Nutrient Conditions for Different Levels of Ecological Status and Biological Quality in Surface Waters (Phase I)

R&D Technical Report P2-260/4

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EXECUTIVE SUMMARY

The Water Framework Directive (WFD) requires the establishment of relationships between the concentrations of physico-chemical quality elements, such as nutrient conditions, and biological quality. As a component of this, Member States are required to establish reference nutrient conditions for different water body types and to link nutrients with indicators of biological quality.

This report summarises the results of the first phase of a project to identify nutrient conditions for different levels of ecological status in UK surface waters. The main objective of phase I was to identify areas where additional research is required before nutrient conditions and biological quality indicators can be linked with an acceptable degree of reliability.

To achieve this objective, literature reviews were carried out summarising the current state of knowledge in two main topic areas:

1. Information on, and approaches to, establishing relationships between biological quality and nutrient conditions in the four types of surface water body, outlined in the WFD (lakes, rivers, transitional waters, coastal waters) with specific reviews of those biological elements requiring monitoring and classification in the WFD (phytoplankton, phytobenthos, macrophytes, benthic invertebrates and fish).

2. Information on, and approaches to, establishing procedures and methods for determining reference nutrient conditions. Specifically, reviews of the three approaches to establishing reference conditions outlined in the WFD: spatial state schemes, palaeoecological methods and modelling approaches.

In all surface water types, the literature reviews conclude that the biological assemblages are shaped by both natural environmental factors (physical, chemical and biological) and a number of “pressures” from human activity (pollution, disturbance, species introductions, etc.). Nutrient pollution may occur simultaneously with other pressures, and it is often, therefore, difficult to identify changes in ecological status solely due to nutrient conditions.

Of the natural environmental factors, alkalinity/pH and salinity have more direct biological significance than nutrients for all biological communities in all systems (rivers, lakes, transitional, coastal). Nutrients tend to be a secondary and indirect driver of community composition but are clearly a significant driver of productivity. If variation due to nutrient conditions is to be assessed, the influential effects of alkalinity and salinity must be adequately addressed through the ecotyping process.

For rivers, the most established UK schemes that target nutrient conditions are the Trophic Diatom Index and the macrophyte Mean Trophic Rank scheme. Both have been shown to be sensitive to nutrient conditions and have standardised methodologies and sampling protocols. Another, less established, approach that merits attention is the use of chlorophyll *a* as a simple, practical measure of phytoplankton abundance in lowland rivers. Additionally, there is a need to assess the value gained by including measures from invertebrate and fish communities to provide a more holistic ecosystem response to nutrient conditions over a range of spatial and temporal scales.

The influential effect of flow and substrate are two critical factors in rivers and are almost certainly of primary importance in determining macrophyte and benthic invertebrate assemblages. Ecological thresholds in rivers in response to nutrient conditions will be particularly influenced by flow. In low-flow conditions, major ecological changes can result from small changes in nutrient concentrations. The influence of these factors can be partly taken into account in the ecotyping process, although short-term flow events can have a significant influence on community composition and abundance.

For lakes, the most established biological indicator schemes of nutrient conditions are those developed for chlorophyll *a*, diatoms and macrophytes. All have established significant relationships with nutrient conditions. All require development in terms of the WFD and standardised methodologies. There is also a need to develop measures for phytoplankton (specifically bloom-forming species) and value in developing indicators from invertebrate (particularly chironomids) and fish communities to provide a more holistic ecosystem response to nutrient conditions over a range of spatial and temporal scales.

The influential effect of depth is a critical factor in lakes. Deep lakes are more predictable in terms of nutrient effects on phytoplankton abundance and the indirect impacts on profundal invertebrate and fish communities. In shallow lakes, ecological thresholds in response to nutrient conditions are much more difficult to define due to significant effects of macrophytes and fish on lake community structure.

One advantage lake systems have over other surface waters is the ability to link long-term historical datasets for phytoplankton, macrophyte and fish assemblages that exist at specific sites with diatom-inferred changes in TP from sediment records. These analyses could be used to identify ecological reference conditions (as opposed to chemical reference conditions) and to assess community changes, or ecological thresholds, in response to changing nutrient conditions. Expert judgement of a site's history can be used to identify characteristic biology associated with high (reference), good and moderate status classes

Transitional and coastal waters are naturally nutrient-rich environments. In both environments the greatest impacts of increasing nutrient conditions have been at sites with restricted water exchange with increases in phytoplankton and macroalgal blooms. There is, however, a marked lack of standardised monitoring or classification schemes examining transitional and coastal waters in relation to nutrient conditions. The most suitable candidates for development are chlorophyll *a* and macrophytes (angiosperms and macroalgae) due to their more established and direct relationship with nutrient conditions. Invertebrate and fish species could be equally sensitive through indirect effects on oxygen and substrate conditions, although their response to changing nutrient conditions requires much greater research. The understanding of 'reference' conditions (chemical or biological) is particularly poorly known, compared with rivers and lakes.

In transitional waters, the primary influence of salinity on community structure will need to be established independently of nutrients, although this can be overcome in the WFD through the ecotyping process.

In terms of defining reference conditions, there appears to be no single method applicable to all surface water types.

Some waterbody types have been very under-researched and present greater problems than others, such as coastal and transitional waters. Likewise, methods for establishing reference conditions for some biological elements are less well developed than others, e.g. spatial-state schemes are well developed for benthic invertebrates in rivers but poorly developed for fish. This review highlights those techniques that are best suited to determining reference conditions in the different waterbody types in the absence of long term datasets (Table 6.1). However, given the original project brief, the focus is on methods for defining *nutrient* rather than *ecological* reference conditions (see Section 6.1).

There are two basic methods:
i) Site-specific methods that use historical documentary data, palaeoecology or other modelling techniques to derive site-specific data.
ii) Spatial-state methods that use contemporary datasets and can be divided into two approaches, (a) selected reference sites approach, where reference sites are chosen using expert knowledge, mapped information and existing data, and (b), percentile data selection of 'best available sites' from a population distribution of sites in a region.

The latter method is most widely applicable but it is difficult to choose the cut-off between reference and non-reference sites. A combination of site-specific and spatial-state methodologies is probably the best approach, as each has advantages and disadvantages. Spatial-state schemes can be scaled up more easily for use at a national level than the site-specific approach, while site-specific methods are of value for supporting and validating results from the former.

Palaeoecology is a powerful technique for defining both nutrient and ecological reference conditions but can only be effectively employed in environments where sediments continually accumulate. Of the surface water types relevant to the WFD, palaeoecological techniques are already available for determining nutrient reference conditions in lakes and transitional waters (coastal lagoons), and under development for coastal waters.

Export-coefficient models are already operational in both England and Wales, and Scotland. They do not incorporate any biological elements, but are useful for establishing reference nutrient loads and concentrations in both rivers and lakes. The technique requires further development for application to transitional and coastal waters. An alternative method of estimating nutrient reference conditions for lakes is the morphoedaphic index (MEI). This is a fairly straightforward method that has been used successfully on lakes in the USA and would benefit from further testing on waterbodies in the UK. It is unlikely that this approach could be developed for use in rivers, transitional or coastal waters.

RIVPACS is a well-established, spatial-state reference scheme for evaluating the biological condition of river sites by comparing the observed macro-invertebrate fauna at each site with the fauna predicted from its measured environmental characteristics. The system is widely used by the British water industry, including the EA, and has been shown to be operationally effective. Future research might involve the development of similar schemes for other taxonomic groups (e.g. macrophytes and fish). PSYM integrates the multivariate prediction methods used in RIVPACS and the multimetric approach adopted by the US EPA to link environmental characteristics of lowland ponds to their macrophyte and macroinvertebrate assemblages. However, the scheme is based on very small, shallow waterbodies and would need to be adapted for lakes. COASTPACS is under development at the University of Hull and aims to generate a model analogous to RIVPACS to predict macrobenthos in transitional and coastal waters.

In general, a great deal of further research is required for the identification of nutrient conditions for different levels of ecological status in all surface waters. To achieve a consistent approach to monitoring and assessment for the Great Britain Ecoregion, the EA needs to progress future research developments in collaboration with SEPA and SNIFFER. Awareness of, and involvement in, EC research programmes is also required to develop standardised and comparable approaches.

1. INTRODUCTION AND PROJECT OBJECTIVES

1.1 Background to Project

The Water Framework Directive (Council of the European Communities 2000), hereafter referred to as the WFD, requires the establishment of relationships between the concentrations of physico-chemical quality elements, such as nutrient conditions, and biological quality. This is needed for a number of aspects of the Directive such as for the:

- classification of ecological status in terms of physico-chemical elements;
- definition of type specific reference conditions for these elements;
- assessment of the significance of point and diffuse source pollution containing or affecting these elements; and
- identifying appropriate (regulatory or non-regulatory) actions to improve ecological status of water bodies impacted by the physico-chemical elements.

The Environment Agency (EA) is not currently in a position to fulfil these requirements as regards nutrient conditions for England and Wales. Further work is required to establish reference nutrient conditions for different water body types and to link nutrients with indicators of biological quality.

This report describes the results of the first phase of Environment Agency R&D Project P2-260/4 "*Nutrient conditions for different levels of ecological status and biological quality in surface waters*". The main aim of phase I is to identify areas where additional research is required before nutrient conditions and biological quality indicators can be linked with an acceptable degree of reliability.

1.2 Project Objectives

The project has two key objectives:

1. To review existing methodologies and approaches to establishing relationships between biological quality and nutrient concentrations and identify areas/techniques for further investigation and development. Where possible, make recommendations on approaches to determining nutrient conditions in different waterbody types equating to high, good and moderate ecological status.

2. To undertake a review of existing methodologies and approaches to establishing procedures and methods for determining reference nutrient concentrations and identify areas/techniques for further investigation and development. Identify data requirements for different approaches and assess current data availability.

1.3 Organisation of the report

To achieve these project objectives, literature reviews were needed to summarise the current state of knowledge in the two main topic areas:

1. Information on, and approaches to, establishing relationships between biological quality and nutrient conditions in the four types of surface water body, outlined in the Water Framework Directive (lakes, rivers, transitional waters, coastal waters).

Specifically, individual reviews of those biological elements requiring monitoring and classification in the WFD (phytoplankton, phytobenthos, macrophytes, benthic invertebrates and fish)

2. Information on, and approaches to, establishing procedures and methods for determining reference nutrient conditions in the four types of surface water body, as outlined in the WFD. Specifically, individual reviews of the three approaches to establishing reference conditions outlined in the WFD. These include spatial state reference schemes, palaeoecological methods and modelling approaches.

Experts were selected from relevant fields to carry out the literature reviews on each specific topic (Tables 1.1 and 1.2).

Table 1.1 The relationships between biological quality and nutrient conditions: literature review topics and the relevant experts undertaking the work

	Rivers	**Lakes**	**Transitional**	**Coastal**
Phytoplankton	Carvalho	Carvalho	Johnston	Johnston
Phytobenthos	Carvalho	Carvalho	-	-
Macrophytes (angiosperms/macroalgae)	Dawson	Luckes/ Monteith	Taylor	Taylor
Benthic Invertebrates	Winder/ Furse	Gunn	Hughes/ Trimmer	Hughes/ Trimmer
Fish	Maitland	Maitland	Hughes/ Trimmer	-

Table 1.2 Procedures and methods for establishing reference nutrient conditions: literature review topics and the relevant experts undertaking the work

	Rivers, lakes, transitional and coastal waters
Spatial-state reference schemes	Furse/Winder
Palaeoecological methods	Bennion
Modelling approaches	May

Following the production of a draft report, a workshop was held of the full project team, the EA project board, and external experts to discuss the findings, summarise the key conclusions and highlight research requirements for Phase II.

This final report follows the review structure. Chapters 2-5 review information on, and approaches to, establishing relationships between biological quality and nutrient conditions in rivers, lakes, transitional waters and coastal waters, respectively. Chapter 6 reviews information on, and approaches to, establishing procedures and methods for establishing reference nutrient conditions. Each chapter concludes with summary sections detailing the research requirements for Phase II.

2 RIVERS

2.1 Introduction

Over the nineteenth and twentieth century widespread enrichment of river systems occurred worldwide, associated with industrialisation and the use of rivers as conduits for sewage effluent disposal. Improvements in sewage treatment have seen large improvements in gross organic pollution, but even secondary treated effluent still contains high concentrations of inorganic nutrients such as nitrogen (N) and phosphorus (P). Historical records of nitrate concentrations in UK rivers show clear increases since records began in the 1930s (Environment Agency 1998). Only in recent years has river monitoring of phosphorus been carried out extensively in the UK and long-term historical trends are generally not available. Hind casting of nutrient concentrations for UK rivers highlights significant enrichment in both nitrogen and phosphorus (See Chapter 6).

Changes in freshwater biological communities as a response to pollution or physical stress have long been recognised (Abel 1989; Davies and Walker 1986; Gaufin 1958; Gray 1989; Hart and Fuller 1974; Hawkes 1979; Hynes 1960 and 1970; Mason 1981; Richardson 1987; Whitton 1975). Mostly research has focused on impairment to water quality resulting from pollution of either a physical, chemical or organic nature. Gradually there has been recognition of the role of both point- and non-point (or diffuse) sources of nutrient enrichment of surface waters. Studies of this cultural eutrophication have historically concentrated on lentic and transitional waters of estuaries and coasts and their associated flora (Collingwood 1977) but since the 1960's interest has also turned to the importance of nutrient enrichment from increasing levels of nitrogen and phosphorus in lotic systems or flowing waters (Arnold 1989; Environmental and Heritage Service Northern Ireland 2000; English Nature 1992; Environmental Change Network 2001; Furse 1998; Harper 1992; Irving 1993; Leaf and Chatterjee 1999; Newbold 1992; MAFF 1992; Muscutt and Withers 1996; Ryther 1981; Smith *et al.* 1999; USEPA 2000a; Vollenweider 1968).

The impact of nutrient enrichment on rivers is complicated by their dynamic nature, but symptoms such as excessive phytoplankton and filamentous algae development, weed growth and changes in macrophyte communities have clearly impacted water supply, fisheries and conservation value. In the UK, a large number of rivers have been designated as Sensitive Areas (Eutrophic) under the EC UWWT Directive. Despite the general decline in river water quality, a few large rivers remain relatively undisturbed. Cardoso et al. (2001) outlines nutrient ranges for near pristine rivers in Europe, North America and Australia and for large rivers in general (Table 2.1).

Table 2.1 Nutrient ranges for near pristine rivers in Europe, North America and Australia and for large rivers >105 km^2 (Cardoso et al., 2001)

	TP	SRP	TN	Nitrate
	(µg/l)	(µg/l)	(mg/l)	(mg/l)
Europe	5-50	0-10	0.2-1.0	0.1-1.0
North America	<25	-	1.0	0.6
Australia	10	-	0.1-0.2	-
Large rivers	-	2-25	0.1-1.2	0.05-0.2

This review of rivers considers approaches taken to quantify the relationship between nutrient conditions and biological quality for the five riverine biological quality elements outlined in Annex V of the WFD: phytoplankton, phytobenthos, macrophytes, benthic invertebrates and fish.

The requirement within the WFD to define nutrient conditions for high ecological status and then define moderate and good status as deviations from high, requires a more specific quantitative understanding of how nutrient conditions relate to biological quality in individual river ecotypes. It is not possible within this review to consider the different responses in individual ecotypes, as there are potentially 36 river ecotypes within England and Wales and 72 within the British Isles (Table 2.2: System A categories). Consideration is given of generalised river types where appropriate e.g. lowland vs. upland rivers. Approaches to quantitative definition of the relationships between nutrient conditions and biological quality for specific biological elements are considered.

Table 2.2 Obligatory river ecotyping factors/categories

System B	*System A*
Altitude	Altitude: High (>800 m), mid (200 – 800 m), or low (<200 m)
Latitude/longitude	Ecoregion
Geology	Geology: calcareous, siliceous or organic
Size	Catchment size (km^2): 10-100, 100-1000, 1000-10000, >10000

2.2 Phytoplankton

Phytoplankton only forms a significant component of primary production in slow-moving rivers in lowland regions where the retention time of water is longer than the generation time of the plankton (Lampert and Sommer 1997). As well as flow rate, turbidity is a critical factor in limiting the development of a phytoplankton community. For these reasons, there has been only limited research aimed at developing phytoplankton as a tool for monitoring river water quality, with much greater interest focused on the phytobenthos (Section 2.3). Much of the information available on phytoplankton in lakes may be applicable (Section 3.2) but has not been explicitly considered for rivers.

