 1969) in order of decreasing tolerance. Algal phyla: Cyanophyta (Cy), Chlorophyta (Ch), Euglenophyta (Eu) and Bacillariophyta (Ba).
Pollution index – see text.

pollution was originally pioneered by Kolkwitz and Marsson (1908).

Palmer (1969) carried out an extensive literature survey to assess the tolerance of algal species to organic pollution, and to incorporate the data into an organic pollution index for rating water quality. Algal genera and species were listed separately in order of their pollution tolerance (Table 3.5), and included a wide range of taxa (euglenoids, blue-greens, green algae and diatoms) as well as planktonic and benthic forms. The assessment of genera was determined as the average of all recorded species within the genus, and is perhaps less useful than the species rating – where single, readily identifiable taxa can be directly related to pollution level.

The species organic pollution index developed by Palmer uses the top 20 algae in the species list (top 10 shown in Table 3.5). Algal species are rated on a scale 1 to 5 (intolerant to tolerant) and the index is simply calculated by summing up the scores of all relevant taxa present within the sample. In analysing the water sample, all of the 20 species are recorded, and an alga is considered to be ‘present’ if there are 50 or more individuals per litre.

Examples of environmental scores are given in Table 3.6, with values of >20 consistent with high organic pollution and <10 signifying lack of nutrient enrichment. Palmer’s index was used by Cattaneo *et al.* (1995) in their studies on a fluvial lake of the St. Laurence River system (Canada), with values from

Table 3.6 Organic Pollution at Selected Sites: Application of Palmer's (1969) Indices

Aquatic Site	Recorded Genera	Pollution Rating	High Organic Pollution
Sewage stabilization pond	*Ankistrodesmus*, *Chlamydomonas*, *Chlorella*, *Cyclotella*, *Euglena*, *Micractinium*, *Nitzschia*, *Phacus*, *Scenedesmus*	25	Clear supporting evidence
Greenville Creek, Ohio	*Euglena*, *Nitzschia*, *Oscillatoria.*, *Navicula*, *Synedra*	18	Probable
Grand Lake, Ohio	*Anacystis*, *Ankistrodesmus*, *Melosira*, *Navicula*, *Scenedesmus*, *Synedra*	13	No evidence
Lake Salinda, Indiana	*Chlamydomonas*, *Melosira*, *Synedra*	7	No organic enrichment

Data from Palmer (1969) for four sites in the United States. See text for calculation of pollution rating.

15–24 at different sampling sites indicating moderate to high levels of organic pollution. Care should be taken in applying this index, since many sites with high organic pollution (e.g. soluble sewage organics) also have high inorganic nutrients (phosphates, nitrates), and algae characteristic of such sites are typically tolerant to both.

In addition to the general application of Palmer's index using a wide range of algal groups, the index may also be more specifically applied to benthic diatoms in the assessment of river water quality (Section 3.4.5).

Acidity

Acidity becomes an important aspect of lake water quality in two main situations – naturally occurring oligotrophic waters and in cases of industrial pollution. Algal bioindicators have been important for monitoring lake pH change both in terms of lake sediment analysis (fossil diatoms, Section 3.2.2) and contemporary epilimnion populations – see below.

Oligotrophic waters The tendency for oligotrophic lakes to be slightly acid has already been noted in relation to inorganic nutrient status (bioindicator species), and for this reason many algae typical of low nutrient waters – including various desmids and species of *Dinobryon* (Table 3.3) – are also tolerant of acidic conditions. Acidic, oligotrophic waters tend to be low in species diversity. In a revised classification of British lakes proposed by Duker and Palmer (2009), naturally acid lakes include highly acid bog/heathland pools (group A), acid moorland pools and small lakes (group B) and acid/slightly acid upland lakes (group C).

Industrial acidification of lakes Industrial atmospheric pollution during the last century led to acid deposition and acidification of lakes in various parts of central and northern Europe.

Central Europe In Central Europe, regional emissions of S (SO_4)and soluble inorganic N (NO_3, NH_4) compounds reached up to $\sim$280 mmol m^{-2} $year^{-1}$ between 1940 and 1985, then declined by $\sim$80% and $\sim$35% respectively during the 1990s (Kopacek *et al.*, 2001). This atmospheric deposition led to acid contamination of catchment areas and the resulting acidification of various Central European mountain lakes, including a group of eight glacial lakes in the Bohemian Forest of the Czech Republic (Vrba *et al.*, 2003; Nedbalova *et al.*, 2006).

Studies by Nedbalova *et al.* (2006) on chronically acidified Bohemian Forest lakes have demonstrated some recovery from acid contamination. This is now beginning, about 20 years after the reversal in acid deposition that occurred in 1985, with some lakes still chronically acid – but others less acid and in recovery mode (Table 3.7). Chronically-acid lakes have low primary productivity, with low levels of phytoplankton and zooplankton and domination by

Table 3.7 Bioindicator Algae of Acid Lakes: Comparison of Chronically Acidified and Recovery-Mode Oligotrophic Bohemian Forest Lakes (Nedbalova *et al.*, 2006)

	Chronically-Acidified Lakes	Recovery-Mode Lakes
Lakes	Cerne jezero, Certova jezero, Rachelsee	Kleiner Arbersee, Prasilske jezero, Grosser Arbersee, Laka
pH and buffering of surface water	pH 4.7–5.1 Carbonate buffering system not operating	pH 5.8–6.2 Carbonate buffering system now operating
Total plankton biomass	$\sim$100 μg C l^{-1} Very low, dominated by bacteria	$\sim$200 μg C l^{-1} Higher, dominated by phytoplankton and crustacean zooplankton
Phytoplankton biomass (Chl-*a* concentration)	0.6–2.8 μg l^{-1}	4.2–17.9 μg l^{-1}
Dominant algae	No differences in species composition of phytoplankton: Dinoflagellates: *Peridinium umbonatum*, *Gymnodinium uberrimum* Chrysophyte: *Dinobryon* spp.	
Other algae typically present in all lakes	Blue-green: *Limnothrix* sp., *Pseudanabaena* sp. Dinoflagellates: *Katodinium bohemicum* Cryptophytes: *Cryptomonas erosa*, *Cryptomonas marssonii* Cryptophytes: *Bitrichia ollula*, *Ochromonas* sp., *Spiniferomonas* sp., *Synura echinulata* Green algae: *Carteria* sp., *Chlamydomonas* sp.	
Phytoplankton biodiversity (total taxa)	No significant differences in biodiversity: 19–22 taxa in chronically acidified lakes, 15–27 in recovery-mode ones.	

All data obtained during a September 2003 survey of the lakes.

heterotrophic bacteria. Lakes in recovery mode have a higher plankton standing crop, which is dominated by phytoplankton and zooplankton rather than bacteria.

Phytoplankton species composition is characterized by acid-tolerant oligotrophic unicellular algae. Lakes in recovery mode are still acid, and have a phytoplankton composition closely similar to chronically acid standing waters. These are dominated by two dinoflagellates (*Peridinium umbonatum*, *Gymnodinium uberrimum*) and a chrysophyte (*Dinobryon* sp.), which serve as bioindicators. Other algae present in the Czech acid lakes (Table 3.7) included many small unicells (particularly chrysophytes and cryptophytes). Diatoms were not present, presumably due to the chemical instability of the silica frustule under highly acid conditions.

Northern Europe Acidification of lakes in southern Sweden follows a similar pattern in terms of algal species, with domination of many acid lakes by the same bioindicator algae seen in central Europe – *Peridinium umbonatum*, *Gymnodinium uberrimum* and *Dinobryon* sp. (Hörnström, 1999). In an earlier study of acid Swedish lakes (typically total phosphorus $<5\ \mu g\ l^{-1}$, pH < 5), Rosen (1981) also identified *Mougeotia* spp., *Oocystis submarina* and small naked chryso- and dinoflagellates as typical of such conditions.

In addition to atmospheric pollution, lake acidification has also been caused by industrial effluents – where it is frequently linked with heavy metal pollution.

Heavy metal pollution

Planktonic algae are considerably influenced by heavy metal pollution (Whitton, 1984), which can arise in a variety of ways – including sewage discharge (Seidl. *et al.*, 1998), resuspension of toxic sediments (Nayar *et al.*, 2004) and industrial effluent discharge.

Cattaneo *et al.* (1998) studied the response of lake diatoms to heavy metal contamination, analysing sediment cores in a northern Italian lake (Lake d'Orta) subject to industrial pollution.

Environmental changes Lake d'Orta had been polluted with copper, other metals (Zn, Ni, Cr) and acid (down to pH 4) for a period of over 50 years – commencing in 1926 and reaching maximum pollutant levels (30–100 μg Cu l^{-1}) between 1950 and 1970. Lake sediment cores collected after 1990 were analysed for fossil remains of diatoms, thecamoebians (protozoa) and cladocerans (zooplankton), all of which showed a marked reduction in mean size during the period of industrial pollution.

Diatom response to pollution The initial impact of pollution, recorded by contemporary analyses, was to dramatically reduce populations of phytoplankton, zooplankton, fish and bacteria. Subsequent sediment core analyses of diatoms showed that heavy metal pollution:

- Caused a marked decrease in the mean size of individuals. The proportion of cells with a biovolume of $<10^2$ μm^3 increased from under 10% of the total population in 1920 to over 60% in 1950.

- The decrease in mean diatom size was caused primarily by a change in taxonomic composition from assemblages dominated by *Cyclotella comensis* (10^2–10^3 μm^3) and *C. bodanica* (10^3–10^4 μm^3) to populations dominated by *Achnanthes minutissima* ($<10^2$ μm^3).

- The change in mean size was also caused by a shift in the size within a single taxon – *Achnanthes minutissima*. The mean length of this diatom decreased significantly from about 14 μm before pollution to a minimum value of 9 μm during 1950–1970 (Fig. 3.7).

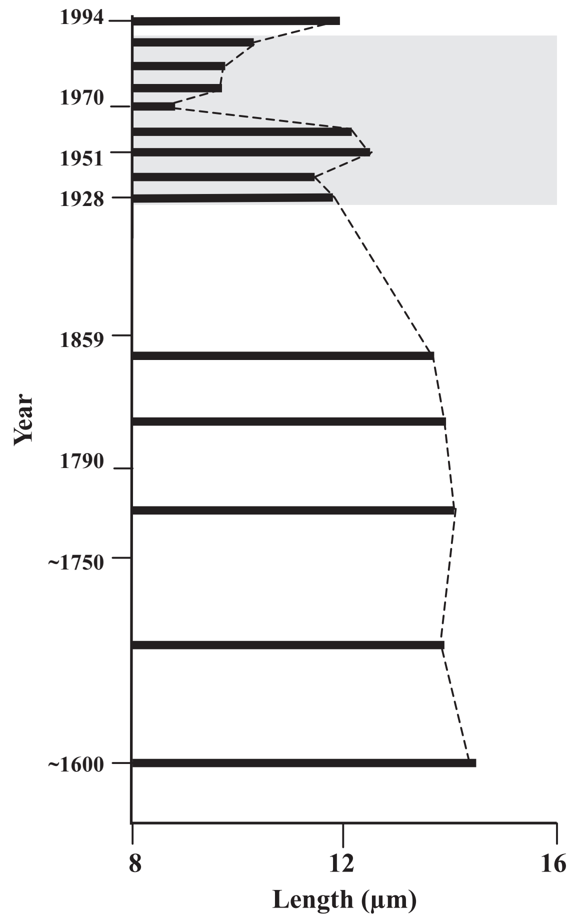

Figure 3.7 Changes in the average valve length of the diatom *Achnanthes minutissima* measured along a sediment core collected in Lake d'Orta (Italy). The size of this heavy metal-tolerant diatom showed a marked decrease during the period of industrial contamination (shaded area), falling to 9 μm by the end (1970) of the most acute period of toxicity. Figure adapted and re-drawn from Cattaneo *et al.*, 1998.

Achnanthes minutissima as a bioindicator Dominance of Lake d'Orta under conditions of heavy metal pollution by *A. minutissima* is in agreement with the known ability of this diatom to withstand strong metal stress. Other studies (Deneiseger *et al.*, 1986, Takamura *et al.*, 1990) have shown it to be dominant in streams subject to heavy metal contamination. The diatom is cosmopolitan, however, often common in benthic assemblages of neutral waters, and its domination of a particular environment is not therefore directly indicative of heavy metal pollution. In spite of this, the presence of dominant populations (coupled with a decrease in mean cell size) is consistent with severe environmental stress – and would corroborate other environmental data indicating heavy metal or acid contamination.

3.3 Wetlands

Wetlands comprise a broad range of aquatic habitats – including areas of marsh, fen, peatland or open water, with water that is static or flowing and may be fresh, brackish or salt (Boon and Pringle, 2009). Many wetlands form an ecological continuum with shallow lakes (Sigee, 2004), with standing water present for the entire annual cycle (permanent wetlands) or just part of the annual cycle (seasonal wetlands). Because the water column of wetlands is normally only 1–2 m in depth, it is not stratified and the photic zone (light penetration) extends to the sediments – promoting growth of benthic and other attached algae.

Wetlands are typically dominated by free-floating and rooted macrophytes, which are the major source of carbon fixation. Although growth of algae may be limited due to light interception by macrophyte leaves, leaf and stem surfaces frequently provide a substratum for epiphytic algae – and extensive growths of periphyton may occur. Wetlands tend to be very fragile environments, liable to disturbance by flooding, desiccation (human drainage), eutrophication (agriculture and waste disposal) and increased salinity (coastal wetlands). Algal bioindicators of water quality are particularly important in relation to eutrophication and changes in salinity – such as those occurring in Florida (United States) coastal wetlands

Case study 3.1 Salinity changes in Florida wetlands

In recent decades, wetlands in Florida have been under particular threat due to extensive drainage, with many of the interior marshlands lost to agricultural and urban development. This has resulted in a shrinkage of wetland areas to the coastline periphery. In addition to their reduced area, coastal marshes in south-east Florida have also suffered a rapid rise in saltwater encroachment due partly to freshwater drainage, but also to rising sea levels resulting from global warming.

Studies by Gaiser *et al.* (2005) have been carried out on an area of remnant coastal wetland to quantify algal communities in three major wetland ecosystems – open freshwater marshland, forested freshwater marshland and mangrove saltwater swamps. The study looked particularly at periphyton (present as an epiphyte and on soil sediments) and the use of diatom bioindicator species to monitor changes in salinity within the wetland system.

Effects of salinity The major microbial community throughout the wetland area occurred as a cohesive periphyton mat, composed of filaments of blue-green algae containing coccoid blue-greens and diatoms. Periphyton biomass, determined as ash-free dry weight, was particularly high (317 $g\,m^{-2}$) in open freshwater marshes, falling to values of 5–20 $g\,m^{-2}$ in mangrove saltwater swamps. Salinity had an over-riding effect on algal community composition throughout the wetlands. The filamentous blue-greens *Scytonema* and *Schizothrix* were most abundant in freshwater, while *Lyngbya* and *Microcoleus* dominated saline areas. The most diverse algal component within the periphyton mats were the diatoms, with individual species typically confined to either freshwater or saline regions. Dominant species within the separate ecosystems are listed in Table 3.8, with freshwater diatoms predictably having lower salinity optima (2.06–4.20 ppt) compared to saltwater species (11.79–18.38 ppt). Salinity tolerance range is also important, and it is interesting to note that that dominant diatoms in freshwater swamps had higher salinity optima and tolerance ranges compared to other freshwater diatoms – suggesting that the ability to tolerate limited saltwater contamination may be important. The converse is true for the saltwater swamps, where dominant species were not those with the highest salinity optima.