Annex V of the Water Framework Directive does, however, list the phytoplankton community as one of the biological quality elements that needs to be considered for rivers. Specifically, it outlines the following criteria that need to be related to type-specific, undisturbed conditions:

- Phytoplankton composition
- Average phytoplankton abundance and its effect on transparency
- Planktonic bloom frequency and intensity

2.2.1 Composition

Phytoplankton composition in rivers includes species that complete their life cycles whilst in suspension, the ‘true’ plankton and benthic species that are frequently resuspended (Belcher and Swale 1979). Characteristic river communities include many species that commonly occur in lakes, with motile (e.g. *Chlamydomonas*) and non-motile (e.g. *Cyclotella, Scenedesmus*) genera being present.

Diatoms are usually the dominant phytoplankton group in rivers (Genkal 1997) and an increase in dominance of small-celled species has been noted as nutrient concentrations increase (Miheeva 1992, cited in Genkal 1997). Excepting large East European rivers, little research has been carried out detailing river phytoplankton composition and no quantitative relationships between composition and nutrient conditions have been developed. One study has been carried out on River Thames data to assess prediction of phytoplankton composition (Ruse and Love, 1997).

In Australia, increasing incidences of riverine cyanobacterial blooms are a problem in low-flow conditions in nutrient-rich lowland rivers; the River Ouse (Anglian Region) has also experienced similar problems (Love A, personal communication).

2.2.2 Abundance

In general, as nutrient concentrations increase, phytoplankton abundance should increase and become more sustained throughout summer. In a study of 21 years of monitoring data from the River Nene, the relationship between phytoplankton abundance and nutrient conditions was weak, being strongest for spring periods (Balbi, 2000). Discharge, temperature and light were significant predictors of spring chlorophyll *a* concentrations. Phytoplankton abundance was generally lower in summer compared with the spring peaks, despite increases in nutrient concentrations, a pattern that appeared to be related to variations in submerged macrophyte abundance (Balbi, 2000). Another explanation for these observations is that filter feeding benthic bivalves become more abundant during summer, and can remove large quantities of phytoplankton from the water column (Love A, personal communication). More research is, however, required to validate these theories.

Phytoplankton monitoring in the rivers Rhine and Meuse, in The Netherlands, does suggest that mean annual biomass has increased over recent decades as a result of eutrophication. It, however, similarly revealed that year-to-year variations in densities were correlated with variation in water discharge, rather than nutrient concentrations (Ibelings *et al.* 1998).

Despite all this, annual mean chlorophyll *a* concentrations are widely used as a measure of phytoplankton abundance and as a general symptom of eutrophication in all surface waters. Dodds *et al.* (1998) outlined a preliminary classification of streams in the USA based on chlorophyll *a*, TP and TN. In Europe, threshold chlorophyll *a* concentrations for rivers subject to eutrophication have been set in a few EC countries in response to the UWWTD (Table 2.3).

Table 2.3 Threshold criteria used in European member states to designate rivers subject to eutrophication (Cardoso et al., 2001)

Parameter	UK	Ireland	France	JRC Ispra
TP (mean) (µg/l)	>100	>50	<100	75-200
Chl a (mean) (µg/l)	>25	>60	>60	-
Chl a (max.)(µg/l)	>100	-	-	-

Chlorophyll *a* concentrations do represent a very simple and integrative measure of the phytoplankton community response to eutrophication. Concentrations are, however, very seasonal. Variability in response to enrichment can also be due to different sensitivities to increased nutrient loading. In particular, flow rates or turbidity levels will greatly affect phytoplankton development in rivers. From the few detailed studies that have been carried out, spring appears to represent the ideal time of year to collect riverine phytoplankton samples if measures of peak abundance are required.

Measurements of chlorophyll *a* can be problematic in that concentrations vary depending on algal composition and their physiological state (Reynolds 1984). In general, cyanobacteria have less chlorophyll *a* per unit biomass than Chlorophyta. Direct counts and measurements of algal biovolume are, therefore, a more accurate measure of phytoplankton abundance and provide informative compositional information, but are much more time-consuming. Transparency is not used in rivers as a measure of enrichment as non-algal suspended particulates are often responsible for turbidity and may predominantly represent catchment disturbance or bank erosion.

2.2.3 Bloom frequency and intensity

Annex V of the WFD specifically characterises moderate status rivers as those in which persistent phytoplankton blooms may occur during summer months. This wording in the WFD appears to be more applicable to lakes, as in rivers spring blooms are more characteristic. The development of dense “bloom” populations is, however, generally unpredictable with bloom frequency and intensity being particularly affected by flow rate and weather conditions (temperature and wind resuspension). For these reasons, it is unlikely that this measure could be practically developed for widespread application.

2.2.4 Influence of factors other than nutrients

Flow rate and turbidity are the two critical factors limiting the development of a phytoplankton community in rivers. Development is only really possible in slow-moving lowland rivers (Lampert and Sommer 1997). In these systems, year-to-year variation in abundance has been shown to be correlated with variation in water discharge rather than nutrient concentrations (Ibelings *et al.* 1998). Models are being developed for the Seine (RIVERSTRAHLER) and Rhine (Love A, personal communication) and could be applied to phytoplankton data from lowland rivers in the UK to assess how physico-chemical parameters affect phytoplankton dynamics.

2.2.5 Monitoring and classification schemes

No monitoring or classification schemes have been extensively developed in Europe for river phytoplankton. One major difficulty if developing a consistent river monitoring and classification methodology, is the fact that phytoplankton are largely absent from many river ecotypes, particularly smaller upland rivers.

Phytoplankton are, however, often responsible for a significant proportion of primary production in large lowland rivers, which merits their inclusion in monitoring Europe's principle lowland rivers. Chlorophyll *a* concentrations represent a very simple and effective measure of phytoplankton abundance, a key ecological response to nutrient conditions. An additional advantage of using chlorophyll *a* is that it integrates the phytoplankton community response to all potentially limiting resources, irrespective of whether it is phosphorus, nitrogen, silicon or light. Sampling is also relatively straightforward compared with sampling for invertebrates, fish or macrophytes in large lowland rivers. The disadvantage of riverine phytoplankton is that it is a highly dynamic assemblage and at least monthly sampling is recommended, or even fortnightly sampling during spring, if peak biomass needs to be measured.

Classification schemes could only be developed for certain river ecotypes and would require identification of reference or undisturbed sites. Identification of undisturbed, lowland reference sites will be highly problematic, although phytoplankton datasets do exist for a few UK rivers dating back to 1930s. The use of long-term historical datasets from large Eastern European rivers may be the only reliable source of true reference conditions. It could, however, be relatively easy and cheap to produce regional and ecotype-specific classifications based on spatial-state reference schemes (See Section 6).

2.2.6 Recommendations for Phase II

If phytoplankton is a required quality element for rivers, much research will be needed to develop robust monitoring and classification schemes. There will be a particular need to assess the confidence limits associated with the timing and frequency of monitoring, as abundance is strongly affected by flow rate, temperature and turbidity. Models developed for the Rivers Rhine and Seine could be applied to British River phytoplankton data to assess their relevance and usefulness and help improve our understanding of how physico-chemical and other factors (e.g. Bivalve filtration) affect phytoplankton dynamics. Phytoplankton may in fact be a better indicator of the combined effects of high nutrient status and low flow conditions on ecological status.

2.2.7 Summary

There is a question over whether phytoplankton is a required quality element in the WFD for rivers, it being absent from the list of quality elements in Annex V, paragraph 1.1.1, but present in paragraph 1.2, Table 1.2.1. (Council of the European Communities 2000).

If it is required, it is likely to be only applicable to slow-moving, lowland rivers and canals where it is often responsible for a significant proportion of primary production, and even in these systems it could be strongly limited by turbidity from resuspended particles. The definition of large British rivers and large mainland European rivers needs to be considered carefully due to big differences in scale. If required, a great deal of research will be needed to develop effective classification schemes for individual river ecotypes.

2.3 Phytobenthos

Phytobenthos is a term used to describe algal communities associated with submerged substrates, including epiphyton (macrophyte-associated), epilithon (rock-associated), episammon (sand-associated) and epipelon (mud-associated) (Round 1981). "Periphyton" and "aufwuchs" are terms that are more or less synonymous with the term "phytobenthos".

Benthic algae are considered to be the main source of primary production in many stream and river systems (Wetzel 2001), but particularly so in mid-sized (third to sixth order) streams (Vannote *et al.* 1980). In addition to their primary production role, they are functionally important in sequestering and transforming many inorganic nutrients into organic forms (Stevenson 1996). Several species of cyanobacteria or diatoms with endosymbiotic (symbiosis in which one symbiont lives within the body of another) cyanobacteria (e.g. *Epithemia* and *Rhopalodia* species) are capable of converting atmospheric nitrogen into ammonia (NH_3) or amino acids (Borchardt 1996). The phytobenthos themselves constitute a significant source of energy for higher trophic levels with most invertebrate grazers feeding on benthic algae, rather than macrophytes.

The phytobenthos has also received considerable attention in terms of its use as an indicator of river water quality, in recent years specifically in terms of nutrient conditions (see Section 2.3.4). Annex V of the Water Framework Directive specifically outlines phytobenthos composition and abundance as two criteria that need defining for type-specific, undisturbed conditions in rivers. The response of the phytobenthos to nutrient conditions will, therefore, be considered in these terms, followed by consideration of other factors affecting the community and finally by discussion of the potential application of established phytobenthos-based monitoring and classification schemes to the WFD.

2.3.1 Composition

Most benthic algae in freshwater habitats are green algae (Chlorophyta), diatoms (Bacillariophyta), blue-green algae (cyanobacteria), or red algae (Rhodophyta), although most other algal groups can occur in benthic habitats. Many species are characteristic of the phytobenthos, and are absent from or rare in phytoplankton communities, including filamentous green algae, such as *Cladophora* and *Vaucheria*, benthic cyanobacteria, such as species of *Rivularia/Calothrix* and many pennate diatoms (e.g. *Cocconeis*, *Achnanthes*, *Gomphonema* and *Navicula*).

If a lack of disturbance permits, benthic algae can develop mixed communities, or algal mats. Initially, colonisation by tightly bound adnate (attached along entire length) algae occurs, composed mainly of diatoms such as *Cocconeis*. If currents are slow, and light and nutrients sufficient, these are then overgrown by apically (attached at top point) attached algae, such as the diatom *Synedra*. Further community development can proceed to stalked diatoms, such as *Gomphonema*, and filamentous algae. The development of phytobenthos communities towards maximum biomass varies with conditions, but is commonly in the range of 1-2 weeks (Wetzel 2001).

Even though the physiological requirements and responses of attached algae are similar to those of the phytoplankton, the relationship between nutrient conditions and phytobenthos community composition is much more complex. Nutrients diffuse much more slowly into attached communities, with strong gradients through boundary layers and the attached communtiy. The thicker the community the more isolated the algae are from water column nutrient conditions. Benthic algae can also obtain nutrients from the substrates that they are attached to. Clear differences in community composition have been observed between natural and artificial substrates (Cattaneo 1978). Nutrient enrichment in the overlying water does, however, result in clear changes in community composition. Species associated with low phosphorus (the diatoms *Achnanthidium minutissimum* and *Gomphonema tenellum*) and low nitrogen (nitrogen-fixing species, such as the diatoms *Epithemia adnata* and *Rhopalodia gibba* and cyanobacteria such as *Anabaena* spp.) waters have been shown to disappear following nutrient enrichment (Fairchild, Lowe and Richardson 1985). In slower-moving lowland rivers, as nutrient enrichment proceeds further, phytoplankton can shade the phytobenthos, and light-limitation becomes more significant; community composition then becomes dominated by species tolerant of low light levels. Low-light specialists are often filamentous and can outgrow less competitive species. The increasing dominance of filamentous green algae, such as *Cladophora glomerata* and *Stigeoclonium tenue*, over diatom species is well recognised as a response to abundant nutrients and low light levels. *Stigeoclonium tenue* in particular indicates heavily enriched waters (Borchardt 1996).

The use of multivariate statistical techniques to explore variability in community composition is beginning to highlight significant relationships between guilds of benthic algae and nutrient conditions (Carrick, Lowe and Rotenberry 1988). These relationships, however, do not necessarily illustrate cause and effect. Kelly (1998) explores the question of whether inorganic nutrients, particularly phosphorus, determine algal community structure in rivers. He concluded that it is often difficult to attribute ecological changes specifically to nutrients, rather than other components of waste discharges such as organic pollution and excessive silting of the stream bed. Nonetheless, the complementary use of independent indicators of organic pollution (e.g. benthic invertebrates) can identify where organic pollution may have a compounding effect.

2.3.2 Abundance

In general terms, numerous studies have demonstrated that nutrient enrichment in the overlying water usually results in enhanced growth of attached algae. In slow-moving lowland rivers, however, at very high nutrient concentrations, the phytoplankton can shade out the phytobenthos community all together.

Biggs (1995) summarised chlorophyll *a* data from 16 New Zealand streams that spanned a range of nutrient conditions. Concentrations ranged from 0.5 to 3.0 mgm^{-2} in un-enriched streams with forested catchments; 3.0 to 60 mgm^{-2} in moderately enriched streams; and 25 to 260 mgm^{-2} in enriched streams with highly developed agricultural catchments. High chlorophyll *a* concentrations (>100 mgm^{-2}) occurred for approximately 40% of the year in enriched streams compared with <1% of the year in moderately enriched streams.

Excessive accumulations of benthic filamentous algae, such as *Cladophora*, occur under high nutrient concentrations and low flows. Biggs and Price (1987) observed that when filamentous algae exceed a cover of 40% (approx. 160 mgm^{-2} chlorophyll *a*) the community becomes very conspicuous from the bank and if cover was 55% or greater (>300 mgm^{-2} chl. *a*) it usually resulted in extensive smothering of the sediments. Nordin (1985) recommended threshold biomass values for different uses in streams of

British Columbia, Canada: <50 mgm^{-2} chlorophyll *a* to protect recreational usage and <100 mgm^{-2} to protect aquatic life. Horner *et al.* (1983) similarly suggest that chlorophyll *a* concentrations >100-150 mgm^{-2}, or cover >20% by filamentous algae is unacceptable. In less enriched streams, stalked diatom communities of much lower biomass thresholds can cause significant ecological changes by completely smothering the substrate (Biggs 1996).

As stated in the last section, the relationship between nutrient conditions and the phytobenthos community is complex due to nutrient gradients through the attached community and interactions with their substrate. Measures of phytobenthos abundance are, therefore, not simply a response to ambient water column nutrient concentrations. Productivity in thick mats is maintained more by efficient internal recycling of nutrients than by external diffusion (Wetzel 2001). The role of the substrate as a source of nutrients for the phytobenthos community is particularly marked in oligotrophic waters (Wetzel 2001).

Water currents also have consequences for nutrient uptake and utilisation, making it difficult to interpret or predict patterns in phytobenthos abundance. For this reason, nutrient mass transport (flow velocity x nutrient concentration) is often a better predictor of phytobenthos abundance than concentrations alone (Borchardt, 1996).

2.3.3 Influence of factors other than nutrients

Substrate

Substrate has a major effect on species composition. Epilithic and epiphytic communities have many species in common, but tend to have a very different composition from communities found on epipelic habitats, or on other loose substrates (Wetzel 2001). Epipelic communities on fine, organic sediments largely comprise motile algae, as mobility is necessary to move to the surface following disturbance of the sediments. Episammic communtities tend to be dominated by algae attached firmly to crevices in sand-grains.

Light availability

In streams and small rivers, shading by bankside vegetation can greatly affect species composition and abundance, with biomass levels 5-times higher in unshaded stretches. Resuspended sediment can also severely limit light levels in rivers, similarly, affecting phytobenthos composition and abundance.

Grazing

Epiphyte communities in particular are affected by grazers (mainly snails and insect larvae). Moderate grazing can enhance algal growth, as it can lead to improved access to nutrients and light (Wetzel 2001), but heavy grazing pressure usually results in reduced phytobenthos biomass. In heavily grazed areas, nutrient-enhanced algal production that is not expressed in phytobenthos biomass is often instead reflected in increased grazer abundance. Intense grazing pressure has also been shown to affect species composition and reduce algal diversity (Mulholland *et al.* 1991).

Disturbance

In rivers, water currents and other physical disturbance events have severe effects on the phytobenthos community and are probably the primary reason for its highly dynamic nature. Attached communities are well adapted to flow rates up to about 15 cm s^{-1}

(Wetzel 2001), but during sudden spates community losses can be great. The frequency and magnitude of spate events is critical. Low biomass values are more likely to be in response to frequent disturbance than low nutrient concentrations. Conversely, less disturbed habitats tend to have higher biomass. Peak biomass is, therefore, the ideal measure of the potential of a river system, but requires frequent monitoring.