Table 3.8 Freshwater and Saltwater Wetland Diatoms (Florida, USA): Salinity Optima and Tolerance (Data from Gaiser *et al.*, 2005)

Species	Salinity Optimum*	Salinity Tolerance*
Diatoms with lowest salinity optima		
Achnanthidium minutissimum	1.80	0.20
Nitzschia nana	1.83	0.72
Navicula subrostella	1.84	0.21
A. Open freshwater marshland		
Nitzschia palea var. *debilis*	2.06	1.24
Encyonema evergladianum	2.25	1.51
Brachysira neoexilis	2.69	1.77
B. Forested freshwater marshland		
Nitzschia semirobusta	2.19	0.86
Fragilaria synegrotesca	3.41	2.68
Mastogloia smithii	4.20	2.75
C. Mangrove Saltwater swamp		
Amphora sp.	11.79	1.57
Achnanthes nitidiformis	17.28	0.51
Tryblionella granulata	18.38	0.92
Diatoms with highest salinity optima		
Caloneis sp.	20.52	1.06
Tryblionella debilis	20.86	1.33
Mastogloia elegans	20.99	1.03

Diatoms are listed in relation to:

- Dominant species in three major wetland ecosystems (A,B,C)
- Three species with lowest salinity optima (Ecosystem A), shaded area – top of table
- Three species with highest salinity optima (Ecosystem C), shaded area – bottom of table.

*Salinity values given as ppt. Optimum and tolerance levels for individual species are derived from environmental analyses (see text).

Predictive model The salinity optima and tolerance data shown in Table 3.8 were derived from environmental samples. Analysis of diatom species composition and salinity (along with other water parameters) was carried out over a wide range of sites. Salinity optima for individual species were determined from those sites where the species had greatest abundance, and salinity tolerance was recorded as the range of salinities over which the species occurred.

The species-related data was incorporated into a computer model that could predict ambient salinity (in an unknown environment) from diatom community analysis. Environmental salinity at a particular site was determined as the mean of the salinity optima of all species present, weighted for their abundances. Predicted values for salinity based on such diatom calibration models are highly accurate (error <10%). Measurement of salinity using a portable meter has a high degree of accuracy, but values often show considerable variation along a time sequence of readings. One advantage of the use of bioindicator species in this context is that such variability is reduced because the effects of salinity on diatom communities are integrated over a period of time.

3.4 Rivers

Until recently, monitoring of river water quality in many countries (Kwandrans *et al.*, 1998) was based mainly on *Escherichia coli* titre (sewage contamination) and chlorophyll-*a* concentration (trophic status). Chlorophyll-*a* classification of the French National Basin Network (RNB), for example, distinguished five water quality levels (Prygiel and Coste, 1996): normal (chlorophyll-*a* concentration $\leq 10\ \mu g\ ml^{-1}$), moderate pollution (10–≤60), distinct pollution (60–≤120), severe pollution (120–≤300) and catastrophic pollution (>300).

The use of microalgae as bioindicators was pioneered by Patrick (Patrick *et al.*, 1954) and has concentrated particularly on benthic organisms, since the rapid transit of phytoplankton with water flow means that these algae have little time to adapt to environmental changes at any point in the river system. In contrast, benthic algae (periphyton and biofilms) are permanently located at particular sites, integrating physical and chemical characteristics over time, and are ideal for monitoring environmental quality. Examples of benthic algae present on rocks and stones within a fast-flowing stream are shown in Fig. 2.23. The use of the periphyton community for biomonitoring normally involves either the entire community, or one particular taxonomic group – the diatoms.

3.4.1 The periphyton community

Analysis of the entire periphyton community clearly gives a broader taxonomic assessment of the benthic algal population compared to diatoms only, but the predominance of filamentous algae makes quantitative analysis difficult. Various authors have used periphyton analysis to characterize water quality, including a study of fluvial lakes by Cattaneo *et al.* (1995 – Section 3.2.1). This showed that epiphytic communities could be monitored both in terms of size structure and taxonomic composition, leading to statistical resolution of physical (substrate) and water quality (mercury toxicity) parameters.

3.4.2 River diatoms

Contemporary biomonitoring of river water quality has tended to concentrate on just one periphyton constituent – the diatoms. These have various advantages as bioindicators – including predictable tolerances to environmental variables, widespread occurrence within lotic systems, ease of counting and a species diversity that permits a detailed evaluation of environmental parameters. The major drawbacks to diatoms in this respect is the requirement for complex specimen preparation and the need for expert identification.

Attached diatoms can be found on a variety of substrates including sand, gravel, stones, rock, wood and aquatic macrophytes (Table 2.6). The composition of the communities that develop is in response to water flow, natural water chemistry, eutrophication, toxic pollution and grazing.

Various authors have proposed precise protocols for the collection, specimen preparation and numerical analysis of benthic diatoms to ensure uniformity of water quality assessment (see below). More general aspects of periphyton sampling are discussed in Section 2.10.

Sample collection

The sampling procedure proposed by Round (1993) involves collection of diatom samples from a reach of a river where there is a continuous flow of water over stones. About five small stones (up to 10 cm in diameter) are taken from the river bed, avoiding those covered with green algal or moss growths. The diatom flora can be removed from the stones either in the field or back in the laboratory. As an alternative to natural communities, artificial substrates can be used to collect diatoms at selected sample sites. These overcome the heterogeneity of natural substrata and consequently standardize comparisons between collection sites, but presuppose that the full spectrum of algal species will grow on artificial media. Dela-Cruz *et al.* (2006) used this approach to sample diatoms in south-eastern Australian rivers, suspending glass slides in a sampling frame 0.5 m below the water surface. Slides were exposed over a 4-week period to allow adequate recruitment and colonization of periphytic diatoms before identifying and enumerating the assemblages.

Specimen preparation

The diatoms are then cleaned by acid digestion, and an aliquot of cleaned sample is then mounted on a microscope slide in a suitable high refractive index mounting medium such as Naphrax. Canada balsam should not be used as it does not have a high enough refractive index to allow resolution of the markings on the diatom frustule.

Cleaning diatoms and mounting them in Naphrax means that identification and counts are made from permanent prepared slides, rather than from volume or sedimentation chambers (Section 2.5.2). It is normal to use a $\times 10$ eyepiece and a $\times 100$ oil immersion objective lens on the microscope for this purpose. Before counting it is always desirable to scan the slide at a $\times 200$ or $\times 400$ magnification to determine which are the dominant species.

Numerical analysis

Diatoms on the slide are identified to either genus or species level and a total of 100–500+ counted, depending on the requirements of the analysis being used. Once the counting has been completed and the results recorded in a standardized format, the data may be processed.

In recent times there has developed a dual approach to analysis of periphytic diatoms in relation to water quality:

- evaluation of the entire diatom community (Section 3.4.3), often involving multivariate analysis
- determination of numerical indices based on key bioindicator species (Section 3.4.4).

Individual studies have either used these approaches in combination (e.g. Dela-Cruz *et al.*, 2006) or separately.

3.4.3 Evaluation of the diatom community

The term 'diatom community' refers to all the diatom species present within an environmental sample. Species counts can either be expressed directly (number of organisms per unit area of substratum) or as a proportion of the total count. Evaluation of the diatom community in relation to water quality may either involve analysis based on main species, or a more complex statistical approach using multivariate techniques.

Main species

Various authors have considered different levels of water quality in terms of distinctive diatom assemblages. Round (1993) proposed the following classification, based on results from a range of British rivers. In this assessment five major zones (categories) of increasing pollution (inorganic and organic soluble nutrients) were described:

Zone 1: *Clean water in the uppermost reaches of a river (low pH)* Here the dominant species were the small *Eunotia exigua* and *Achnanthes microcephala*, both of which attached strongly to stone surfaces.

Zone 2: *Richer in nutrients and a little higher pH (around 5.6–7.1)* This zone was dominated by *Hannaea arcus, Fragilaria capucina* var *lanceolata* and *Achnanthes minutissima. Tabellaria flocculosa* and *Peronia fibula* were also common in some instances.

Zone 3: *Nutrient rich with a higher pH (6.5–7.3)* Dominant diatoms included *Achnanthes minutissima* with *Cymbella minuta* in the middle reaches and *Cocconeis placetula, Reimeria sinuate* and *Amphora pediculus* in the lower reaches.

Zone 4: *Eutrophic but flora restricted through other inputs* Fewer sites in this category were found and more work was considered necessary to precisely typify its flora. However the major diatom associated with the decline in water quality was

Gomphonema parvulum together with the absence of species in the *Amphora*, *Cocconeis*, *Reimeria* group.

Zone 5: *Severely polluted sites, where the diatom flora is very restricted* As with Zone 4 not many sites in this category have been investigated and more work is needed. The flora was frequently dominated by small species of *Navicula* (e.g. *N. atomus* and *N. pelliculosa*). Detailed identification of these small species can be difficult, especially if only a light microscope is available. Round (1993) concluded that identification to species level for these was unnecessary and merely to record their presence in large numbers is enough. If the pollution is not extreme there may be associated species present such as *Gomphonema parvulum*, *Amphora veneta* and *Navicula accomoda.* Other pollution-tolerant species include *Navicula goeppertiana* and *Gomphonema augur.*

Round's diatom assessment for British rivers is compared with those of other analysts in Table 3.9 for categories of moderate and severe nutrient pollution. Although there is substantial similarity in terms of species composition, differences do occur – to some extent reflecting differences in river sizes and geographical location. These differences highlight the problems involved in attempting to establish standard species listings for water quality evaluation.

Multivariate analysis

Multivariate analysis can be used to compare diatom assemblages and to make an objective assessment of species groupings in relation to environmental parameters. Soininen *et al.* (2004) analysed benthic diatom communities in approximately 200 Finnish stream sites to determine the major environmental correlates in boreal running waters. Multivariate statistical analysis was used to define:

- diatom assemblage types
- key indicator species that differentiated between the various assemblage groups
- relative contribution of local environmental factors and broader geographical parameters in determining diatom community structure.

The results showed that the ~200 sites sampled could be resolved into major groupings which corresponded to distinctive ecoregions (Table 3.10). Environmental parameters characterizing these groups related to water quality (conductivity, total P concentration, water humic content), local hydrology and regional factors. The impact of local hydrology is shown by the presence of planktonic diatoms in benthic samples where rivers are connecting lakes (Group D) or have numerous ponds and lakes within the watercourses (Group J). Group H had a very distinct diatom flora characteristic of this extreme subarctic region.

These authors concluded that although analysis of diatom communities provides a useful indication of water quality, hydrology and regional factors must also be taken into account. The different diatom communities identified in this study compared well with those documented for stream macroinvertabrates, suggesting that bioassessment of boreal waters in Finland would benefit from integrated monitoring of these two taxonomic groups.

3.4.4 Human impacts and diatom indices

Diatom indices have been widely used in assessing water quality and in monitoring human impacts on freshwater systems. The latter can be considered either in terms of change from an original state, or in relation to specific human effects.

Change from a 'natural' community

The use of benthic diatom populations to assess anthropogenic impacts on water quality implies comparison of current conditions to a natural original community, with deviation from this due to human activities such as eutrophication, toxic pollution and changes in hydrology. This concept is implicit in some major research programmes, such as the European Council Water Framework Directive (WFD: European Union, 2000) where the baseline is referred to as 'reference conditions.' In the case of lakes (Section 3.2.2), reference conditions can be determined by on-site extrapolation into the past

Table 3.9 Comparison of the Diatom Floras Monitored by Lange-Bertalot, Watanabe and Round for Severely-Polluted and Moderately-Polluted River Sites

Lange-Bertalot	Watanabe	Round
Severe nutrient pollution: most tolerant diatom species.		
Group 1	Saprophilic*	Zone 5
Amphora veneta	***Navicula goeppertiana***	***Amphora veneta***
Gomphonema parvulum	*Achnanthes minutissima*	*Gomphonema augur*
Navicula accomoda	var *saprophila*	***Navicula accomoda***
Navicula atomus	***Navicula minima***	Small *Navicula*
Navicula goeppertiana	*Navicula mutica*	Small *Nitzschia*
Navicula saprophila	***Navicula seminula***	
Navicula seminulum	***Nitzschia palea***	
Nitzschia communis		
Nitzschia palea		
Synedra ulna		
Navicula minima		
Navicula frugalis		
Navicula permitis		
Navicula twymaniana		
Navicla umbonata		
Navicula thermalis		
Moderate nutrient pollution: Less-tolerant diatom species.		
Group IIa	Eurysaprobic*	Zone 4
Achnanthes lanceolata	***Achnanthes lanceolata***	*Amphora pediculus*
Cymbella ventricosa	*Cocconeis placentula*	*Reimeria sinuata*
Diatoma elongatum	*Gomphonema parvulum*	= *Cymbella sinuata*
Fragilaria vaucheriae	*Navicula atomus*	*Gomphonema sp.*
Fragilaria parvulum	*Nitzschia frustulum*	
Navicula avenacea		
Navicula gregaria		
Navicula halophila		
Nitzschia amphibian		
Surirella ovalis		
Synedra pulchella		

Adapted from Round, 1993.
*Saprophilic (preference for high soluble organic nutrient levels), Eurysaprobic (tolerant of a range of soluble organic nutrient levels). Species present in two or more of the lists area shown in bold.

using sediment analysis. Diatom sediment analysis is not generally appropriate for rivers, however, since there is little sedimentation of phytoplankton, the sediment is liable to disturbance by water flow and the oxygenated conditions minimize preservation of biological material.

An alternative strategy, in the quest for a baseline state, is to locate an equivalent ecological site that has a natural original community – unaffected by human activity. This is not straightforward, however, since local variations in environment and species within a particular ecoregion make it difficult to define what is

Table 3.10 Benthic Diatom Community Analysis of Finnish Rivers (Soininen *et al.*, 2004) Showing Some of the Major Community Groups

Group	Ecoregion	Stream Water Quality	Characteristic Taxa
A	Eastern Finland	Polyhumic, acid	*Eunotia rhomboidea* *Eunotia exigua*
B	Eastern Finland, woodland	Oligotrophic, neutral	*Fragilaria construens*, *Gomphonema exilissimum*
C	South Finland, small forest streams	Slightly acid, low conductivity, humic	*Gomphonema gragile* *Achnanthes linearis*
D	South Finland Connect lakes		*Aulacoseira italica** *Rhizosolenia longiseta**
E	Mid-boreal	Mesotrophic, neutral, humic	*Achnanthes bioretii* *Aulacoseira subarctica*
F	North boreal	Oligotrophic, clear water, neutral	*Caloneis tenuis* *Gomphonema clavatum*
H	Arctic-alpine		*Achnanthes kryophila* *Eunotia arcus*
J	South Finland, many small lakes and ponds	Polluted: eutrophic, high organic content	*Aulacoseira ambigua** *Cyclotella meneghiniana**
K	South Finland	Polluted: eutrophic – treated sewage, diffuse agricultural loading	Motile biraphid species – e.g. *Surirella brebissonii* *Nitzschia pusilla*

*Planktonic diatoms. Groups A–K selected from 13 ecoregions.

actually meant by a natural original community. This is evident in the studies of Eloranta and Soininen (2002) on Finnish rivers, for example, where diatom species composition of undisturbed benthic communities varied with river substrate, turbidity and local hydrology (Table 3.11).