2.3.4 Classification and monitoring schemes

The phytobenthos has received considerable attention in terms of its use as an indicator of river water quality. Microscopic benthic algae, in particular, offer the advantages of relatively direct sensitivity to nutrient pollution and ubiquitous distribution. Diatom-based methods have been the main focus of attention as they are a diverse group of organisms found in all river habitats, their ecological requirements are relatively well known, and they are relatively unaffected by flow rates (Horner *et al.* 1990).

The first recorded use of diatoms as a river monitoring tool was in the development of the saprobien system in Germany, used to indicate organic pollution (Kolkwitz and Marsson 1908, cited in Round, 1981). Later developments used diversity indices to indicate water quality (Archibald 1972; Patrick 1954), although these were found to be unreliable and led to the conclusion that compositional data was more relevant. One progression from this was an effective scheme developed by Lange-Bertalot (1979) relating diatom species tolerances to organic pollution (B.O.D.). The development of effective benthic invertebrate-based schemes that indicate organic pollution, such as RIVPACS, has, however, led to the redundancy of many of these algal-based schemes.

Following the significant improvements in river water quality in terms of organic pollution, there has been a shift in attention to assessing the impacts of inorganic nutrient pollution. In particular, the requirement within the UWWT Directive to identify rivers affected specifically by eutrophication has resulted in a need to develop new monitoring schemes. Diatom-based methodologies have been widely adopted across Europe, particularly in France (Prygiel and Coste 1996), Germany (Coring 1996), and Belgium and Luxembourg (Descy and Ector 1996).

In the UK, the Trophic Diatom Index (TDI) has been extensively developed for the specific needs of the UWWT Directive (Kelly and Whitton, 1995; Kelly, 1998) and has been compared with the Mean Trophic Rank (MTR) scheme based on macrophytes and macroalgae (reviewed in section 2.4). The TDI scheme was shown to be particularly useful in situations where the MTR was not applicable (no macrophytes) or inconclusive, and could identify sites influenced more by inorganic nutrient pollution (eutrophication) from those where organic pollution had the most significant effect (Harding and Kelly 1996).

The TDI approach is based on weighted averaging, where the average chemical conditions where a taxon is found is weighted by its abundance at each known site (see Kelly and Whitton (1995), Kelly (1996b) and Kelly *et al.* (2001) for full details). The ‘sensitivity’ of taxa were assigned based upon the phosphorus concentration at which they were most abundant. The TDI and most of the other European diatom-based methods refine this further by designating an ‘indicator’ value for all the taxa (Kelly 1998b). This gives greater weight to taxa that are good indicators of eutrophic status (i.e. show narrow tolerance around their weighted-average optimum). The production of a “User’s Manual” (Bowburn Consultancy 1996; Kelly *et al.*, 2001) allows relatively easy application of the TDI by trained Environment Agency staff, with many taxa only requiring identification to the genus level. The TDI was primarily developed for use on lowland rivers and will require further development for widespread use within a specific-WFD context, particularly for application to upland river stretches.

One problem associated with widespread application of the TDI or the other European diatom indices is the fact that they are based on epilthic diatom communities. Sampling of stones free of filamentous algae and significant siltation is suggested (Kelly and Whitton 1995), which can be difficult in enriched, lowland rivers (Round 1993). Because of habitat variability, artificial substrata of uniform composition are commonly recommended for monitoring schemes (Clesceri, Greenberg and Eaton 1998). Methods for installing and using unglazed roof tiles have been recommended for routine monitoring where natural solid substrates are absent (Kelly *et al.* 1998). In a study of lowland rivers in southern England, Goldsmith (2001) found that only 40% of river sites had a good epilithic community and 23% of sites had no epilithon but that two artificial substrates, unglazed tiles and polyethylene rope, could provide comparable results. The communities that developed on the artificial substrates were substrate-specific, highlighting the need to develop substrate-specific training sets for application to biomonitoring studies. It is, however, unlikely that the use of artificial substrates is applicable to the analysis of ecological status in the WFD as a measure of 'deviation from undisturbed'. In a study of two streams in Ontario, Canada, Winter and Duthie (2000) challenge the need for substrate-specific sampling, finding that the best relationships between diatoms and nutrient concentrations can be achieved by using all available diatom communities: epilithon, epipelon and epiphyton. In fact the latter two communities showed more significant relationships with total phosphorus than the epilithon community. If the TDI method is to be developed further for the WFD, distinct and mixed natural community responses should be investigated to assess the effect of substrate more fully.

Goldsmith (2001) recommended the use of a taxon's actual weighted average optimum and tolerance to phosphorus rather than the 'sensitivity' and 'indicator' classes used in the TDI and other European schemes. This does, however, assume a rather simplistic relationship between phosphorus and diatom species responses that often does not hold true in river systems. In contrast, the TDI and other European diatom indeces offer a more distinct ecological assessment that is more in accordance with the ethos of the WFD and "ecological status" in that it is measuring an effect of eutrophication rather than acting as a proxy for the cause (nutrient conditions).

Kelly (1998a) outlines why a biological community-based index of eutrophication will always have an upper limit of responsiveness. Above certain concentrations, nutrients may no longer affect diatom species composition or limit abundance, with other factors, such as light, becoming the limiting resource. Any scheme is, therefore, likely to be relatively insensitive at very high phosphorus concentration (>300 μgl^{-1}) or if affected by severe organic pollution (Kelly 1998); actual thresholds of insensitivity are dependent on a number of factors and will vary between catchments (Kelly M, personal communication).

In terms of reference conditions, there is a need to collate existing Agency phytobenthos datasets to establish current geographical and environmental coverage. The use of epiphytic diatoms on macrophyte herbarium records could be used to identify reference assemblages for river ecotypes where undisturbed reference sites no longer exist. A pilot study applying the TDI to herbarium records from chalk streams successfully highlighted the potential of this approach (Hourigan, 1999).

Quantitative analyses of phytobenthos communities are much more difficult than qualitative analyses of species composition. The simultaneous collection of debris and dead cells can confound abundance estimates, making wet or dry weights difficult to apply. The most ideal assessment of phytobenthos abundance is a direct biovolume estimate. This is, however, time-consuming to obtain and phytobenthos abundance is generally measured as chlorophyll *a* per unit area of substrate. It is particularly important, however, to correct for pigment degradation products to take into account of decomposing material (Wetzel and Likens 2000). Pigment ratios can also be examined to distinguish different algal groups.

No standardised sampling methods or established classification schemes exist for phytobenthos abundance. Because of its dynamic nature in response to grazing and disturbance, it may be difficult to set typical values for different ecological status classes. For this reason, peak biomass is the ideal measure of the potential of a river system, but requires frequent monitoring. Threshold peak biomass values could, however, be developed between high, good and moderate status classes using chlorophyll *a* concentrations per unit area as a practical measure. It must be recognised that sampling may not represent peak biomass, but if the threshold value is exceeded, then a site would be of lower class.

One major problem with the WFD is that monitoring frequency for the phytobenthos ("other aquatic flora") is outlined as every 3 years. This is certainly inadequate for this community. At a minimum, the 6-monthly frequency stated for phytoplankton should be adopted for phytobenthos too, although a 3-season sampling programme (such as in RIVPACS) may be more appropriate (Kelly M, personal communication).

2.3.5 Summary

The phytobenthos community is the main source of primary production in many stream and river systems. Europe-wide, it is probably the most established indicator of river water quality in terms of nutrient conditions, complimenting the use of benthic invertebrate indices as measures of organic and toxic pollution. The dynamic nature of river environments could lead to misinterpretation of phytobenthos community data, particularly with measures of abundance. Standard sampling and analysis protocols for semi-quantitative diatom indices require acceptance at a European level.

2.3.6 Recommendations for Phase II

There are a number of essential requirements before the phytobenthos can be fully utilised in the WFD ecological classification:

- The established use of phytobenthos as a biological indicator of eutrophication, developed in response to the UWWTD, needs widening. Specifically, current diatom indices, such as the TDI, need to be calibrated and validated for a wider number of river types, particularly upland rivers.
- Distinct and mixed natural community responses should be investigated to assess the effect of substrate more fully and to assess whether a consistent method is applicable to different river types

- In terms of reference conditions, there is a need to collate existing Agency phytobenthos datasets and, if needed, historical records could be extended using epiphytic diatom remains on macrophyte herbarium records.
- Chlorophyll *a* per unit area could be developed as a measure of phytobenthos abundance, but the effect of sampling frequency needs to be established. Threshold peak biomass values could be established to distinguish high, good and moderate status classes.

2.4 Macrophytes

2.4.1 Factors affecting composition and abundance

The habitat requirements of macrophytes (especially substrate, water flow and water chemistry) have been considered in detail over the last century, alongside an appreciating acknowledgement of pollution, particularly the effects of nutrients (Arber 1922; Butcher 1933; Whitton 1975; Westlake 1976; Dawson 1988).

Changes in the abundance of macrophytes and macroalgae are often the most visible sign of change in nutrient conditions of British rivers. In general, the observations are as follows:

- nutrient-poor streams - relatively sparse biomass but a diverse range of higher plants, Bryophytes or algae
- medium nutrient streams - elevated biomass, but fewer species, of higher plants
- nutrient-rich streams - dominated by few species of higher plants or macroalgae, whose populations may alternate or cycle for short or extended periods
- streams with very high levels of nutrients - dominated by colonies of a few species of filamentous algae or microbial films, and where most higher plants have been lost.

All macrophyte species need nutrients in relatively similar proportions for basic growth but may respond differently to additional levels depending upon general water chemistry, substrate, flow and light conditions. For example, in iron-rich waters phosphate may be chemically bound and not available for plant growth. Disproportionately high or low water velocities will also moderate growth either directly through water velocity or through, for example, substrate alteration making it unsuitable for root attachment or penetration.

The presence of different species may be limited by a single factor, or by several in combination, but little data are available on many of these relationships. Thus whilst nutrients may enhance the growth and biomass of a species, competition with other species with greater capacities for growth may result in the loss of the species. Likewise direct or indirect interactions with, for example, moderated seasonal or decreased water flow, channel alteration, modification of water velocities or increased inputs of inorganic or organic compounds may alter species compositions. In general, little is known about combined or synergistic effects. Studies currently available include: three species of *Myriophyllum* in relation to calcium concentration (Hutchinson 1970), heavy metals (Kelly 1989), boat traffic (Murphy and Eaton 1983; Willby and Eaton 1993), pesticides (Frisque *et al.*1981) and detergents (Kohler 1975). In reality, however, many of these interacting factors may result in a broad dispersion of the presence and abundance of any species when compared to one factor such as nutrient conditions. Thus any analysis of such relationships, as undertaken for the assessment of the Mean Trophic Rank method in UK (see Section 2.4.3), must accommodate this when defining ecological status (Dawson *et al*. 1999).

2.4.2 Relationships between biological quality and nutrients

Little is known about the time required for macrophytes to respond to changes in nutrient levels and to establish an equilibrium with these nutrient concentrations and thus establish indicator status. Plants respond to short-term nutrient changes by luxury uptake (Gerloff 1975), which increases their internal concentration, and if conditions allow rapid growth may ensue. However, no direct or causal evidence is available directly relating plant growth or physiological condition to nutrient concentrations. Plant population changes which should occur as a result of increases in nutrients are moderated by the plant species themselves and the presence or not of other more tolerant species which can then increase rapidly and change the population structure. Conversely the changes in species following reductions in nutrient concentrations appear to be less rapid as less tolerant plants need to reinvade from elsewhere. The MTR methodology arbitrarily considers that three years (and four surveys) is sufficient to indicate if significant change has occurred.

There have been several surveys of flowing waters undertaken in order to establish the typical habitats of fluviatile macrophytes over recent decades. Although examples of underlying relationships with habitat and water chemistry have been illustrated, it has not been possible to establish relationships with nutrients on a national scale until recently. The first national surveys were probably initiated by the NCC, and continued by CCW, EN and SNH (‘the Conservation Rivers database’). The data collected were added under contract by NERC-CEH (formerly NERC-IFE) for the EA to meet the requirements of assessing eutrophic waters for the UWWT Directive and have enabled a large database to be assembled for analysis of the effects of nutrient conditions (Dawson 1999).

2.4.3 Classification and monitoring schemes

Several methodologies have been developed over recent decades in attempts either to study the effects of various conditions on the relative growth of different species of water plant, or to use the responses of plants to indicate current or changing conditions (Table 2.4). Many of these studies have been on single rivers or catchments or have compared similar rivers with differing conditions e.g. water chemistry or inputs (Table 2.5). Such catchments may appear to have features in common but the responses of plants may be influenced by unmeasured or unknown factors.

Table 2.4 Methodologies for plant assessment in rivers and nutrient classification with national coverage

Reference		**Methodology overview [basis of assessment]**	**Trophic divisions**
Seddon 1972	Predominantly lake plants and their natural status	Ordination of lake plant assemblages by physico-chemical and water chemistry parameters	5 broad groupings
Haslam 1978	River plants	Descriptive [Expert opinion]	
Newbold and Palmer 1979	Use of plants to reflect general trophic status in national context for open water bodies using P, N, alkalinity	Trophic Ranking number (1-150) given to 150 open water plant species [Expert opinion]	5 ranges <5 , 5-10, 10-30, 30-100, >100
Haslam 1981	Classification of the status of plant communities	Assessment of damage using difference from expected for 8 plant communities in habitats derived for stream size, landscape and rock-type [expert opinion]	
Holmes 1983	Typing rivers according to their flora	Nature conservation evaluation of rivers using indicator species to type river plant assemblages called 'community types' divided on height of source and gross geology [improved analysis - no water chemistry]	
Holmes and Newbold 1984	River plant communities – reflectors of water and substrate chemistry	Evaluation of trophic status of riverine plant communities developing the Trophic Rank of Newbold and Palmer to a Trophic Score [improved analysis - no water chemistry]	5 ranges from oligo- to meso-, eutrophic
Standing committee of analysts 1987	Standard methods for UK	classification for conservation purposes and also 'plant score' for pollution assessment	
Holmes and Newbold 1989	British rivers - a working classification		

Reference		**Methodology overview [basis of assessment]**	**Trophic divisions**
Palmer 1989	Classification of standing waters and method for assessing changes in water quality	Surveys of standing waters with hierarchical analysis and classification with physico-chemistry of waters and site with comparison to other methods [no nutrient chemistry]	10 types
Leglize and Peltre 1991	Method for classification of river plant associations and environmental quality	Method for plant distribution, plant habitat with some geomorphology	
Holmes 1995	Macrophytes for water and other river quality assessments – East Anglia	Water quality assessment through indicator species basis of MTR method [synthesis of expert opinions - no water chemistry]	
Haury, Peltre Muller , Tremolieres, Barbe, Dutartre and Guerlesquin 1995	Macrophyte indices to assess stream water quality : preliminary proposals		
Holmes, Boon and Rowell 1999	Vegetation communities of British rivers	Plant species key to classifying rivers into descriptive groups with trophic status [Correlative analysis of geology, habitat, and plant species - no water or sediment chemistry]	3 ranges oligo - meso- eutrophic
Holmes, Newman, Chadd, Rouen, Saint and Dawson 1999	Mean % cover of individual plant species by their STR rankings	Users manual for Mean Trophic Rank methodology	continuous
Dawson, Newman, Gravelle, Rouen and Henville 1999	Mean % cover of individual plant species by their STR rankings	Assessment of the trophic status indicator plant species and evaluation of the Mean Trophic Rank methodology. [comparison of indicator species with SR phosphate and nitrate]	continuous or 3 broad ranges

Table 2.5 Methodologies or studies of plant assessment with lesser degrees of coverage or extent of nutrient classification.

Author	**Methodology overview [basis of assessment]**	**Nutrient classification**
Kohler Wonneeberger and Zeltner 1973	Assessment of chemical pollution indicators for a German river (indicated that only N and P had indicator value)	Series of floristic-ecological river zones (*c*6)
Wiegleb 1978	Indicator values for plant communities in standing waters of differing water chemistry, Lower Saxony [statistical analysis of surveys]	6 clear community based phytosociological indicator groups
Harding 1981	Plant cover in rivers of NW England	5 ranges of plant species: sensitive to tolerant
Marieux 1982	Use of phyto-sociological groupings as indicators of water quality	5 zones/levels
Caffrey, 1987	Comparison of plant species in three Irish catchments with water quality class	Uses 5 water quality classes
Haury, Peltre, Muller, Tremoliers, Barbes 1991	Comparison of ten French rivers on different geology, conductivity and degree of pollution establishing indices for 200 riparian species [expert opinion plus N, P]	
Kelly, 1996c	Diatoms monitoring eutrophic and organic enrichment	Continuous and broad ranges
Dawson and Szoszkiewicz 1999	Relationships of some ecological factors with the associations of vegetation in British rivers (N, P etc)	Community based - no bands defined but nutrient data given
Thiebaut and Muller	Eutrophication with acidification in Vosges, France	4 communities in sequence
Haslam 2000	Pragmatic method for evaluation of pollution in Malta	

Biological quality using individual plant species, assemblages or phyto-sociological communities have all been used to indicate given nutrient regimes. The Mean Trophic Rank (MTR) is a biotic index based on the presence and abundance of aquatic macrophytes, where species present are assigned a score according to their tolerance to eutrophication (Holmes 1996; Holmes *et al.* 1999). The MTR has been extensively developed and tested in the UK for particular application to monitoring river water quality for the purposes of designating Sensitive Areas (Eutrophic) under the UWWTD.