In an objective assessment of human impact on natural communities, Tison *et al.* (2005) analysed 836 diatom samples from sites throughout the French hydrosystem using an unsupervised neural network, the self-organizing map. Eleven different communities were identified, five corresponding to natural

Table 3.11 Diatom Adaptations to Local Environmental Conditions in Finnish Rivers (Eloranta and Soininen, 2002)

Environment	Typical Diatoms
Substrate	
Hard substrates	Attached epilithic and epiphytic taxa
Soft substrates	*Pinnularia*, *Navicula* and *Nitzschia*
Turbidity	
Clear waters	*Achnanthes*
Clay-turbid waters	*Surirrella ovalis*, *Melosira varians* and *Navicula* spp.
Hydrology	
Lake and pond inflows	*Aulacoseira* spp., *Cyclotella* spp. and *Diatoma tenuis*

(undisturbed) conditions and relating to five different ecoregions (i.e. river types with similar altitude range and geological characteristics). The six other communities were typical of rivers that had been disturbed by human activity. Although natural variability occurred within individual ecoregions (similar to that noted in Finnish rivers, see above), this was greatly exceeded by the effects of pollution at the different sampling sites. The study aimed to identify diatom indicators for different types of anthropogenic disturbance by comparing benthic diatom communities in natural and disturbed sites.

Use of bioindicators

An alternative approach to analysing human impact in terms of general disturbance is to use diatom indices with indicator species that directly reflect particular environmental changes resulting from human activities. Individual species can be numerically weighted to reflect their degree of environmental specificity. Bioindicators may relate to single parameters such as total-P (trophic diatom index – TDI; e.g. Hofman, 1996) or reflect more general aspects of water quality, combining organic loading and inorganic nutrient concentration (IPS: index of pollution sensitivity, GDI: generic diatom index). Diatom indicator lists for other variables such as salinity, trophy, nitrogen metabolism types, pH and Oxygen requirements have also been published (van Dam *et al.*, 1994).

3.4.5 Calculation of diatom indices

As with lake phytoplankton indices (Section 3.2.3) the benefit of river diatom indices is that they reduce complex biological communities (involving a range of species) to a single numerical value. This provides a very simple quantitative evaluation which can be appreciated and used by biologists and non-biologists alike. The disadvantage of an index is that potentially useful information may be lost as counts of individual species are brought together and combined into a general evaluation. Diatom indices, based on bioindicator algae, are either single or multiple taxon assessments.

Single taxon assessment

Indices based on a single species represent a very simple environmental approach.

- Kothe's Index. This is referred to as the 'species deficiency index' (F) and relates the number of cells (N_1) of a particular key species at site 1 to the number (N_x) at site x, where:

$$F = \frac{N_1 - N_x}{N_1} \times 100 \qquad (3.2)$$

This very simple index is assessing environmental impact on a single species. It assumes that site 1 is the typical one for that river and that changes in the numbers of the selected species will occur at other sites (for example a decline with downstream pollution).

Multiple taxon indices

Most diatom indices are based on multiple taxa (genera or species). They are determined either in terms of the presence/absence of key indicator species (e.g. Palmer's index) or are based on the weighted average equation of Zelinka and Marvan (1961):

$$\text{Index} = \frac{\sum_{j=1}^{n} a_j s_j v_j}{\sum_{j=1}^{n} a_j v_j} \qquad (3.3)$$

Where:

a_j = is the relative abundance (proportion) of species j in the sample
s_j = the pollution sensitivity of species j
v_j = the indicator value of species j
n = is the number of species counted in the sample.

Different indices adapt this equation in different ways, and the performance of a particular index depends on which taxa are used, the number of taxa and the values given for the constants s and v for each taxon. The values of individual indices based on this equation vary from 1 to a maximum value equal to the highest value of s. Commonly used indices include the following.

Palmer's Index (Palmer, 1969) Palmer's index, applied to river diatoms, is derived from an extensive literature survey and is based on the occurrence of 20 common diatom species, listing them according to their tolerance to organic pollution. The sequence, from least polluted to most polluted waters, includes:

Fragilaria capucina → *Achnanthes minutissima* → *Cocconeis placentula* → *Diatoma vulgare* → *Surirella ovata* → *Gomphonema parvulum* → *Synedra ulna* → *Nitzschia palea.*

As with the more general version of Palmer's index (Section 3.2.3, Table 3.5) diatom species are rated on a scale 1 to 5 (intolerant to tolerant) and the index is calculated by summing up the scores of all relevant taxa present within the sample. Values >20 indicate high levels of organic pollution.

Descy's Index A frequently used index (Descy, 1979). Values of s_j range from 1 to 5 and v_j from 1 to 3 (equation 3.2) giving index values from 1 to 5. These can then be related to water quality (Table 3.12: Descy, 1979).

Example The example of Descy Index (I_d) calculation shown in Table 3.13 is taken from the River Semois in Belgium (Round, 1993). The part of the river investigated was heavily polluted with domestic sewage and contained the key diatom species *Navicula accomoda*, *Nitzschia palea*, *Achnanthes lanceolata* and *Gomphonema parvulum*. The I_d value obtained (1.0) signals heavy pollution, in agreement with the observed sewage contamination.

Some workers have reported that this index tends to give high values and thus underestimates heavy pollution (Leclercq and Maquet, 1987).

CEE Index: Descy and Coste, 1991 This index of general pollution is based on a total of 223 diatom taxa. The index ranges from 0–10 (polluted to non-polluted water).

GDI Index (generic diatom index): Coste and Ayphassorho, 1991 An index of organic and inorganic nutrient pollution, based on 44 diatom genera. Values range from 1–5 (polluted to non-polluted water).

IDAP Index: Prygiel ***et al.***, 1996 Index based on a combination of 45 genera and 91 diatom species. Values range from 1–5 (polluted to non-polluted water).

IPS Index (specific pollution index): Coste, in CEMAGREF, 1982 This index for pollution sensitivity is based on organic load and nutrient concentrations. Values range from 1–5 (polluted to non-polluted water).

TDI (trophic diatom index): Kelly and Whitton, 1995 In its original formulation, this index was based on a suite of 86 diatom taxa selected for their indicator value (tolerance to inorganic nutrients) and ease of identification. The index was determined as the weighted mean sensitivity (WMS: equation 3.3), with pollution sensitivity values (s_j) from 1–5, and indicator values (v_j) from 1–3. The

Table 3.12 Diatom Index and River Water Quality* (Descy 1979)

Diatom Index (I_d)	River Water Quality
>4.5	No pollution, the best biological quality
4.0–4.5	Very slight pollution, near normal communities
3.0–4.0	Toxic pollution at moderate level or nutrient enrichment (eutrophication). Community changes apparent and sensitive species disappearing
2.0–3.0	Heavy pollution. Only pollution resistant species abundant or dominant. Sensitive species severely reduced
1.0–2.0	Severe pollution. Only a few tolerant species survive. Very reduced diversity

*Water quality considered in relation to toxic pollution (e.g. heavy metal content) or nutrient enrichment (eutrophication).

Table 3.13 Calculation of the Diatom Index (I_d): River Semois (Round 1993)

Diatom	a_j	v_j	$a_j \times v_j$	s_j	$a_j \times v_j \times .s_j$	I_d
Achnanthes lanceolata	0.9%	1	0.9	3	2.7	
Gomphonema parvulum	0.5%	1	0.5	2	1.0	
Navicula accomoda	78.7%	3	235.1	1	235.1	
Nitzschia palea	19.7%	2	39.4	1	39.4	
Others	0.2%					
Total			275.9		278.2	278.2/275.9 = 1.0

See text for symbols and I_d calculation (equation 3.3).

value of TDI ranged from 1 (very low nutrient concentrations) to 5 (very high nutrients).

This index has now been modified (Kelly, 2002) to increase the range from 0–100, where:

$$TDI = (WMS \times 25) - 25 \qquad (3.4)$$

ISL Index (Index of saprobic load): Sladecek, 1986 This index of soluble organic pollution (saprobity) is based on 323 diatom taxa, each with a designated saprobic value. Values range from 4.5–0 (polluted to non-polluted water).

3.4.6 Practical applications of diatom indices

The diversity of available and currently-used diatom indices presents a complex and confusing picture as to which ones should be used to ensure comparability of studies and consistency of approach. A further potential source of confusion is that some indices (e.g. TDI, see above) have been subsequently modified to give a wider range of values in relation to water quality. The range of available diatom indices raises a number of key practical issues, including:

- which index? ease of use
- comparability between diatom indices
- comparability between diatoms and other bioindicator organisms
- differences between indices in response to changes in water quality
- adoption of a standard approach to use of diatoms as bioindicator organisms
- quality assurance: reliability of analyst assessment.