Analysis of the combined database for the assessment of MTR indicated that although several individual species may have broad responses to phosphate or nitrate, the assemblages as a whole were more reliably indicative. Recommendations for future research to progress such indicator status have been made by proposing links with other macrophyte distribution projects using common survey methodologies for sampling of plant communities from a wider range of physical substrate, resolving the role of rare plants, investigating the distribution of those species which are potential indicator species, examining species richness or diversity as an alternative measure of trophic status, and examining the effects of nutrient reduction on plant communities. Community analysis has been almost entirely developed in mainland Europe (Braun-Blanquet 1928; Tuxen 1937; Dierske 1994) but developments have been progressing in the UK (eg. Rodwell, 1995; Reilly and Page 1990) including specific studies in relation to nutrients, such as Dawson and Szozkiewicz (1999) and Kelly and Whitton (1994).

Simple process models do not seem to have been developed but an EA scoping study has been undertaken to develop a predictive element and extend the MTR system called 'PLANTPACS' (Plant Prediction [classification] and Classification System). Further research is required to confirm the precise influence on MTR of depth, cross-sectional area, flow category, substrate size, river slope, solid geology, and the altitude of the source.

2.4.4 Methods for defining reference communities

Several methods have been used to establish reference nutrient concentrations and characteristic communities but the determination of good status has always been a major point of discussion. Some analysis of the presence or extent of species at the individual, assemblage or community level has been undertaken and a variety of responses have been found often dependent upon the habitat and general water chemistry characteristics of the study sites. An initial assessment of the MTR data leads to the conclusion that undisturbed 'high' status could be defined by annual mean SRP concentrations less then about 10 μg l^{-1} (Table 2.6). Experiments in large channels (Dawson 1983; Ladle *et al*. 1988) also support this, showing enhanced plant growth for some species, such as *Ranunculus*, around a 10 μg l^{-1} threshold.

Several ranges of phosphate concentration have been proposed over the last few decades which broadly relate to the OECD definitions established for lakes, which for annual mean concentrations of total phosphorus are:

oligotrophic	<10 μg l^{-1}
mesotrophic	10-35 μg l^{-1}
eutrophic	35-100 μg l^{-1}
hypertrophic	>100 μg l^{-1}

For rivers, the NRA and the UK conservation agencies proposed interim standards for four levels at 20, 60, 100 and 200 μg l^{-1} SRP as the equivalent of oligotrophic, mesotrophic, meso-eutrophic and eutrophic (Environment Agency, 1998).

Table 2.6 Comparison and justification for proposed range limits for class definition of the ecological status for nitrate and phosphate in running waters

Status	**Class definitions**	**Detailed field studies***	**MTR assessment****	**Proposed range limits** (alternative less rigorous value)
High	Undisturbed or minor anthropogenic alterations	10 μg P l^{-1} 3.7 mg N l^{-1}	18-40μg P l^{-1} 0.2-0.8 mg N l^{-1} Plant types: Mostly bryophytes	<10 (<25) μg P l^{-1} 0.2 (0.5) mg N l^{-1}
Good	Slight deviation in biology from undisturbed. No significant undesirable disturbance	49 μg P l^{-1} 4.0 mg N l^{-1}	40-80 μg P l^{-1} 0.8-2.2 mg N l^{-1} Plant types: Mostly bryophytes and submerged species	30-40 (75) μg P l^{-1} 0.8 (1.5) mg N l^{-1}
Moderate	Moderate deviation in biology that may produce a significant undesirable disturbance in other biological and physico-chemical elements	112μg P l^{-1} 2.8 mg N l^{-1} (increased to 8 mg N l^{-1} in 2000)	90-125μg P l^{-1} 2.2-5.5 mg N l^{-1} Plant types: Submerged and emergent species	60 (100) μg P l^{-1} 2 (4) mg N l^{-1}
Poor	Major alterations in biology. Substantial deviation from undisturbed		125-*c*450 μg P l^{-1} 5.5-8.0 mg N l^{-1} Plant types: Includes *Cladophora* agg.	100 (200) μg P l^{-1} 6 (8) mg N l^{-1}
Bad	Severe alterations in biology. Undisturbed communities largely absent		>450(-*c*6000) μg P l^{-1} >8.0 (- *c*12) mg N l^{-1} Plant types: Mostly emergent spp. but includes *Enteromorpha*	>500 μg P l^{-1} > 8 mg N l^{-1}

*Data of the detailed field studies are derived from Westlake *et al*. (1972)

**MTR assessment is derived from the 129 MTR species ordered by their relationship with mean soluble phosphate-P or nitrate-N and divided equally with proposed range limits for P and N based upon consideration of dispersion of values.

2.4.5 Recommendations for Phase II

National surveys on the distribution and habitat of many species of fluviatile aquatic plant are becoming increasingly available on large databases. There is in general, sufficient data for the broad analysis of the relationship of most species with nutrient conditions. However, some targeted sampling is required to cover all river ecotypes and to clarify the relationship for rare plant species. Preliminary further analysis with species of *Ranunculus, Potamogeton* and *Callitriche* confirms general relationships with water chemistry but shows that species responses to phosphorus are affected by other factors, such as dominant substrate (from RHS surveys) and so require analysis in terms of natural ecotype-specific assemblages.

Many surveys (including MTR) do not, however, always exactly match sample sites for macrophyte and water chemistry data. This leaves us with the comparison of experimental data on optimum growth and habitat conditions being compared with field site data. The latter, however, includes sites degraded by a range of modifications from reduced, moderated or modified seasonal flow regimes, enrichment by nutrient or other substances or plant management regimes. Analysis of such data could falsely ascribe mean conditions as average and the basis for definitions of 'high' or 'good' status. For example for *Ranunculus penicillatus* the 30 or 60 $\mu g\ l^{-1}$ proposed by English Nature is not in agreement with experimental data which has shown no inhibition of growth at 10 $\mu g\ l^{-1}$. This lower concentration would currently be impractical to achieve but perhaps represents excellent conditions i.e. the upper end of 'high' status. It should be noted that this value is also likely to require regular monitoring to a lower level of detection limits than is currently standard. Conversely the use of algae, even filamentous algae, as indicators of elevated nutrients has to be used cautiously. For example, in some streams, particularly in shaded forest clearings, the absence of higher plant species can lead to opportunistic growth of filamentous algae.

For these reasons several areas of long-term research are recommended:

- The interactive effects with general water chemistry, flow, substrate and management
- The source of nutrients to macrophytes, i.e. proportions from water and sediment
- Establishment of minimum uptake levels. Do plants needs levels above 5 $\mu g\ l^{-1}$?
- The extent of excess or 'luxury' uptake. Does it apply to all nutrients equally?
- The proportions of each nutrient required by different species

The influence and importance of flow and sediment nutrient loads is of particular importance and potential relevance to the WFD. It has long been considered that aquatic plants of rivers derive nutrients and particularly phosphorus from wherever it is more readily available, frequently the sediment. In rivers, nutrient concentrations in the sediment reach an equilibrium state with the overlying water and can, however, only be considered a better predictor of ecological status than water concentrations if a greater understanding of the relationships is established. Phase II R&D should, therefore, probably focus on how the MTR approach needs to be modified to accommodate these interactive factors and what implications this has for the the ecotyping process in the WFD.

2.4.6 Summary

Macrophytes have considerable ecological importance as they provide refuges, substrates and a source of food for many other riverine organisms. Their distribution is affected by both water and sediment nutrient conditions as well as a multitude of other factors, such as flow, substrate type and light conditions.

Data does now exist, in the form of the MTR database, to associate distributions with nutrient concentrations in the water column and these other habitat factors. This database could be used to define nutrient conditions associated with 'high' status, but classes below this are less definable. There are concerns with associating absolute phosphorus concentrations with different status classes, as shown in Table 2.6, as regional or other environmental factors will affect where boundaries between classes should be set (Holmes N, personal communication). Further analysis of the MTR database examining the interactive effects of general water chemistry, flow and substrate type is required and may help establish natural ecotype categories and status class thresholds.

2.5 Benthic Invertebrates

2.5.1 Background

The response of benthic macroinvertebrate communities in streams and rivers to stressors of various types has been acknowledged and utilised as a means of assessing water quality since the beginning of the twentieth century in the idea of saprobity, or response to organic pollution, developed by Kolkwitz and Marsson (1909). The more specific response of benthic invertebrates to nutrient enrichment has been studied since the early seventies (Cole 1973). Despite the statement by Mol (1980) that 'invertebrate species with a clear preference for a specific nutrient level are not very numerous as compared to phytoplankton or macrophytes', the body of work on the relationship between freshwater benthic invertebrate communities and nutrient enrichment is considerable.

2.5.2 Relationships with nutrient concentrations

Much research demonstrates the complexity of response of benthic invertebrates to changes in water quality and the difficulties in isolating responses resulting from changes in physical factors (e.g. light levels and river flows) and locality effects (e.g. shading and substrate characteristics) from water quality variations. The response of the benthic invertebrate community to a particular stressor will be different under various conditions such as geographical location, geology, ambient water chemistry, hydrological regime, season, etc. There is no apparent evidence for linear continua in nutrient-invertebrate relationships because of the many concomitant factors influencing benthic invertebrate communities.

There is potential for ecological thresholds to occur, e.g. as nutrient concentrations increase there is the potential for loss of macrophytes, an important habitat for invertebrates, resulting in significant changes in abundance or composition of the invertebrate fauna. This is, however, an indirect effect. More direct threshold effects may be through a changing food resource, affecting each species individually, with no obvious direct threshold effect on the invertebrate assemblage as a whole.

Most research has been directed at standing waters but recent work demonstrates the growing knowledge on nutrient effects on riverine biology as a whole (algae, macrophyte and invertebrates) (Wright *et al.* 2001; Neal *et al.* 2001).

Neal *et al.* (2001) bring together information on the water quality functioning of the River Kennet and other parts of the upper River Thames in the south east of England. The Kennet represents a groundwater fed riverine environment impacted by agricultural and sewage sources of nutrient pollution. Models are developed and applied to assess key processes involved in such a highly dynamic system and to provide estimates of the likely responses to environmental change. Modelling is, however, targeted at macrophytes, rather than benthic invertebrates. Modelling simulations indicated that flow has the most important control on macrophyte biomass in the Kennet, and that the change in phosphorus concentrations due to the removal of phosphorus from effluent at a sewage treatment works has a relatively minor effect (Wade *et al.* 2001a,b). The paper concludes with a discussion on the present state of knowledge, key issues and future research on the science and management of groundwater fed nutrient impacted riverine systems.

Much research has been concerned with very general effects of eutrophication on benthic invertebrate assemblages (Omernik 1977; Bargos *et al.* 1990; Rader and Richardson 1994; Brown 1995; Blumenshine *et al.* 1997). Bournaud and Keck (1980) demonstrated that eutrophication could be detected by the mean density of benthic invertebrates. Bournaud *et al.* (1980) found that Ephemeroptera and Plecoptera are sensitive to eutrophication. Poor family richness resulted from organic enrichment in streams on cultivated land in lowland Greece (Giannakou *et al.* 1997). Breitburg *et al.* (1999) found that most taxa increased in abundance in response to nutrient additions, while Harding *et al.* (1999) found no change in species richness but species composition changed with nutrient concentrations.

The community changes are generally consistent and distinct enough to be used to detect eutrophication and have been assimilated into water quality assessment programs e.g. the nutrient pollution source assessment of Clark Fork River (Ingman and Kerr 1990); the National Water Quality Assessment used in the Kentucky River Basin (Smoot *et al.* 1995), and the macroinvertebrate indices used by the US EPA (1998) to indicate eutrophication.

North American case-study

In the United States, Miltner and Rankin (1998) at the Ohio Environmental Protection Agency, studied primary nutrients and biotic integrity in rivers and streams. Biotic integrity refers to the extent to which a community has a species composition, diversity and functional organisation comparable to that expected for the natural habitat of the region (Karr and Dudley, 1981). The relationship between nutrients and biotic integrity in rivers and streams was tested using biological (fish and benthic invertebrates), physical and chemical information collected over an extended time-span from similar locations in streams throughout Ohio using standard procedures. A negative correlation between nutrient concentrations, especially total phosphorus, and biotic integrity was found and potential threshold concentrations of total inorganic nitrogen (TIN) and total phosphorus (TP) beyond which macroinvertebrate communities are likely to be degraded, were defined.

The correlative approach of Miltner and Rankin (1998) was used to determine any relationship between nutrients and fish or benthic invertebrates; to find the amount of variation explained by either nitrogen or phosphorus compared to other variables such as habitat; to quantify potential threshold concentrations of nutrients; and to investigate the relationship between fish and macroinvertebrate community structure and increasing concentrations of nutrients. Macroinvertebrate community structure was measured by the Invertebrate Community Index (ICI, DeShon, 1995), which is a multimetric measure of the community composed of ten metrics based on structure and composition. The individual metrics were scored against expectations derived from least impacted reference sites. They included:

- the number of Emphemeroptera, Plecoptera and Trichoptera (EPT) taxa
- the percentage composition of mayflies
- the percentage composition of dipterans in the midge tribe Tanytarsini
- the percentage composition of other dipterans and non-insects (e.g. oligochaetes, snails, leeches)
- abundance of scrapers and abundance of all organisms as number per square metre on the artificial coloniser block.

In wadeable streams, the number of EPT taxa and the relative abundance of Tanytarsini midges decreased relative to increasing nutrient concentration, while other dipterans and non-insects were positively associated with increasing nutrient concentration. The relative abundance of mayflies was highest at TIN and TP concentrations falling between the 50th and 75th percentiles in wadeable streams. Abundance of scrapers was higher in headwater streams having either TP or TIN concentrations exceeding the 25th percentile while in wadeable streams, scraper abundance was significantly higher in lower nutrient concentration streams than at streams with high nutrient concentrations.

Biotic integrity in low- to mid-order streams is negatively correlated with nutrients. Though nutrients may serve as surrogates for other variables or act indirectly in structuring biotic communities, some evidence for cause and effect was inferred because the abundance of benthic invertebrates was generally highest at intermediate nutrient concentrations, and the abundance of grazers in headwater streams was highest at enriched sites which might be predicted from the river continuum concept (Vannote *et al.* 1980).

United Kingdom case-study

In the United Kingdom, a small-scale and simple study of the relationship between benthic invertebrates and nutrient enrichment has recently been published (Hooda *et al.*, 2000). A water quality study was carried out in three streams in a predominantly dairy farming region, with the aim of assessing the effects of diffuse- and point-sourced inputs on a number of water quality parameters and benthic invertebrates.

Significant increases in streamwater BOD, NH_4-N and SRP concentrations were recorded between upstream and downstream reaches of farms. These changes in water quality appeared to be largely due to point-source inputs from farmyards, and had a significant effect on the ability of the water to support a healthy and diverse community of invertebrates. Drainage from grassland fields and cattle entering the water to drink had little obvious effect, although species diversity may have been reduced, allowing a reduced number of sensitive species such as mayflies to coexist with oligochaetes and leeches. The two most important parameters were concentrations of dissolved oxygen (DO) and ammonium, which were inversely related to the invertebrate scores.

European case-study

In Sweden, a broad-scale and sophisticated study has been undertaken by Sandin and Johnson (2000) investigating the statistical power of selected indicator metrics using benthic invertebrates for assessing acidification and eutrophication of running waters. The aim of this study was to examine how well the different indices performed in detecting anthropogenic impact, and to determine the number of independent samples (either temporal or spatial) needed to detect change with a known degree of certainty (i.e. statistical power).

Ten benthic macroinvertebrate indicator metrics commonly used in the assessment of running waters (taxa richness, total density, number of EPT taxa, Shannon's index, Simpson's index, ASPT, DFI, and three acidity indices) were evaluated for effect size, standardised effect size, statistical power and the number of samples needed to detect an impact of either eutrophication or acidification, The eutrophication and acidity indices were also evaluated for type I and type II errors. Indices were evaluated using the coefficient of variation of data taken from a spatial dataset of some 700 sites distributed across Sweden, and a temporal dataset of five streams sampled over a time period of 6-11 years. Richness measures (taxa richness and number of EPT taxa) and the two eutrophication indices (ASPT and DFI) had the highest standardised effect sizes as well as a high statistical power and therefore needed fewer samples to detect changes caused by perturbation. Conversely, total density of individuals was the least informative metric with the lowest standardised effect size and the highest spatial, temporal and sample variability.