Which index? Ease of use

One practical problem with the use of diatoms as bioindicators is the large number of taxa encountered in environmental samples. This can be overcome in two main ways.

- Use indices based on genera (e.g. GDI) rather than species (e.g. SPI, TDI). Various researchers (e.g. Case study 3, below) have found no significant differences between the two.
- Restrict identification to the most abundant species. Round (1993), for example, used about 20 key species for monitoring rivers in the United Kingdom.

Comparability between indices

Recent projects using diatoms as bioindicators have tended to use groups of indices, to eliminate any irregular conclusions that might have arisen from use of a single index. Different indices have been compared for assessment of water quality within different river systems, where there is variation in trophic status (inorganic nutrients – particularly phosphates and nitrates) and a range of other factors such as salinity, organic pollution (related to biological oxygen demand – BOD) and industrial contamination (metal pollution and acidity).

A summary of studies is shown in Table 3.14, with some detailed examples below. In general, different diatom indices give broadly comparable results. Various studies (e.g. Case studies 3 and 4 below) do indicate, however, that the IPS index is particularly useful for monitoring general changes in water quality. This index best reflects the combined effects of eutrophication, organic pollution and elevated salt concentrations, since it usually integrates all diatom species recorded within the samples.

Case study 3.2 Field studies using different diatom indices (Table 3.14)

1. *Greek rivers: variation in trophic status, organic and inorganic chemical pollutants.* Iliopoulou-Georgudaki *et al.* (2003) used IPS, Descy 1979 and CEE indices as they were considered more representative of environmental conditions. The indices gave exactly comparable results.

2. *Selected rivers in England and Scotland.* Ranging from nutrient-poor upland streams to lowland rivers subject to varied eutrophication and contamination with organic pollutants, pumped minewater, heavy metals and agricultural run-off.

   Kelly *et al.* (1995) assessed water quality using four indices based on diatom genera (GDI) and species (SPI, TDI-P and TDI-NP). The high correlation between indices across the different river sites suggested that any could be used individually for routine monitoring and that diatom recognition to genus rather than species level was adequate.

3. *Metal pollution in lowland river*. De Jonge *et al.* (2008) assessed diatom populations in relation to metal (ZN) contamination and related physicochemical variables. The IPS index best reflected changes in water quality (pH, conductivity, oxygen concentration, inorganic nutrients), and was the only diatom index that indicated a significant difference between control and contaminated sites.

4. *Rivers of southern Poland: variation in trophic status and organic pollution.* Kwandrans *et al.* (1998) used the suite of 8 diatom indices contained in the OMNIDIA database software to evaluate water quality in this river system. Except for Sladek's index, indices typically showed significant correlations with each other and also with parameters of water quality: organic load (BOD), oxygen concentration, conductivity, measured ion concentrations and trophic level (NH_4-N, PO_4-P). Two particular indices – IPS and GDI, gave the best environmental resolution in terms of correlation with water quality variables (see Table 3.6) and showing clear differences between the separate river groups.

Although the diatom indicator system emerged as a useful tool to evaluate general water quality, some indices were not able to differentiate between adverse effects of eutrophication (inorganic nutrients) and organic material pollution. Abundance of key indicator species, however, such as *Achnanthes minutissima* (highly sensitive towards organic pollution) and *Amphora pediculus* (eutrophic species, sensitive to organic pollution) can be useful in evaluating the type of pollution involved.

Table 3.14 Comparability of Diatom Indices in the Assessment of River Water Quality

Site	Descy, 1979	CEE	GDI	IDAP	IPS	L&M	SHE	TDI	SLAD	Comments	Reference
Rivers Alfeios and Pineios in Greece	+	+			+					Indices gave exactly similar quality assessments (bad to very good.)	Iliopoulou-Georgudaki *et al*., 2003
Rivers in southern Poland		+	+	+	+	+	+	+	+	Most diatom indices (except SLAD) correlated with each other & water quality	Kwandrans *et al*., 1998
English and Scottish rivers			+		+			+		Good correlation between indices	Kelly *et al*., 1995
Finnish rivers			+		+			+		Good correlation between indices: rivers ranging from poor to high quality	Eloranta and Soininen, 2002
Metal-contaminated Belgian rivers		+	+		+	+	+	+	+	Good correlation with Zn concentrations, but only IPS gave good indication of general water quality	De Jonge *et al*., 2008

Shaded area: OMNIDIA database software (Lecointe *et al*., 1993) containing a range of diatom indices.
[1]CEE (Descy and Coste, 1991), GDI (Coste and Ayphassorho, 1991), IDAP (Prygiel and Coste, 1996), IPS (Coste, in CEMAGREF, 1982), L & M (Leclercq and Maquet, 1987), SHE (Schiefele and Schreiner, 1991), TDI (Kelly and Whitton, 1995), SLAD (Sladacek, 1986).

Case study 3.3 Comparisons of diatom indices with other bioindices

1. *Use of different bioindicators for assessing water quality in Greek Rivers.* Iliopoulou-Georgudaki *et al.* (2003). This study was carried out on river sites with water quality ranging from very poor to very good and used bioindices based on diatoms (see previously) plus four other groups of organisms. Sampling of fish and macrophyte vegetation gave only limited environmental assessment, with macrophytes in particular being incapable of a graded response to varying degrees and kinds of stress. Macroinvertebrates and diatoms recorded the full range of pollution conditions, but indices based on macroinvertebrates were considered to be the most sensitive – responding more readily than diatoms to transient changes to environmental state.

2. *Selected rivers in England and Scotland.* Kelly *et al.* (1995) found that their diatom indices were significantly correlated with two benthic macroinvertebrate indices – BMWP (Biological Monitoring Working Party scores) and ASPT (Average Score Per Taxon), both used routinely for water quality monitoring in the United Kingdom.

3. *Metal pollution in lowland river.* Comparison of macroinvertebrates and diatoms as bioindicators by De Jonge *et al.* (2008) showed that diatom communities most closely reflected changes in metal concentration, with distinct taxa being associated with low (e.g. *Tabellaria flocculosa*) moderate (*Nitzschia palea*) and high (*Eolimna minima*) zinc levels. In contrast to diatoms, macroinvertebrate communities most closely followed physical-chemical variables and the effects of metal pollution. The combined use of both groups for biomonitoring was considered to be more appropriate than separate use of either.

Comparison to other bioindicator organisms

Although routine monitoring of all but the deepest rivers has been based for many years on macroinvertebrates (e.g. De Pauw and Hawkes, 1993), a range of other bioindicator organisms is available. These include diatoms, other algae, fish, aquatic and riparian macrophytes. Various authors have made simultaneous comparisons of some or all of these to assess the relative usefulness of particular groups of organisms – with particular emphasis on diatoms and macroinvertebrates. In the examples below (continued from the previous section), diatom communities showed comparable changes to macroinvertebrates in relation to water quality, but (with the exception of the IPS index) were generally less sensitive.

Response to changes in water quality

One important aspect of algal bioindicators is that they are able to detect a rapid change in water quality. Because of their shorter generation time, diatom communities are potentially able to respond more rapidly than other bioindicator groups (e.g. macroinvertebrates, fish), which integrate water quality over longer time frames.

The time sequence of diatom change can be investigated by transferring diatom biofilms from polluted to non-polluted waters, and record:

- how different diatom indices compare over fixed time periods
- the time needed for different indices to indicate a significant change in water quality.

Biofilm studies by Rimet *et al.* (2005), showed that some indices (e.g. CEE, TDI – high sensitivity) responded more rapidly than others (GDI, ILM, SLA – intermediate sensitivity) to environmental change. All indices showed significant change (integration interval) within 40–60 days of biofilm transfer.