Data were obtained from the National Stream Survey (Wilander *et al.* 1998) in which 694 stratified and randomly selected sites were sampled for benthic macroinvertebrates using a handnet in riffles according to Swedish standard (SIS 1994). Sites moderately affected by eutrophication ($15 < \text{TP}\ \mu\text{g l}^{-1} < 50$, n = 246) were removed. The remaining streams were divided into those not significantly affected by anthropogenic stress (referred to as spatial reference sites $\leq 15\ \mu\text{g TP l}^{-1}$, n = 246) and sites affected by eutrophication ($\geq 50\ \mu\text{g TP l}^{-1}$, n = 78).

Among the ten biological indices evaluated, two indices for eutrophication were assessed: Average Score Per Taxon (ASPT) (Armitage *et al.* 1983) and the Danish Fauna Index (DFI) (Kirkegaards *et al.* 1992). The DFI is similar to the Trent Biotic Index (Woodiwiss1964).

The statistical power to detect a difference between the nutrient enriched and non-impacted sites was >0.99 for taxa richness, total density, and number of EPT taxa. ASPT and DFI also had relatively high power ≥ 0.95. The diversity indices had the lowest statistical power to detect eutrophication. The number of samples needed to detect the effect of eutrophication were in almost all cases higher using linear regression than a t-test. For a t-test, the number of samples needed was lowest for taxa richness, total density and number of EPT taxa.

2.5.3 Approaches to establishing relationships between benthic invertebrates and nutrient concentrations

Background

The history of the way in which benthic invertebrates have been used for assessing stream and river water quality has been covered by several authors including The Monitoring and Assessment Research Centre (1985), Metcalfe (1989), Reynoldson and Metcalfe-Smith (1992), Brinkhurst (1993), and Marshall (1993).

The concept of biological indicators of environmental conditions in running water, particularly with regard to organic/sewage pollution, is discussed by Wilhm (1975). The use of pollution indicator species in streams and rivers originated with the Saprobien system (Kolkwitz and Marsson, 1908, 1909). While the Saprobien system has been generally accepted, with certain caveats, for use in European countries, the indicator organism concept was initially widely rejected in North America (Roback, 1974) in favour of physicochemical methods of pollution assessment.

Empirical approaches

Methods of biomonitoring, bioassessing, or surveying involve qualitative and quantitative techniques, indicator species, indicator groups, score systems, biotic indices, diversity indices, metrics, multimetrics, and multivariate techniques.

Unfortunately, in many instances, these approaches to establishing the relationship between benthic invertebrates and nutrient conditions utilise methods that determine unspecified impairment (stress, impact, pollution, enrichment, degradation) from anthropogenic influences, or determine impacts of organic pollution in general. The methods have not been designed to detect the specific effects of nutrient enrichment as distinguished from the effects of other concomitant variables. Complicating factors include natural variations resulting from physical geography and geology, seasonality and water flow, all of which introduce spatial and temporal variability that must be distinguished from culturally induced variations.

The need to understand natural or near natural conditions and variability for the accurate evaluation of individual site conditions resulted in the reference condition concept (see Section 6.1). The use of reference conditions depends on the classification of water bodies and can be incorporated into prediction systems. This kind of spatial-state reference scheme is described in greater detail in section 6.3. To date, these systems have not been designed specifically to detect or predict eutrophication effects in benthic invertebrate communities but could potentially be modified in order to do so by the incorporation of appropriate metrics.

Although concentrations of phosphorus and nitrogen were not routinely incorporated in the variables used in the development of the RIVPACS program (Wright *et al.* 1981), such data may still be available in Environment Agency databases for the RIVPACS sites for the years in which the samples were collected. In the Environment Agency Management Strategy on Eutrophication (Environment Agency 2000) a stipulated objective is the use of historical data to improve understanding of cause-effect relationships.

Other approaches to the establishment of relationships between benthic invertebrates and nutrient conditions are: direct experimentation; a consideration of functional feeding groups in the affected benthic community; and the use of artificial intelligence.

Experimental approaches
Experiments to directly determine the relationship between benthic invertebrates and increasing concentrations of nutrients are not numerous. Brown (1995) is studying the impacts of nutrients on periphyton growth and benthic invertebrates in the Thredbo River. This is an ongoing experimental project, which is using nutrient diffusing substrata to establish which nutrients, or combinations, are limiting in the Thredbo River, Australia. Other studies include Cooper *et al.* (1996-7), Culp and Podemski (1996) and Kiffney and Richardson (in press). The 'Cumulative Impacts on Aquatic Diversity Project' at Environment Canada's National Water Research Institute (NWRI) is using artificial streams to pinpoint effects of aquatic stress and to develop ecological effects indicators for river ecosystems.

The functional feeding group approach
The experimental work exploring the relationships between benthic invertebrates and nutrient enrichment in rivers incorporates a consideration of the role of certain functional feeding groups (FFG) as potential indicators of eutrophication. Kelly and Whitton (1998b) state that a likely effect of changes in the amounts or composition of phototrophs due to eutrophication may be a change in the relative proportions of invertebrate functional groups in a river. The number of epiphyte grazers may increase, numbers of filter feeders may respond to increased phytoplankton, and increased amounts of decaying macrophytes would increase the abundance of shredders. Trends in functional feeding groups were difficult to detect with their existing data but questions were posed as to the probability of detecting grazer family increases in enriched rivers and whether any broad patterns of invertebrate functional groups would be apparent between major RIVPACS classes, in a way that could be related to nutrient concentrations.

Subsequent to the paper by Kelly and Whitton (1998), Clarke *et al.* (2001) have produced an unpublished appraisal of RIVPACS for evaluating trophic structure. Their approach may offer an alternative viewpoint from which to interpret the particular form of stress or stresses to which a site is exposed. Clarke *et al.* (2001) showed that, once averaged across a very wide range of types of site, the percentage abundance of the various functional feeding groups varies only weakly with the environmental characteristics of sites, and only changes dramatically for very poor quality sites. In theory, the proportion of grazers (scrapers) that feed on periphyton should increase in eutrophicated sites but, in this work, for very poor quality sites (source of problem unspecified) the O/E ratio for the % of gatherers is considerably greater than 1.0 while for all other FFGs it is less than 1.0. There are other critics of the concept of FFG and pollution. Quinn and Hickey (1993) found no general relationships between relative densities of FFGs and water quality variables. They took the view that early warning of enrichment is more easily seen at species level than at the functional level.

In view of the limited practical application of FFGs based on RIVPACS samples, it is recommended that no attempt is made to incorporate procedures for calculating the expected relative abundances of each FFG for any given site with stated environmental features into the next version of RIVPACS. However, the possibility that this approach might have some practical application at a future date should be kept under review.

Resh *et al.* (2000) examined the selection of benthic macroinvertebrate metrics for monitoring water quality in the Fraser River, British Columbia and its implications for both multimetric approaches and multivariate models. The rapid bioassessment protocols in the United States use a multimetric approach in which scores for individual metrics are evaluated against thresholds developed from reference sites. Comparisons were made between this approach and a combination of multimetric and multivariate approaches, like those used in RIVPACS, AUSRIVAS and the BEAST. In 40–60% of metric comparisons made between unimpaired sites located in different ecoregions, with unimpaired sites in different streams in the same ecoregions, and with unimpaired sites in the same stream, incorrect indications of impairment were shown. Using functional feeding measures, 60% gave incorrect indications of impairment in sites from different ecoregions, 90% in sites from different rivers, and 60% in sites from the same rivers. The general term ‘impairment’ was used, however, with no specific mention of eutrophication.

Modelling approaches
Walley and Fontama (2000) commend artificial intelligence (AI) for biological classifications of river quality. Two techniques, Bayesian reasoning and neural networks, are shown to be well suited to the modelling of river quality classes. See Section 6.3.3.

Integrated approaches
Two significant papers concerning assessment were published last year, both emanating from North America. These papers by Barbour *et al.* (2000) and Hughes *et al.* (2000) represent the culmination of all that has been learnt in the previous century about assessment methods to attain biological and ecological integrity in the United States. However, what they describe sounds familiar when read in the knowledge of the Water Framework Directive proposals, and the paper by Hughes *et al.* (2000) actually states that the proposed ‘approach to sampling design and indicators has distinct advantages for monitoring and assessments that may be applicable to European Communities seeking to assess the ecological integrity of waters’. Both papers refer to benthic invertebrates, excess nutrients and running waters.

According to Barbour *et al.* (2000), the concept of ecological integrity has become a worldwide phenomenon that has become entrenched into the regulatory structure of environmental law in the United States. The attainment of ecological integrity requires the attainment of its three elements: physical, chemical and biological integrity. Because biological communities integrate the effects of different stressors such as reduced oxygen, excess nutrients, toxic chemicals, increased temperature, excessive sediment loading, and habitat degradation, the advent of bioassessment in regulatory programs has provided a more comprehensive and effective monitoring and assessment strategy.

The US Environmental Protection Agency (USEPA) has produced technical guidance for developing effective bioassessment programs; they include crucial elements such as defining objectives, classifying water bodies according to expected biological attributes, deriving the reference condition of the site classes, developing standardised protocols for sampling and data analysis, and implementing a quality assurance plan. Approaches to bioassessment in the USA follow a basic design of incorporating various attributes of the elements and processes of the aquatic community into a multimetric index or a series of multivariate analyses.

The Environmental Monitoring and Assessment Program – Surface Waters (EMAP-SW), is a survey of ecological integrity in the United States (Hughes *et al.* 2000). Data analysed on a broader base at national, multistate or state scales often reveal more serious deterioration of various biological assemblages than that suspected from site specific studies simply because the impacts are observed regionally rather than locally. Unfortunately many regional assessments are based on data collected with different sampling designs and methods, making their representativeness, accuracy and precision questionable.

In 1989, the USEPA began EMAP-SW, a program for developing methods to monitor and assess status and trends in the nation's lakes and rivers in a statistically and ecologically rigorous manner. EMAP's conceptual design focuses on biological integrity through the use of multiple biological assemblages and includes physical and chemical habitat and landscape characteristics. When coupled with appropriate biological indicators, a probability-based design enables the provision of accurate, precise and unbiased assessments of biological conditions, along with quantitative estimates of sampling uncertainty.

Regional EMAP-SW surveys indicate the importance of assessing multiple biological assemblages because each assemblage is differentially sensitive to different stressors and at different spatial scales. Synthesising multiple metrics from multiple assemblages allows the detection of effects of multiple anthropogenic disturbances. The value of using historical reconstruction and palaeolimnological data for determining reference conditions where disturbance is extensive is also illustrated. The conclusion is that an EMAP approach to sampling design and indicators (recognising natural ecoregional differences) has distinct advantages for monitoring and assessments that are considered potentially to be applicable to EC Member States seeking to assess the ecological integrity of waters.

2.5.4 Monitoring and classification Schemes

Within the UK, the 1990, 1995 and 2000 GQA (General Quality Assessment) surveys conducted by the EA, SEPA, and the Industrial Research and Technology Unit in Northern Ireland (IRTU) provide the most comprehensive source of biological and environmental data currently available in the UK. The EA data covers approximately 6000 sites. The Centre for Ecology and Hydrology has biological data for 2000 reference sites.

The biological data is benthic invertebrate data to BMWP family level both in terms of presence/absence and log categories of abundance. The environmental data consists of a limited set of GQA determinands, comprised of biochemical oxygen demand (BOD), $O_{2,}$ and ammoniacal nitrogen. A wider range of other chemical determinands, including nutrients such as nitrate, nitrite and orthophosphate, are also available for many of the sites. There are, however, problems with matching the biological and chemical data sites, as demonstrated by the difficulties encountered in the Dynamic RIVPACS project (RIVPACS DYNAMO), which is the development of RIVPACS procedures for predicting the impact of environmental stresses.

Studying the early onset of eutrophication may require identification of benthic invertebrates to species level. Currently, however, there are no extensive datasets at species level, which means that more work needs to be done.

In other temperate regions of Europe, North America, and Australia there is a substantial body of information relating benthic macroinvertebrates to nutrient conditions. However, most of this research has been of a general nature in that the relationship is recognised, and also utilised, without the details of the precise effects of specific concentrations or loadings of nutrients on the benthic fauna being understood.

Present UK approaches

Currently in the UK, benthic invertebrates in running waters are used for water quality and ecological quality assessment through the use of standardised three-minute pond-net multi-habitat samples. These are generally identified to BMWP family level (although increasingly to species level) and the data assessed using the River InVertebrate Prediction And Classification System (RIVPACS). Details of RIVPACS and similar spatial-state reference schemes are presented below. GQA audits carried out by the EA in 1990, 1995 and 2000 depend on the RIVPACS system.

The Environmental Change Network (Sykes *et al.* 1999), was formed with the concept of integrated monitoring as a key principle (http://www.ecn.ac.uk/protocols). The Environmental Change Network has collected data for a number of river and lake sites across the UK since 1993. Data relating to eutrophication have been analysed and are presented on a series of Web pages: (http://www.ecn.ac.uk/freshwater/pressures_eutrophication.htm). Pages present a summary of: chemical and biological parameters for the freshwater sites; time series data for nitrate; time series data for phosphate; time series data for freshwater invertebrate (FIN) diversity and relative abundances of FIN, and indicator taxa of eutrophication. It must, however, be stressed that the taxa offer only a crude indication of nutrient conditions.

2.5.5 Recommendations for Phase II

Insufficient analysis has been carried out to quantify the relationships between benthic invertebrates and nutrient conditions in streams and rivers although it is now an active field of interest. The nearest to this approach, at the moment, is the Dynamic RIVPACS project which hopes to determine primary indicators of organic enrichment (but not elevated nutrient status) and develop quantitative predictions of the consequences of specific changes at a site based on its benthic invertebrate assemblage. There are other systems, however, that should also be considered, such as the AI approach described earlier being developed at Staffordshire University (Walley and Fontama, 2000).

There is a real need to clearly define what is meant by the terms of organic enrichment, nutrient enrichment and eutrophication. These terms are often used as if they were synonymous.

There is a potential need for macroinvertebrate identifications at species level. There is also a clear requirement for biological samples to be accompanied by environmental and chemical variables, including nitrogen and phosphorus concentrations, for each site and sampling occasion. Retrospective matching of biological and chemical data could be difficult or impossible to obtain. However, in future, the simultaneous sampling and recording of these variables could be considered as a standard procedure that would enable a better understanding of the relationships between macroinvertebrates and nutrient conditions.

Currently there are no practical protocols and no metrics designed specifically to detect eutrophication by using benthic invertebrates despite the fact that at least one piece of research refers to ASPT and DFI scores as being suitable indicators (Sandin *et al.* 2000). Work concerning eutrophication effects on benthic invertebrates in flowing waters has often disregarded intermediate nutrient concentrations and only considered clean and severely enriched sites, in order to clarify the response of the metrics to the eutrophicated state (Sandin *et al.* 2000). Other assessment methods, such as the application of the BEAST in Fraser River catchments (Rosenberg *et al.* 2000), have not been able to distinguish intermediate levels of nutrient enrichment: only severely impacted sites were distinguishable by these metrics.

In order to apply the principles of the Water Framework Directive consistently throughout Europe, the clear need to harmonise and standardise existing methods of assessing the ecological status of rivers is recognised (the proposed EU 5th Framework Project, STAR (Standardisation of river classifications: Framework method for calibrating different biological survey results against ecological quality classifications to be developed for the Water Framework Directive. Proposal number: EVKI-2001-00034).

2.6 Fish

In relation to fish, the definitions of High, Good and Moderate ecological status in the WFD are:

High status	**Good status**	**Moderate status**
Species composition and abundance correspond totally or nearly to undisturbed conditions. All the type-specific disturbance-sensitive species are present	There are slight changes in species composition and abundance from the type-specific communities attributable to anthropogenic impacts on physico-chemical and hydromorphological quality elements	The composition and abundance of fish species differ moderately from the type-specific communities attributable to anthropogenic impacts on physico-chemical or hydro-morphological quality
The age structure of the fish communities show little sign of anthropogenic disturbance and are not indicative of a failure in the reproduction or development of any particular species	The age structures of the fish communities show signs of disturbance attributable to anthropogenic impacts on physico-chemical or hydromorphological quality elements, and, in a few instances, are indicative of a failure in the reproduction or development of a particular species, to the extent that some age classes may be missing.	The age structure of the fish communities shows major signs of anthropogenic disturbance, to the extent that a moderate proportion of the type specific species are absent or of very low abundance.

In developing techniques for assessing reference conditions for fish in running waters for the Great Britain ecoregion, it is impossible to ignore the effects of history and geomorphology. Probably the most important event in the evolution of Britain's environment over the last 30,000 years was the development of the ice sheet which completely buried most of the mainland and surrounding island groups. This, last, ice age, which came to a close in the British Isles some 10,000 years ago, can certainly be taken as the starting point for any consideration of the present freshwater fish fauna of Great Britain.

After the ice age there was a relatively easy colonisation of Britain’s fresh waters from the sea by those fish species with marine affinities, followed by a slow natural dispersal and an increasingly faster rate of transfers by humans of other, purely freshwater, species from the south. Additionally, alien species from abroad have been introduced. All of this has resulted in somewhat of a north/south divide in the distribution of species and the composition of fish communities in Britain. Whilst the situation is more complex than a single dividing line on the map, it is certainly true to say that the fish communities in the north and on islands have many fewer species than comparable systems in the south.

Thus, for example, typical northern running waters in the north of Scotland like the Rivers Naver, Thurso and Helmsdale have only about six or seven species of freshwater fish, whereas comparable waters in southern Scotland, for example the Rivers Nith, Annan and Tweed, have sixteen to nineteen species. Further south still, in England, the Rivers Severn, Avon and Thames may have twenty five to thirty species. In terms of species composition, many of the northern communities have probably remained the same for thousands of years and are relatively stable, whereas those in the south have changed substantially over the last two centuries due to human impacts and continue to do so with introductions by anglers and others. Each new introduction can produce instability in its new community, as studies of the River Endrick have shown in recent years (Maitland 1966, Doughty and Maitland 1994).

The essence of this, in the present context, is that in the south the high range of species available allows changes in fish communities from, say salmond to cyprinid, as nutrients increase (see below). In the north, however, where there are virtually no cyprinids, such a succession is impossible and so sensitive species may be gradually eliminated, with no replacement by others.

A second major consideration for this review is the nature of the habitat concerned and the ability of different species to survive there. Thus, in depositing lowland rivers as eutrophication increases, the succession from salmonids to percids to cyprinids is perfectly possible. However, in faster flowing eroding upland streams, few percids or cyprinids can maintain themselves and so again sensitive species are gradually eliminated, with no replacement by others. A generalised scheme for both rivers and lakes may be summarised in the following simple diagrammatic classification of freshwater habitats (Maitland and Morgan 1997). The diagram represents a continuum - which is actually the commonest situation, for most water bodies interconnected with others. In some large catchments, all these different water types may be found.

GEOLOGY (rock and soil)	SIZE (area/depth)	ALTITUDE (and latitude)	FISH (community)	DIVERSITY (species)
POOREST	SMALLEST	HIGHEST	PERHAPS	NONE
dystrophic	shallow	cooler	stenothermic	very low
and	pools	waters	species (e.g.	
oligotrophic	and	often	Salmonidae)	low
systems	trickles	frozen	may dominate	
mesotrophic				medium
systems				
eutrophic	deeper	warmer	eurythermic	high
and	lakes	waters	species (e.g.	
hypertrophic	and	rarely	Cyprinidae)	medium
systems	rivers	frozen	may dominate	
RICHEST	LARGEST	LOWEST	SOME	BRACKISH

2.6.1 Composition and abundance

In general, the composition of fish communities in Great Britain is, as indicated above, related more to historical events and a complex range of environmental factors than concentrations of nutrients per se. Nevertheless, nutrient concentrations do play a part in determining community composition, usually indirectly through food chains or changing of water quality (e.g. lowering of oxygen content because of excessive algal or macrophyte growths). Oxygen depletion as dead algae and macrophytes decompose has resulted in substantial fish kills in some lowland rivers (Harper 1992) and the fish communities of these waters are now limited to a few species characteristic of slow-flowing eutrophic waters, such as Bream *Abramis brama* and Roach *Rutilus rutilus* (Lucas and Marmulla 2000). Though emphasis was given to the study of lakes during the intensive research on eutrophication during the 1970s and 1980s, there is some useful information available from studies on rivers.

Ryder *et al.* (1981) have pointed out that eutrophication and other changes to the aquatic environment may have dire effects on fish genetic stock composition. Depending on the spatial distribution of any stresses imposed and their temporal duration, ‘fish communities may be altered almost imperceptibly or with unrelenting selection against the survival of certain species or stocks’. This selection operates most intensively at the vulnerable links in the metabolic and behavioural chain (Loftus and Regier 1972) - i.e. the early life stages. Thus fish species which form aggregations in shallow streams may be vulnerable to cultural stresses emanating from the land. Spawn deposited in the same areas may be affected similarly and all the early larval stages are vulnerable because of changes in the structure of the food web arising from excess nutrient input.

Case studies: composition

Matthews *et al.* (1992) used a 10-year compilation of fish data and 14 water quality variables from 70 streams in Arkansas to carry out a principal component analysis and detrended correspondence analysis. This showed the spatial distribution of stream fishes to be significantly related to an aggregate of water quality conditions.

Twenty sites in the San Joaquim River in California were sampled by Brown (2000) to characterise fish communities and their associations with water and habitat quality. Of 31 fish species captured, only 10 were native to the catchment. The conclusion was that fish are responsive to environmental conditions (which included here specific conductance) and that changes in water management and water quality could result in changes in species distributions.

Case studies: abundance

A study of 30 Michigan streams sampled across a strong nutrient gradient showed that nutrient enrichment resulted in increased populations of insects and fish (Riseng *et al.* 2000), though the former showed a muted response to nutrients because of strong top-down regulation by fish.

The average fish community production at 55 river sites was found by Randall *et al.* (1995) to be three times greater than at 22 lakes (273 to 82 kg/ha/year) due to higher densities of fish (14x) and greater biomass (2x). Average fish weights and P/Biomass ratios were inversely correlated. Fish production was positively correlated with phosphorus in both rivers and lakes. The authors suggest that the productive capacity of freshwater habitats can be predicted using a multiple regression model where fish production is calculated from average fish biomass and body mass.

There are numerous studies showing that the addition of some nutrients can increase growth and production in riverine fish populations (Portt *et al.* 1986, Muller 1992). Even sensitive species such as Atlantic Salmon *Salmo salar* and Brown Trout *Salmo trutta* can respond positively to small additions of nutrients (Maitland 1962).

Influence of factors other than nutrients

Many factors other than nutrient input affect the composition and abundance of freshwater fish in rivers and these include pollution, acidification, physical barriers, habitat destruction, land use, alien introductions, inappropriate fishery management, etc. In Great Britain alone, many hundreds of fish populations have been eliminated from the impact of these anthropogenic factors and several species have shown severe declines during the 20th century. Often, individually, these may be much more important than nutrients in determining the nature of a fish community or its production, and frequently one or more of these may act together to produce change. A summary of the most important anthropogenic factors and their effect on fish populations in both rivers and lakes world-wide is as follows:

FACTOR	EFFECT
Industrial and domestic effluents	Pollution, poisoning, blocking of migration routes
Acid deposition	Acidification, release of toxic metals
Land use (farming and forestry)	Eutrophication, acidification, sedimentation
Industrial development (including roads)	Sedimentation, obstructions
Warm water discharge	Deoxygenation, temperature gradients
River obstructions (weirs and dams)	Blocking of migration routes, sedimentation
Infilling, drainage and canalisation	Loss of habitat, shelter and food supply
Water abstraction	Loss of habitat and spawning grounds
Fluctuating water levels (reservoirs)	Loss of habitat, spawning and food supply
Fish farming	Eutrophication, introductions, diseases
Angling and fishery management	Elimination by piscicides, diseases, introductions
Commercial fishing	Overfishing, genetic changes
Introduction of alien species	Elimination of native species, diseases, parasites
Global warming	Loss of southern or low altitude populations
Water transfer, canals	Transfer of species and disease
Canalisation and dyking	Habitat loss, flooding
Use of pesticides	Loss of biodiversity
Water recreation	Disturbance, habitat loss

There are many thousands of studies available concerning the effects of various anthropogenic impacts on freshwater fish.

Case studies: factors other than nutrients

The effect of improved water quality on fish assemblages in streams in southern Sweden was assessed by Eklov *et al.* (1998) by comparing species composition at 161 sites and water quality at 29 sites in the 1960s and the 1990s. Water quality had improved and there was an increase in the number of sites with Brown Trout, Stone Loach and Eel. The response was best explained by improved oxygen concentrations. In contrast, the numbers of Nine-spined Stickleback *Pungitius pungitius*, Orfe *Leuciscus idus* and Brook Lamprey *Lampetra planeri* decreased, possibly due to competition with Brown Trout. It was concluded that improving water quality was an effective method for rehabilitating fish populations in streams where natural colonisation was possible.

Spatial variation of quality of fish communities in the basin of the River Seine and nearby coastal streams in France were examined by Belliard *et al.* (1999) using an Index of Biological Integrity (IBI). A trend of fish community degradation was found from the edge to the centre of the Seine basin and from upstream to downstream. IBI scores in headwaters indicated these scores were significantly related to water quality and substrate clogging, suggesting that fish community quality is linked to catchment land use. Downstream, IBI scores were mainly linked with habitat diversity, suggesting that fish community quality depends on river bed alterations.

The impact of removing riparian vegetation, channel straightening and fluctuations in flow regime were found by Schlosser *et al.* (1982a, 1982b) to be major factors responsible for recent changes in large river fish communities in the midwest of the United States, from insectivore and insectivore-piscivore species to omnivore and herbivore-detritivore species.

Penczak *et al.* (1984) found that upstream of a new reservoir the species composition changed slightly but biomass increased by 3x - most of it caused by immigration from the reservoir. Downstream of the reservoir, new species appeared and four previously dominant species disappeared - probably because of cold hypolimnetic water from the reservoir. Production here however, increased by 3.2x.

Virbickas (1998) found that fish community production in rivers has a reliable correlation with both primary production and the concentration of ‘biogenic elements (phosphorus and nitrogen) directly influencing primary (autochthonous) production’. He found also that fish community production has a reliable positive correlation with the input of allochthonous organic material from elsewhere. In an extensive study of fish in river systems in Lithuania, he showed that, in natural systems the three most important factors affecting the qualitative and quantitative nature of fish communities were, in decreasing order of significance: river size, thermal regime and biotope variety. He and co-workers (Kesminas and Virbickas 1995a, 1995b) also showed that anthropogenic factors, notably river regulation, eutrophication and pollution, can cause change in fish communities and their production. For example, as eutrophication increases, fish species variety diminishes and rheophylic and benthic species are often eliminated. The following riverine factors were found by Virbickas (1998) to be of significance in relation to fish community production.

FACTOR	REGRESSION COEFFICIENT	SIGNIFICANCE
River size	$r = 0.73$	$p < 0.05$
Thermal regime	$r = 0.05$	$p < 0.01$
Flow velocity	$r = 0.53$	$p < 0.05$
BOD_5	$r = 0.84$	$p < 0.0006$
Phosphorus	$r = 0.72$	$p < 0.008$
Phytoplankton production	$r = 0.89$	$p < 0.0005$
Zooplankton production	$r = 0.43$	$p < 0.029$

Classification and monitoring schemes

One of the problems of classifying rivers in relation to their native fish communities is the natural variation in the zoogeography of the individual fish species themselves. As indicated above, even in as small an area as the United Kingdom there are substantial differences between riverine fish communities found in the south of England and those in the north of Scotland (Maitland and Campbell 1992). General attempts to classify running waters have been of two kinds: firstly, the division of the system into definable zones (from source to mouth), and secondly, the separation of river systems into definable types. Each type of classification presents its own problems.

Thienemann (1925)	**Carpenter** (1928)	**Huet** (1959)	**CORINE** (1991)
Springs	Head stream	-	rivulets
Trout zone	Trout beck	Trout zone	Trout zone
Grayling zone	Minnow reach	Grayling zone	Grayling zone
Barbel zone	Upper lowland reach	Barbel zone	Barbel zone
Bream zone	Lower lowland reach	Bream zone	Bream zone
Brackish zone	-	-	-

Various schemes of zonation for running waters have been devised (Hawkes 1975); most relate to just one or a few factors - for example, a single group of plants or animals. Harrison and Elsworth (1958) based their initial scheme of zonation on physical features such as substrate, while Tansley (1939) had previously classified rivers into zones on the basis of their vegetation. Schmitz (1955) recognised various invertebrate zones in running waters, while one of the common methods of differentiating zones in rivers is the distribution of common fish species. Such schemes, originally described for European rivers by Thienemann (1912), were unsuccessfully adapted for rivers in the British Isles by Carpenter (1928). A similar, but more elaborate, scheme for Europe was developed by Huet (1946, 1959) and this has been adopted as part of the CORINE Biotope classification (Devillers *et al.* 1991), a standardised classification of both freshwater and terrestrial ecosystems. The main categories of rivers are: rivulets, trout zone, grayling zone, barbel zone and bream zone. For the zoogeographic reasons outlined above, this scheme does not work in northern Britain, where grayling, barbel and bream are absent.

In general, any rigid scheme of zonation for organisms in running waters, however clear-cut it may be, is rarely valid for other groups there, or even the same group in another water. The general theme of change in biotic associations from source to mouth in a running water is one of transition (Maitland 1966) rather than zonation and this theme has been further developed by Vannote *et al.* (1980) as the river continuum concept. The main value of characterising zones is that, in a general sense, they may be useful for descriptive purposes.

There have also been various attempts to classify whole running water systems; (Ricker 1934) classified on physical and chemical characteristics, and Carpenter (1927) on the type of origin of each system. Lagler (1949) suggested a very arbitrary system based on the average density and weight of invertebrates in a standard area. One of the most valuable discussions on the classification of running waters is that of Berg (1948), who points out that running waters cannot be classified like standing waters because they are not uniform entities, but are systems which change from source to mouth. It is not possible, therefore, to undertake ecological groupings of running waters but merely of certain reaches within them, and the unit in such a system will not be an entire running water channel, but a stretch of one within which the environment is uniform, i.e. a habitat. The problem of classifying running waters is thus one of describing these habitats and their organisms. In relation to such studies, the concept of stream order hierarchy is a useful one. The Environment Agency's fish classification scheme for rivers, HABSCORE, is of some relevance here, although does not make any direct reference to nutrient conditions (North R, personal communication)

Of major relevance in a European context is the EEC Directive 659 (1978) 'on the quality of waters needing protection or improvement in order to support fish life.' This Directive aims to protect or improve the quality of running or standing fresh waters designated by Member States as needing protection or improvement in order to support fish life belonging to 'indigenous species offering a natural diversity', or 'species the presence of which is judged desirable for water management purposes'. It does not apply to waters in natural or artificial fish ponds used for intensive fish farming. For the purposes of the Directive waters are divided into salmonid and cyprinid waters, defined as follows:

Salmonid waters are 'waters which support or become capable of supporting fish belonging to species such as salmon (*Salmo salar*), trout (*Salmo trutta*), grayling (*Thymallus thymallus*) and whitefish (*Coregonus*)'.

Cyprinid waters are 'waters which support or become capable of supporting fish belonging to the cyprinids (Cyprinidae), or other species such as pike (*Esox lucius*), perch (*Perca fluviatilis*) and eel (*Anguilla anguilla*)'.

The Directive lays down the minimum quality criteria to be met by such waters, involving physical, chemical and microbiological parameters; binding limit values and indicative values for these parameters; and minimum frequency of sampling and reference methods of analysis for such waters. The Directive is amended by Council Directives 656 (1990) and 692 (1991).

Case studies: classification and monitoring schemes

Harris (1995) reviewed the use of fish in ecological assessments and concluded that the Index of Biological Integrity can be used as a predictive model of aquatic environmental quality. The IBI is claimed to be 'a quantitative biological tool with a strong ecological foundation that integrates attributes from several levels of ecosystem organisation .. its robustness, flexibility and sensitivity can cope effectively with the low diversity of the Australian fish fauna and the dominance of ecological generalists.'

Yang and Sykes (1998) analysed eutrophication control in shallow streams from the viewpoint of a trophic chain. A trophic-dynamic model was established to resolve the interactions between all trophic species. The results showed that algae are limited by nutrients in one-link and three-link systems and by predators in a two-link food chain. These conclusions are in good agreement with other theoretical predictions. As a result, biomanagement of predators to regulate excessive algae only works in two-link and four-link food chains.

In South Africa, Kleynhans (1999) is developing a Fish Assembly Integrity Index (FAII) based on fish species expected to be present in river segments with homogeneous habitat. It was concluded from initial work that the index 'provides a broad, synoptic estimation of the biological integrity of a river but further refinement of the technique is required'.