Standardization of approach

The above studies indicate that benthic diatoms provide the basis for a standard approach to river monitoring, able to be used as an alternative (or addition) to macroinvertebrate sampling. Individual commonly-used diatom indices appear to correlate significantly with macroinvertebrate data, and also with each other (see above). Studies by Kelly *et al.*(1995) have also shown that benthic diatom indices do not change significantly either with season or with major flow events (both of which can influence invertebrate populations) – suggesting that diatom indices are robust and that consistent results can be obtained throughout the year.

Standard procedures are important in ensuring comparability of results. These include standardization of sampling procedures (Kelly *et al.*, 1998), adoption of analyst quality controls (see below), access to common data bases and use of comparable diatom indices.

Access to common databases Access to web-based data sites such as the European Diatom Database Initiative (EDDI) promotes a standard approach to identification, data acquisition and manipulation. Although this site has particularly application for palaeolimnology (Section 3.2.2), other databases specifically on benthic diatoms (Gosselain *et al.*, 2005) may be more applicable to river studies.

Comparable diatom indices Although a single diatom index (coupled with the identification of key diatom indicator taxa) would be adequate for environmental monitoring, the trend is for diatom counts to be fed into a database for determination of multiple indices. The general availability of the OMNIDIA database software (Lecointe *et al.*, 1993), incorporating indices summarized in Table 3.14, is particularly useful in this respect.

The indices contained in the OMNIDIA software are based on European diatom communities and clearly apply most directly to European rivers. Application of this software in other parts of the world requires the incorporation of local (endemic) species, as emphasized by Taylor *et al.* (2007) in their studies on South African rivers. It is also important to check that cosmopolitan species have similar ecological preferences in different parts of the world. Dela-Cruz *et al.* (2006), for example, assessed the suitability of 'northern hemisphere' ecological tolerance/preference data for periphytic diatoms in south-eastern Australian rivers before using bioindicator indices.

Quality assurance

Although techniques for standardized sampling of diatoms (Section 2.10.1), and different types of indices (Section 3.4.5) are well defined, the final environmental assessment ultimately depends on the analyst's ability to accurately record species composition from the environmental samples. Quality assessment of analyst performance is important, since regulatory agencies must be confident that data produced by their staff are relevant and accurate. In Europe, for example, the requirement of water companies to install nutrient removal facilities in certain large sewage treatment works (Urban Wastewater Treatment Directive – European Community, 1991) is determined by assessment of water quality and is highly expensive. The use of benthic diatoms in the implementation of this directive (Kelly, 2002) must be reliable.

In the case of chemical analyses of water quality (including soluble nitrates and phosphates) the situation is relatively simple, since chemical parameters are relatively few, with comparisons that are univariate and amenable to conventional parametric statistics. In contrast to this, biological monitoring is more complex – based on field samples containing many species, all of which may contribute to the assessment. These species counts may be assessed as presence/absence or in terms of relative abundance.

- *Presence/absence.* Invertebrate analyses in the United Kingdom are carried out on this basis, with water quality being determined in relation to the presence/absence of key benthic invertebrate data (Mason, 1996). Reliability of assessment by individual analysts can be measured as a 'quality audit,' where the number of taxa 'missed' by an (inexperienced) analyst can be compared to the sample assessment by an experienced auditor.

- *Relative abundance.* A more complicated situation is presented by diatom-based monitoring, where the relative abundance of taxa, rather than their presence or absence, is being recorded. In this situation, comparison of analyses by different analysts can be carried out on the basis of 'similarity measures'. The higher the value of the 'measure' the greater the similarity, rising to a point at which the two sets of data can be considered derived from the same population.

Kelly (2001) has used the 'Bray–Curtis similarity measure' to assess analyst performance, with levels of >60% indicating good agreement between primary analyst and auditor. Evaluation of about 60 comparisons showed that reliability of assessment varied with species diversity, and that samples with large numbers of species had lower levels of similarity compared to those with low numbers. The use of such an audit measure, providing an objective measure of analyst performance, has clear application within regulatory organizations such as Water Authorities.

Figure 3.8 View across the Mersey Estuary (United Kingdom) at low tide, showing the extensive mudflats with main river channel in the distance. Freshwater drainage into the mudflats (foreground), with saltwater flooding at high tides, leads to complex localized variations in salinity and nutrient concentrations. Some epipelic diatoms (present in surface biofilms) have wide tolerances to variations in water quality while others have clear environmental preferences (see text).

3.5 Estuaries

Estuaries are aquatic zones that interface between freshwater rivers and saline seas. As such, they tend to be dominated by saltwater conditions, but also have major freshwater inputs. Algae have been widely used as bioindicators of environmental change (Bortone, 2005) in these highly complex aquatic systems.

3.5.1 Ecosystem complexity

Estuaries are highly complex ecosystems in relation to habitats, hydrology, effects of weather, constituent organisms and human activity.

Habitats

Estuaries can be divided into two main regions – a central river drainage channel (or channels) and a surrounding expanse of mudflats (Fig. 3.8). Major populations of phytoplankton are present within the drainage channel, and an extensive biofilm of euglenoids, diatoms and filamentous blue-greens can occur across the surface of the mudflats (Underwood and Kromkamp, 1999). In addition minor drainage channels also discharge freshwater into the mudflats/main channel (Fig. 3.8) and freshwater/saline wetlands are also frequently associated with the main estuarine system.

In many estuaries, water from the catchment area also has a major influence on the local marine environment, flowing as a freshwater plume into the surrounding ocean.

Hydrology

Patterns of water circulation are complex. Water movements within the main channel depend on input from the sea (tidal currents), river (freshwater discharge), surface drainage and ground sources (freshwater discharge). Mixing of freshwater and saltwater within the main channel results in an intermediate zone of brackish water – the 'salt wedge,' where dense sea water underlies an upper freshwater layer. The salt wedge is a permanent feature that moves up and down the main channel with advancing and receding tide. Periodic movement water out of the estuary into the surrounding ocean leads to a plume of fresh or brackish water in the bay area.

Weather

Estuaries are markedly affected by local weather events such as droughts, high rainfall (flooding) and winds. During the autumn of 1999, for example, hurricanes inundated North Carolina (United States) with as much as 1 m of rainfall, causing a major flood in the watershed of Pimlico Sound (Paerl *et al.*, 2005). Sediment and nutrient-laden water displaced more than 80% of the Sound's volume, depressed salinity by >70% and accounted for half the annual load of nitrogen in this nitrogen-sensitive region.

Estuarine organisms

Many algae living in or around estuaries are typically adapted to either freshwater or saline conditions, and have restricted habitats (e.g. Section 3.3 – diatoms in coastal wetlands). Other estuarine organisms have become tolerant of a range of conditions. Diurnal and seasonal changes in tidal flow mean that planktonic organisms in the main water channel are subjected to wide periodic fluctuations in salinity, and biota occurring on the surface of mudflats are exposed to extremes of salinity change, desiccation and irradiation. The typically high nutrient level of river inflow also signals a further difference between freshwater and saline organisms in terms of productivity, with a marked increase in carbon fixation from freshwater algae when saline water is displaced (Section 3.5.2).

Algal diversity: the mudflat biofilm Organism diversity within the complex estuarine environment is illustrated by the area of mudflats, where localized drainage and inflow (Fig. 3.8) lead to numerous small-scale salinity and nutrient gradients (Underwood *et al.*, 1998). Epipelic diatoms are the main group of algae inhabiting these intertidal sediments, forming surface biofilms that have high levels of productivity. Algae associated with estuarine sediments are also important in substrate stabilization (Section 2.7.2) and are in dynamic interchange with planktonic populations (Section 1.1.4).