SERCON (System for Evaluating Rivers for Conservation) is a broad-based technique for river evaluation, designed to be applied with greater consistency than present methods, and to provide a simple way of communicating technical information to decision makers (Boon *et al.* 1996, 1997). SERCON evaluates data on 35 attributes, grouped within six conservation criteria - Physical Diversity, Naturalness, Representativeness, Rarity, Species Richness and Special Features. Attributes concerning fish feature in all but the first of these and the scoring systems are based on geographic information on the observed complement of fish species found in a river (or lake) compared to that expected. Tables have been produced to show which native species are expected in each zone. Scores for Naturalness are reduced when alien species are present. This way of evaluating 'departure from naturalness' is highly relevant to the concept of 'ecological status' adopted in the EU Water Framework Directive.

Schmutz *et al.* (2000) have proposed a multi-level concept for fish-based assessment (MuLFA) of the ecological integrity of running waters designed for large-scale monitoring programmes such as required for the Water Framework Directive. The principle of MuLFA is based on an assessment of the deviation from undisturbed reference conditions. Reference conditions have to be complied for every distinct river type using historical fish and abiotic data, present river-type-specific reference sites and reference models. The final assessment procedure compares the assessment reach with the reference conditions using a five tiered scheme and assigning that reach to the level of highest coincidence. The benefit of MuLFA is claimed to be its potential for consistent sensitivity to low and high dose human alterations and its adaptability to all river types.

Fish were among the key criteria used in Austria by Chovanec *et al.* (2000) to assess the ecological integrity of running waters. A classification was developed based on the assessment of individual criteria by means of a comparison between a river-type-specific reference state and the current conditions. This approach has been laid down in the Austrian Standard M 6232 'Guidelines for the ecological study and assessment of rivers', which also meets the general requirements for the classification of ecological status of running waters as described in the EU Water Framework Directive.

2.6.2 Disturbance-sensitive species

Several riverine species in Great Britain can be regarded as disturbance-sensitive species and these include River Lamprey *Lampetra fluviatilis*, Brook Lamprey *Lampetra planeri*, Sea Lamprey *Petromyzon marinus*, Allis Shad *Alosa alosa*, Twaite Shad *Alosa fallax*, Atlantic Salmon *Salmo salar*, and Smelt *Osmerus eperlanus*. All of these have undergone decline over the last century in Great Britain and elsewhere in Europe. However, very few of the studies of these declines have implicated nutrients in running waters, though it is likely, especially in lowland rivers, that in some cases excessive algal and macrophyte growths have lead to low oxygen concentrations and smothering of spawning grounds, with resultant problems for sensitive species.

Influence of factors other than nutrients

As indicated above, many factors other than nutrients have lead to the decline and extinction of sensitive fish species in rivers and there are numerous studies describing such events.

Case studies: influence of factors other than nutrients

Hybrids between Twaite *Alosa fallax* and Allis Shad *Alosa alosa* have been found in a number of rivers (e.g. River Loire) and in some estuaries (e.g. Solway Firth). In some systems (e.g. the Rivers Loire and Lima) these hybrids may form a significant proportion of the population (Quignard and Douchement 1991) and, since hybrids are probably fertile (Alexandrino *et al.* 1996), this is bound to have an impact on the genetic integrity of the stocks of both species. It has been suggested that extensive hybridisation is a result of man-made obstacles to migration, thus forcing both species to use the same spawning grounds. Boisneau *et al.* (1992) found that the highest percentages of hybrids in the River Loire were collected at shared spawning grounds which had converged after habitat disturbance upstream. Similarly, in the River Lima, major dams have denied shads access to traditional spawning sites upstream (Alexandrino *et al.* 1996).

Though mild pollution can increase nutrients in a river or lake, and so enhance the growth of some fish species, severe pollution can eliminate entire fish populations and, in rivers form a complete barrier to all migratory fish species, thus eliminating them from that river. This has happened in several rivers in Great Britain (e.g. Rivers Clyde and Thames), thus reducing their biodiversity by about 50% (Maitland 1987). In the River Clyde, Atlantic Salmon *Salmo salar* and several other diadromous species were absent from the river for about 100 years, but with increasingly successful pollution control several species are now returning to the river and restoring the original native fish population.

The population of Smelt *Osmerus eperlanus*, currently found in the River Cree and its estuary, is the sole survivor of several populations which formerly occurred along the south and west coasts of Scotland (Maitland and Lyle 1996). The main threats to the Smelt, which account for its demise in previous locations and present dangers for present stocks, are overfishing and pollution, obstruction of spawning migrations by dams and weirs, and physical destruction of spawning and nursery areas by engineering works. High concentrations of oxygen are known to be of importance during the period of spawning and larval development. However, the main cause of the extinction of the Solway stocks, with the exception of the Cree, was almost certainly overfishing (Maxwell 1897): 'There is no close time provided for these fish by law; hence in some rivers - the Annan and the Nith, for example - where they were plentiful in former times, they have been netted to the verge of extinction, and the industry, once profitable, no longer pays'.

Classification and monitoring schemes

Disturbance-sensitive species are included in various classification schemes but there are no schemes dealing specifically with such fish. Some species (e.g. Atlantic Salmon *Salmo salar*, Brown Trout *Salmo trutta* and Grayling *Thymallus thymallus*) are also involved in the regular monitoring programmes outlined above (i.e. those of the Environment Agency and the Scottish Fisheries Trusts).

2.6.3 Age structure

Though there are many studies indicating a positive relationship between increased growth of fish and increasing nutrients in rivers (e.g. Cooper 1967, Johnston *et al.* 1990, Hoyer and Canfield, 1991), there is little evidence of any significant changes to age structure. Theoretically, it is likely that age structure could be affected where (a) sudden increases in food supply initiated a rapid increase in juvenile recruitment, thus skewing the age structure towards young fish, or (b) deoxygenation, resulting from increased plant growths eliminated all or part of one or more age classes, but not others.

Influence of factors other than nutrients

Many factors other than nutrients are known to affect the age structure of fish in rivers (see summary list of anthropogenic factors affecting fish above). Notable among the impacts which are likely to affect fish population age structure are pollution, acidification, river obstructions, fluctuating water levels and fisheries. Any of these which impact on one age group more than another or which affect recruitment by stopping access to or damaging spawning grounds is likely to result in significant changes in the age structure of the fish populations concerned.

It must not be forgotten that many natural factors affect the age structure of fish in rivers, notable among which is climate. It is well established that favourable climatic conditions during spawning, egg incubation, hatching and early feeding can produced strong year classes which may dominate a population for many years.

Classification and monitoring schemes

Though there are considerable data in the literature which would provide the basis for schemes of classification of fish populations on the basis of age structure (Regier and Henderson 1973), no comprehensive scheme has yet been attempted for the freshwater fish species occurring in rivers in Great Britain.

2.6.4 Summary

The main points emerging from this review are as follows:

(a) The composition and abundance of fish populations in rivers can be affected by increased (and decreased) nutrients, usually indirectly through ecological pathways.

(b) Some riverine fish species are particularly sensitive to disturbance, though few have been affected directly by increased nutrient concentrations alone.

(c) Though growth rates are frequently increased by higher nutrient concentrations (and vice versa), these rarely affect age class structure unless change in concentrations (up or down) occurs rapidly or changes consequent upon increased nutrients affect some age classes more than others.

(d) In the majority of cases involving damage to fish populations in rivers, factors other than nutrients are more important (e.g. Pollution, barriers to migration, river engineering).

(e) Present schemes of classification are unsatisfactory in relation to the Great Britain ecoregion and there is little monitoring in relation to reference conditions, especially in large rivers.

(f) Considerable research is still required in order to provide satisfactory ecological classification and referencing systems for fish in rivers if the requirements of the Water Framework Directive are to be met.

2.6.5 Recommendations for Phase II

The following are recommendations for Phase II research, which would significantly increase the ability to define, classify and assess reference conditions for riverine fish populations in the Great Britain ecoregion.

(a) Develop a more sophisticated version of the SERCON assessments for fish, which will operate effectively for running waters across the whole of Great Britain.

(b) Initiate a comparative study of riverine fish populations, comparing stocks (i) within rivers above and below significant anthropogenic nutrient inputs, and (ii) between comparable rivers which have either high or low anthropogenic nutrient concentrations.

(c) Carry out an analysis of available data on fish communities in rivers in Great Britain, which will provide a basis for their classification in relation to physicochemical parameters.

(d) Carry out an analysis of available data on age structure in natural populations of fish species in rivers in Great Britain in order to provide a basis for their classification in relation to habitat and geography.

(c) Review the status and impact of alien species in running water systems in Great Britain, with due regard for alien species from abroad and 'alien' species outwith their natural distribution here.

2.7 Summary

2.7.1 How sensitive are different groups of organisms to increased nutrient concentrations?

No integrated analysis has been carried out on rivers to assess which biological element is most sensitive to nutrient conditions. All biological elements contain taxa that are potentially sensitive to changing nutrient status, providing nutrients are limiting productivity e.g. specific algae, macrophytes, benthic invertebrates and fish. Primary producers represent the most direct, and therefore immediate, response. Other biological groups may, however, be extremely sensitive to indirect effects, such as reduced light or oxygen conditions.

2.7.2 Which existing techniques hold the most potential for further investigation and development, or are new techniques required?

The most established UK schemes and their advantages and disadvantages are listed below (Table 2.7):

Table 2.7 Monitoring and classification schemes applicable to the assessment of biology-nutrient relationships in UK rivers

River Schemes	Advantages	Disadvantages
Phytoplankton: chlorophyll *a* (EA/EC schemes)	Highly sensitive to nutrients Simple, rapid response Integrates limiting factors	Highly dynamic Not consistently present
Phytobenthos: TDI	Highly sensitive to nutrients Rapid response Present in all watercourses	Relatively specialist training required
Macrophytes: MTR	Practical relevance Data rich Historical records for defining reference conditions	Response difficult to interpret in terms of nutrients alone. Species poor Not consistently present
Benthic Invertebrates: RIVPACS	Data rich Well developed EA technique Present in all watercourses	Response difficult to interpret in terms of nutrients alone.
Fish: SERCON	Practical relevance Data rich Historical records for defining reference conditions	Response difficult to interpret in terms of nutrients alone. Species poor

A comparison of TDI and MTR methods did show that the TDI scheme was shown to be particularly useful in situations where the MTR was not applicable (no macrophytes present) or inconclusive, and could identify sites influenced more by inorganic nutrient pollution (eutrophication) from those where organic pollution had the most significant effect (Harding and Kelly 1997).

Ideally the optimal classification scheme would incorporate the most sensitive indicators from all biological elements. This would have the advantages of indicating a response over a range of temporal and spatial scales. For example chlorophyll *a* may provide the most direct and rapid response to nutrient enrichment in lowland rivers, compared with phytobenthos and macrophytes, but it is also more dynamic and may indicate change when nutrient concentrations are not changing. Conversely, phytobenthos and macrophytes have less seasonal variability. All three could be used in combination and if changes associated with nutrient enrichment occur in all three then more confidence could be attributed to a ‘eutrophication-response’ interpretation. A multi-phyletic scheme would also better represent an ‘ecosystem’ response, Community indices, such as the TDI or MTR, could be used in combination with specific species quality indicators e.g. presence/absence of blanket weed *Cladophora* spp. (bad) or Atlantic Salmon *Salmo salar* (good).

2.7.3 Is ecological status influenced more by factors other than nutrients?

In rivers, flow and substrate are critical factors and almost certainly of primary importance in determining macrophyte and benthic invertebrate assemblages. Their influence can be partly taken into account in the ecotyping process, although short-term flow events can have a significant influence on community composition. Ecological thresholds in rivers in response to nutrient conditions will be influenced by flow in particular. Major ecological changes can occur at very low nutrient concentrations in low-flow situations. Nutrient mass transport (flow x concentration) has been shown to be a better predictor of community performance in rivers than concentrations alone.

2.7.4 To what extent can current EA monitoring and assessment practices fulfil the requirements of the WFD with respect to nutrients (e.g. differentiating between ecological status classes)?

Approx. 8000 river sites are routinely monitored throughout England and Wales as part of GQA surveys. Benthic invertebrate and chemistry (not necessarily nutrient) data are available, although there are issues related to matching the biological and chemical data sites. Additionally, CEH holds invertebrate data for approx. 2000 potential 'reference' sites, although there is uncertainty over whether undisturbed reference sites exist in the UK for large lowland rivers

Up to 1000 sites have been sampled in TDI and MTR development and testing, including 230 paired sites used in comparitive analysis. Sites sampled were biased towards nutrient-rich, alkaline lowland rivers and there is an urgent need to extend sampling upstream from potential sensitive areas.

In terms of monitoring nutrient conditions, measures are most comparable between sites and year-to-year in the form of annual means, even though seasonal measures (winter loading, growth season mean) may be more ecologically relevant. The 3-monthly monitoring frequency outlined in the WFD is generally inadequate for accurately representing annual means. Monthly monitoring is undoubtedly preferable and in fact fortnightly monitoring during spring is recommended if measurements of peak phytoplankton biomass is required for rivers. The WFD will require confidence limits to be assessed for the 3-monthly monitoring frequency. The use of existing long-term datasets to assess cofidence of varying monitoring frequency is recommended.

2.8 Recommendations for Phase II

If possible carry out an integrated analysis of riverine classification schemes (TDI, MTR, RIVPACS, SERCON) to identify the most effective scheme in terms of nutrient conditions that integrates an ecosystem response over a range of temporal scales.

Practically, however, the EA may want to focus R&D on those organisms that are already being used as, or are widely recognised as, sensitive indicators of nutrient status, such as chlorophyll *a*, the TDI and the MTR. All require modification or specific calibration for WFD purposes and the possibility exists to combine them in a form of multi-metric index to nutrient pollution. Benthic invertebrates and fish will also need to be monitored under the WFD but are not necessarily worth pursuing in terms of representing an ecological response to nutrient conditions.

Routine EA monitoring will need to be extended for phytobenthos and macrophytes. Monitoring of phytoplankton composition and/or abundance (chlorophyll *a*?) may be needed in lowland rivers to fulfil WFD requirements.

To achieve a consistent approach to monitoring and assessment for the Great Britain Ecoregion, the EA needs to progress future research developments with SEPA and SNIFFER, preferably through co-ordinated European programmes, such as the WFD Common Implementation Strategy. Particular EC projects related to river quality assessment include REFCOND, PAEQANN, FAME, AQEM and DIAMON. Analysis of existing, frequently monitored, long-term datasets needs to be carried out to assess confidence limits associated with varying sampling frequencies.

3 LAKES

3.1 Introduction

Of all surface waters, the impact of nutrient enrichment on lakes (and reservoirs) has probably received the greatest attention. The loss of macrophytes and increasing incidence of toxic cyanobacteria blooms has become a widespread problem in the UK having considerable environmental, social and economic implications (Environment Agency 1998). Lowland oligotrophic and mesotrophic lakes have become extremely rare with a high proportion of even our most protected sites (NNRs and SSSIs) showing clear symptoms of eutrophication (Carvalho and Moss 1995). Based on numerous documented studies and experimental evidence, qualitative schemes of how nutrient enrichment affects biological quality in lakes in general have been outlined (e.g. Hartmann, 1977, modified in Moss, 1998).

The requirement within the WFD to define nutrient conditions for high ecological status and then define moderate and good status as deviations from high, requires a more specific quantitative understanding of how nutrient conditions relate to biological quality in individual lake ecotypes. It is not possible within this review to consider the different responses in individual ecotypes, as there are potentially 108 lake ecotypes within England and Wales and 216 within the British Isles (c.f. Table 3.1: System A categories). Consideration is given of generalised lake types where appropriate e.g. shallow/deep and acid/alkaline. Approaches to quantitative definition of the relationships between nutrient conditions and biological quality for specific biological elements (for individual lake ecotypes) are considered.

Table 3.1 Obligatory lake ecotyping factors/categories

System B	*System A*
Altitude	Altitude: High (>800 m), mid (200 – 800 m), or low (<200 m)
Latitude/longitude	Ecoregion
Depth	Mean depth: shallow (<3 m), mid (3-15 m), or deep (>15 m)
Geology	Geology: calcareous, siliceous or organic
Size	Size: 0.5-1 km^2, 1-10 km^2, 10-100 km^2, or >100 km^2

Much of the nutrient data presented relates to phosphorus concentrations as it has long been considered the principal limiting nutrient in lakes (Schindler 1977) and often originates from controllable, point sources. Nitrogen limitation of phytoplankton biomass in particular was thought to be less common because of the potential for nitrogen fixation by certain species of cyanobacteria. Nitrogen limitation is, however, now accepted as being more widespread (Moss *et al.* 1997), although probably as a consequence of elevated phosphorus concentrations. In general, studies often consider a N:P ratio (in weight) >10 suggests phosphorus-limitation and a ratio of <5 suggests nitrogen-limitation is more likely.