Although many epipelic diatoms are cosmopolitan in their distribution, indicating broad tolerance of environmental variations, others are more specific. Analysis of diatom distribution within local salinity gradients led Underwood *et al.* (1998) to classify some diatoms as oligohaline (0.5–4% of full salinity: *Navicula gregaria*), mesohaline (5–18%: *Navicula phyllepta*) and polyhaline (18–30%: *Pleurosigma angulatum*, *Plagiotropis vitrea*). Variations along a nutrient gradient indicated some species (*Nitzschia sigma*) typical of high nutrient conditions, while others (*Navicula phyllepta*, *Pleurosigma angulatum*) were more common at low nutrient levels. Similar studies by Agatz *et al.* (1999) on tidal flat epipelic diatoms also demonstrated nutrient-tolerant (*Nitzschia sigma*) and nutrient-intolerant (*Achnanthes*, *Amphora*) taxa. The above algae serve as bioindicators for microenvironmental (local) conditions of pore water quality.

Human activity

Human activity has a major and varied effect on estuaries, and is a dominant source of stress and change. At least half the world's population resides in estuarine watersheds (Paerl *et al.*, 2005), greatly increasing sediment and nutrient loads to downstream estuarine and coastal systems. This results in deterioration of water quality and an overall decline in the ecological and economic condition of the coastal zone, including loss of fisheries habitat and resources.

3.5.2 Algae as estuarine bioindicators

Algae have been used to monitor changes in water quality of estuaries and coastal systems in relation to two main aspects: changes in salinity and increases in inorganic nutrient levels (eutrophication). The potential role of epipelic diatoms as monitors of local porewater salinity/nutrient concentrations has been noted above, and diatom bioindicators of coastal wetland salination were discussed in Section 3.3.

Detection of large-scale eutrophication is a more pressing and general problem, since it is a feature of almost all estuaries. Nutrient increases are accelerating with rising levels of human population, and have important economic impacts in relation to fisheries. The use of algae as bioindicators of general estuarine eutrophication is considered in relation to two main aspects: monitoring algal populations using pigment concentrations and molecular detection of freshwater species in river plumes.

Eutrophication: analysis of pigment concentrations

Analysis of algal populations as bioindicators in estuarine and related coastal systems typically involves monitoring a large area of water surface. The speed with which environmental changes can occur also means that relatively frequent sampling may be necessary. Because of these requirements, many estuarine monitoring programmes have combined rapid sample analysis (using pigments as diagnostic markers) with automated collection (from fixed sites or by remote sensing).

The combined use of HPLC (pigment separation), spectrophotometry (pigment quantitation) and a matrix factorization program such as ChemTax® (calculation of biomass proportions) is described in Section 2.3.3 and provides a speedy and accurate assessment of the algal community. Although pigment analysis only resolves phytoplankton communities to the major group (phylum) level, the results obtained give clear information on the algal response to environmental change and enable these organisms to be used as bioindicators.

The use of HPLC/ChemTax® analysis is reported by Paerl *et al.* (2005) for various southern United States estuarine systems (Table 3.15), including the Neuse River Estuary (North Carolina), Galveston Bay (Texas) and St. Johns River (Florida). In all of these cases, eutrophication occurs due to flushing of high volumes of nutrient-rich freshwater into the lower reaches of the estuary and into the surrounding ocean. The source of nutrients primarily results from agricultural, urban and industrial activities in the watershed, and flushing occurs due to high rainfall – which may be seasonal (summer wet season) or episodic (hurricane and storm effects).

Neuse River Estuary This aquatic system is subject to alternations of high river discharge (seasonal and hurricane rainfall) with periods of low discharge (no rainfall). Algal bioindicators indicate phases of eutrophication resulting from high freshwater flow in relation to biomass increase (up to 19 μg chl-*a* l^{-1}) and a switch in phytoplankton populations – promoting growth of chlorophytes and diatoms, but reducing levels of blue-green algae and dinoflagellates. This apparent reversal of the normal effects of eutrophication is due to the conditions of high water flow (reduced residence time), where algae with rapid growth and short generation time (r-selected organisms) are able to dominate more slow growing algae (K-selected organisms).

Galveston Bay Eutrophication resulting from tropical storms in 2000 led to increased algal growth in the bay, with HPLC analysis indicating growths particularly of cryptophytes and peridinin-containing dinoflagellates. The location of the algal blooms overlapped with commercial oyster reefs, leading to ingestion of phycoerythrin-containing cryptophytes and a commercially disastrous red/pink coloration of the oysters.

St Johns River System This is a 300 mile-long estuarine system composed of lakes, tributaries, riverine segments and springsheds. Eutrophication was indicated by extensive blooms of blue-green algae, which dominated the relatively static parts of the system such as freshwater lakes and various oligohaline (low salinity) sites. These blooms have been associated with fish kills, loss of macrophytes and wildlife mortalities.

Eutrophication: molecular detection of indicator algae

Freshwater plume Outflow of freshwater from rivers creates a fluctuating zone of fresh (brackish)

Table 3.15 Algal Bioindicators of Estuarine Pollution: Episodic and Seasonal Events in Southern USA Estuaries (Paerl *et al.*, 2005)

Estuarine System	Source of Eutrophication	Algal Analysis: Results*
Neuse River estuary *North Carolina*	High rainfall – hurricanes in 1999 Pollution source – agricultural urban and industrial activities	River discharge into estuary – elevated biomass (chl-*a*), increased chlorophytes/diatom populations, seasonal changes-reduced dinoflagellate bloom
Galveston Bay. *Texas*	High rainfall – tropical storm in 2000	Freshwater flushed into bay, resulting in reduced salinity, increased DIN plus blooms of cryptophytes and dinoflagellates. Adverse effects on oyster beds
St Johns River estuarine system, *Florida*	High summer rainfall Accelerating point and diffuse nutrient loading from watershed	Seasonal nutrient enrichment from watershed leading to blue-green algal blooms, fish kills, submerged-vegetation loss, wildlife mortalities

*HPLC diagnostic photopigment determinations coupled to ChemTax® analysis.

water within the surrounding marine environment. This plume of water extends into the surrounding ocean, and is characterized by reduced salinity, high levels of inorganic nutrients, high levels of suspended material and a distinctive phytoplankton population. The freshwater tends to lie on the surface of the seawater (forming a 'freshwater lens'), so that the location of the freshwater plume can be considered both in terms of horizontal and vertical distribution.

A good example of this is the Amazon River plume, which extends into the Western Tropical North Atlantic (WTNA) ocean (Foster *et al.*, 2007). The Amazon River represents the world's largest fluvial outflow, releasing an average $1.93 \times 10^5\ m^3\ s^{-1}$ of water to the WTNA. The influence of the Amazon river plume is geographically far-reaching, with freshwater lenses containing enhanced nutrients and distinct phytoplankton populations reported as far away as >1600 km from the river mouth (Borstad, 1982). The Amazon plume brings silica, phosphorus, nitrogen and iron into the WTNA, promoting high levels of phytoplankton productivity within the plume area. This productivity tends to be nitrogen limited, promoting the growth of nitrogen-fixing (diazotrophic) blue-green algae.

Molecular detection The detection of diazotrophic blue-greens can be used to map regions of eutrophication resulting from freshwater outflow. Studies by Foster *et al.* (2007) on the occurrence of nitrogen-fixing algae in the WTNA targeted the vertical and horizontal distribution of seven diazotrophic populations, including *Trichodesmium* (typical of open ocean environments), three symbiotically-associated algae and three unicellular freeliving organisms (phylotypes). Algal distributions were examined in terms of the presence of the *nif*H gene, which encodes for the iron protein of the nitrogenase enzyme (responsible for nitrogen fixation), and is highly conserved. The presence of species-specific *nif*H gene sequences was determined using DNA quantitative polymerase chain reaction (QPCR) technology.

The studies showed that one particular algal symbiont (*Richelia*), associated with the diatoms *Hemiaulus hauckii* and *Rhizosolenia clevei*, was distributed within the freshwater lens of the Amazon plume – with abundances of *H. hauckii–Richelia nif*H genes being particularly prominent. The QPCR study showed the dominance of *H. hauckii–Richelia* symbioses in the Amazon plume waters, implying that these associations had an ecological advantage over other diazotrophs and establishing them as indicator organisms of the plume environment. Free living unicellular blue-green diazotrophs were particularly abundant in the saline waters outside the plume, where inorganic nutrients were at minimal levels of detection.