Despite the lack of existing data on biological relationships with nitrogen concentrations, it is important that nutrient criteria are established for both nitrogen and phosphorus.

3.2 Phytoplankton

The phytoplankton community forms a key component of primary production in lakes; its relative importance, compared with phytobenthos and macrophytes, increasing with lake depth. The fact that phytoplankton are short-lived and derive their nutrients from the water column makes this biological quality element the most direct and earliest indicator of impacts of changing nutrient conditions on lake ecosystems. Phytoplankton are, therefore, ideal indicators of deteriorating ecological status associated with increasing nutrient status (eutrophication), or, of ecological recovery following reductions in nutrient loads.

There are numerous socio-economic problems associated with eutrophication-related increases in phytoplankton abundance, particularly with increasing frequency and intensity of toxic cyanobacteria blooms. These include detrimental effects on drinking water quality, filtration costs for water supply (industrial and domestic), water-based activities, and conservation status (submerged macrophytes and sensitive fish species, such as salmonids and coregonids). In one or two limited contexts, increasing phytoplankton abundance could be considered as a positive feature. For example, increasing fishery productivity or a potential sink for increasing concentrations of atmospheric carbon.

In summary, the phytoplankton community is a key indicator of ecological status in lakes, and particularly represents nutrient conditions and the impacts of eutrophication on environmental, social and economic value. This response of the phytoplankton community to nutrient conditions is considered in terms of the three phytoplankton-related quality elements, outlined in Annex V of the WFD, that need defining for type-specific, undisturbed conditions:

- Phytoplankton composition
- Phytoplankton abundance and its effect on transparency conditions
- Planktonic bloom frequency and intensity

3.2.1 Composition

Reynolds (1998b) outlines a provisional scheme of phytoplankton compositional changes across a nutrient gradient that merges seasonal effects. Most algal groups, including cyanobacteria, span the entire nutrient gradient. The only exceptions to this are chrysophyte algae that are characteristic of more nutrient poor (and acid) waters. Compositional changes due to nutrient enrichment become more apparent at the generic and species level. For example, of the diatoms, *Cyclotella* species are frequently associated with nutrient poor lakes and *Stephanodiscus* species tend to dominate following enrichment. Nuisance cyanobacteria such as the large colonial and filamentous species *Microcystis*, *Aphanizomenon* and *Anabaena* also tend to increase in dominance and abundance in response to increasing nutrient concentrations, often resulting in dense, mono-specific blooms during summer in eutrophic waters (Reynolds 1984).

Explanations for nutrient-related compositional shifts are not straightforward. Direct phosphorus- or nitrogen- limitation of particular species is probably rarely responsible for compositional changes, with the exception of diatom declines and silica-limitation (Lund 1950) or in the case of ultra-oligotrophic lakes (rare in the UK). Nutrient resource-ratios (Si:P or N:P) also offer limited explanation, particularly in lakes where nutrient concentrations are rarely depleted (Reynolds 1998b) although N-limitation is considered to promote dominance by cyanobacteria (Schindler 1977). Compositional responses to nutrient conditions may be explained more by factors such as phytoplankton growth rate, surface area to volume ratio, or CO2-concentrating capacity. Empirical models have been developed, based on phytoplankton functional groups incorporating these characteristics (Elliott, Reynolds and Irish 2001; Reynolds 1999). These models can be used to predict which species should dominate under particular environmental regimes and explore how community composition will qualitatively change along an increasing nutrient gradient under particular temperature and stratification states (or altitude/latitude and depth ecotypes).

3.2.2 Abundance and transparency

In general, as nutrient concentrations increase, phytoplankton abundance shows more frequent and sustained peaks throughout summer and transparency declines. The specific exceptions to this, shallow macrophyte-dominated lakes, are considered later in section 3.2.4. Reynolds (Reynolds 1984: 113-115) describes general seasonal patterns in phytoplankton abundance in a range of six lake types. In nutrient poor lakes, phytoplankton abundance increases over spring and summer to a peak in July in response to increasing water temperature, then decreases to a winter minimum as a result of nutrient deficiency. In other larger, nutrient poor lakes phytoplankton biomass may peak earlier in spring associated with blooms of planktonic diatoms, decline due to silica limitation, with other algae peaking later in summer. A clear pattern of spring and autumn peaks is most frequently characteristic of shallow (<3m), temperate mesotrophic lakes (Hutchinson 1967). In very shallow lakes abundant phytoplankton can be sustained throughout much of the year.

Chlorophyll *a* concentrations are extensively used as a measure of phytoplankton abundance and as a general symptom of eutrophication. Secchi disc depth is frequently used as a simple, practical measure of transparency. Both have been widely adopted in UK, European and international lake monitoring and classification schemes (see Section 3.2.5). Measurements of chlorophyll *a* can be problematic in that concentrations vary depending on algal composition and their physiological state (Reynolds 1984). In general, cyanobacteria have less chlorophyll *a* per unit biomass than Chlorophyta. Direct counts and measurements of algal biovolume are, therefore, a more accurate measure of phytoplankton abundance and provide informative compositional information, but are much more time-consuming. Transparency, or secchi disc depth, is also a problematic measure of phytoplankton abundance as light levels can be affected by suspended solids (particularly in shallow lakes) and water colour (in dystrophic lakes).

3.2.3 Bloom frequency and intensity

Annex V of the WFD specifically characterises high status lakes as those where the frequency and intensity of blooms occur consistent with the type-specific physico-chemical conditions [they may occur naturally more frequently in deeper waters where stratification is more stable]. It also characterises moderate status lakes as those in which “persistent phytoplankton blooms” may occur during summer months.

Nuisance blooms in lakes are generally associated with toxic cyanobacteria, in particular the genera *Microcystis*, *Aphanizomenon* and *Anabaena*. These species are commonly associated with nutrient enrichment, but the development of dense “bloom” populations is unpredictable with bloom frequency and intensity being particularly affected by physical factors such as stratification intensity and the depth of light penetration. As cyanobacteria tend to be relatively slow-growing, long periods of relatively stable stratification are required for large populations to develop. If nutrient concentrations are high, greater population sizes can be achieved. If very stable summer stratification develops green algae, such as *Scenedesmus*, can dominate whereas in summers of greater mixing, diatoms and cyanobacteria of the genus *Oscillatoria* can dominate (Reynolds and Bellinger 1992). The specific development of surface blooms and scums is associated with rapidly changing weather conditions. Many cyanobacteria species can regulate their buoyancy. If, however, a period of calm weather occurs abruptly following a period of wind-induced mixing, they may not be able to adjust their buoyancy rapidly enough and become trapped at the surface (National Rivers Authority 1990).

As cyanobacteria bloom formation is highly dependent on prevailing weather conditions, typical frequencies or intensities cannot be given for specific ecological quality classes. Threshold densities for bloom potential do, however, exist for particular nuisance species (Reynolds 1998a) and could be used to differentiate between ecological status classes.

3.2.4 Influence of factors other than nutrients

Seasonality, and even relatively short-term variations in thermal stratification and zooplankton grazing intensity, can have major influences on phytoplankton composition and abundance. Phytoplankton communities may respond over a period of days in response to a changing environment (weather, zooplankton grazer numbers). For this reason it is impossible to accurately predict what specific species composition or abundance would be found under a particular nutrient regime.

Strong altitudinal and latitudinal effects on ice cover will alter the temperature regime, affecting the seasonal succession of phytoplankton community. At a UK scale, however, variations due to altitude and latitude are not likely to be that extreme and will only require limited ecotype definition. The greatest variations in phytoplankton composition within the UK will be between lakes of different alkalinity/pH (geology) and depth. Ecological changes in response to increasing nutrient conditions should, therefore, be considered mainly in terms of shallow/deep and acid/alkaline lakes.

Lake Depth

In deep (>3m), stratifying lakes, as nutrient concentrations increase, there is an increasing incidence of filamentous and colonial cyanobacteria or large dinoflagellates, often dominating as mono-specific blooms during summer (Reynolds 1984). In shallow lakes (<3m), smaller algae tend to dominate the community, with green algae, such as *Scenedesmus* and other Chlorococcales, frequently dominating following enrichment.

Shallow Lakes and Alternative Stable States

Shallow lakes represent a more unpredictable situation where the macrophyte and fish communities exert a strong structuring role on the whole system, independent of nutrient concentrations (Jeppesen *et al.* 1998). Scheffer *et al.* (1993) proposed that shallow lakes can exist in two alternative stable ecological states. Supported by a wide range of empirical and experimental evidence, their model predicts that over a wide range of nutrient concentrations (30–1000 $\mu g l^{-1}$ TP), both clear water plant-dominated and turbid algal-dominated lake states can exist, each stabilised by buffering mechanisms. Below 30 μgl^{-1} TP submerged macrophytes tend to be abundant, while above 1000 μgl^{-1} TP phytoplankton dominance is the norm. Between this range, switches from macrophyte-dominance to phytoplankton-dominance are frequently associated with eutrophication, although other factors (water level or turbidity changes, toxic effects on grazers) can be responsible. Defining nutrient concentrations for particular ecological states or community compositions will, therefore, be particularly difficult in shallow lakes. Sayer (2001) identifies problems in the application of diatom-phosphorus transfer functions in shallow lakes where community changes are more associated with changes in habitat availability (macrophyte composition and abundance) than with changes in phosphorus concentrations.

In macrophyte-dominated shallow lakes, zooplankton grazers (particularly *Daphnia*) can exert significant effects on both phytoplankton composition and abundance. Zooplankton can discriminate phytoplankton according to size, shape and texture. Changes in the phytoplankton community composition and abundance, may, therefore, be more related to edibility with larger (e.g *Ceratium hirundinella*, *Volvox* spp.), toxic (e.g. cyanobacteria) or undigestible (e.g. mucilaginous greens) species dominating over small, edible unicellular algae. In periods of peak zooplankton abundance in late spring/summer, grazers can filter 30% of the entire lake volume in a day, a loss rate higher than phytoplankton growth can keep up with (Cyr and Pace 1992).

Alkalinity and pH

Little research has been carried out on the impact of increasing nutrient concentrations in acid lakes, although it is generally considered that acidity tends to have an overriding effect on phytoplankton composition. Acid lakes tend to be associated with an 'oligotrophic' flora which changes very little with increasing nutrient concentrations, until more circumneutral pH conditions are established (Reynolds *et al.* 1998). At the opposite end of the geological spectrum, alkaline marl lakes have a phytoplankton composition typical of eutrophic lakes, even in nutrient-poor conditions (Lund 1961), although phytoplankton abundance remains low.

The composition and proportion of cyanobacteria can also be altered by alkalinity and depth-related factors such as stratification intensity. Nuisance cyanobacteria such as the large colonial and filamentous species *Microcystis*, *Aphanizomenon* and *Anabaena* are favoured by high alkalinity (Shapiro 1990) and relatively stable stratification (Reynolds and Bellinger 1992) and will form a significant natural component of the phytoplankton community in lakes with these characteristics. One such example is the deep, alkaline Shropshire/Cheshire meres in which cyanobacteria have been a prominent feature for thousands of years (McGowan *et al.* 1999). An analysis of 55 Irish lakes, also revealed that the distribution of bloom-forming cyanobacteria is strongly related to the geology of the catchment and the presence of significant bicarbonate buffering (Reynolds and Petersen 2000). Conversely, in low alkalinity lakes with catchments of base-poor geology, bloom-forming cyanobacteria tend to be much less prominent.

The fact that depth and geology can have significant effects on phytoplankton composition and abundance highlights the importance of further research needed on individual ecotypes, to define typical communities and the boundaries between status classes.

3.2.5 Classification and monitoring schemes

No agreed UK-wide or European standard sampling or analysis methods exist. The Environment Agency's Anglian Region has developed a standard methodology for phytoplankton sampling and analysis (Anglian Region 1995). Standard sampling and analysis protocols have also been developed for the UK Environmental Change Network (Sykes, Lane and George 1999). Phytoplankton is monitored at least quarterly at 15 UK lake sites as part of this network. Some of these sites, and other non-ECN sites, have had their phytoplankton community monitored for decades and could provide information on reference conditions and responses to changing nutrient conditions. Monitoring of cyanobacteria populations is also carried out at a large number of lake and reservoir sites by the UK Environment Agencies.

Despite it being widely acknowledged as representing important impacts of eutrophication on lake ecosystems, phytoplankton composition is rarely a component of modern lake classification and monitoring schemes. The requirement of expert skills in identification and the complexity of interpretation are often considered to limit their routine application (Fozzard *et al.* 1999). The publication of a freshwater algal flora of the British Isles (John, Brook and Whitton 2002), specifically designed as a tool for monitoring, could help overcome some of these difficulties.

Direct quantitative relationships between phytoplankton composition and nutrient conditions have, however, been developed for the relationship between diatoms and total phosphorus concentrations (Bennion 1994; Wunsam and Schmidt 1995) (See Section 6.1 for full details). These diatom-phosphorus training sets include phytoplankton and phytobenthos species. They are based on relative abundances within surface sediment sub-fossil assemblages and, therefore, do not represent true ecological optima. Individual species TP optima are also dataset specific and cannot generally be applied outside the region of development or for different lake ecotypes as Table 3.2 illustrates.

Table 3.2 Total phosphorus optima ($\mu g\ l^{-1}$) for selected diatom species

	SE England (shallow/lowland)	**Alps and pre-alps (deep, upland)**
	(Bennion, 1994)	(Wunsam and Schmidt, 1995)
Navicula menisculus	60	12
Cyclotella radiosa	71	18
Fragilaria pinnata	94	21
Aulacoseira ambigua	96	19
Amphora pediculus	114	15
Aulacoseira granulata	127	52
Fragilaria construens f. binodis	150	25
Stephanodiscus parvus	201	27
S. hantzschii f. tenuis	312	111

Sayer (2001) identifies problems in the application of diatom-phosphorus transfer functions in shallow lakes where community changes are more associated with changes in habitat availability (macrophyte composition and abundance) than with changes in phosphorus concentrations. These diatom-phosphorus training sets do, however, provide a general scale of response to increasing phosphorus concentrations that reflect how diatom compositions are likely to change along an increasing nutrient gradient.

The studies also reveal an upper threshold of sensitivity, with very little species compositional change at TP concentrations above 300 μgl^{-1}.

It is possible that these existing diatom datasets could be analysed further to develop classification or monitoring schemes for individual lake ecotypes, although it is likely that larger datasets would be required to provide statistically robust relationships.

In terms of phytoplankton abundance, one of the first classification schemes developed was that of Carlson (1977) who used chlorophyll *a* and transparency (secchi disc depth) as measures of trophic status. The most widely recognised classification in terms of chlorophyll *a* (and transparency) is, however, that developed during the OECD programme on eutrophication (OECD 1982). This developed quantitative regression models relating chlorophyll *a* concentrations to total phosphorus concentrations (Equations 1 and 2).

$$\text{Chlorophyll}_{\text{mean}} = 0.48(\text{P}_{\text{lake}})^{0.87}$$

$$\text{Chlorophyll}_{\text{max.}} = 0.74(\text{P}_{\text{lake}})^{0.97}$$

Where, $\text{Chlorophyll}_{\text{mean}}$ and $\text{Chlorophyll}_{\text{max}}$. are the annual mean and annual maximum in-lake chlorophyll *a* concentrations respectively and P_{lake} is the annual mean in-lake TP concentration (all units µg/l).

A preliminary classification of lakes in terms of chlorophyll *a*, transparency (secchi depth) and nutrient concentrations was also outlined, detailing boundary values between trophic classes (Table 3.3, based on an interpretation by Newman (1988)).

Table 3.3 OECD classification scheme for lake trophic status

	Annual mean TP (µg/l)	**Annual mean chlorophyll *a* (µg/l)**	**Annual maximum chlorophyll *a* (µg/l)**	**Annual mean secchi depth (m)**
Ultra-oligotrophic	<4	<1	<2.5	>12
Oligotrophic	<10	<2.5	<8	>6
Mesotrophic	10-35	2.5-8	8-25	6-3
Eutrophic	35-100	8-25	25-75	3-1.5
Hyper-eutrophic	>100	>25	>75	<1.5

These classes are not directly applicable to the WFD ecological quality classes, as they are based on data that span a large number of lake ecotypes from lake regions across the globe, with a pre-dominance of large, deep lakes in the original dataset. In general, lowland lakes in the UK are likely to have naturally higher TP and chlorophyll *a* concentrations than upland lakes due to naturally more fertile catchments. Lake depth and altitude/latitude (geology/temperature) will also both affect how effectively nutrients are transformed into phytoplankton biomass; different ecotypes will, therefore, show variable sensitivities to nutrient enrichment.

Despite regional and ecotype differences, a common classification has been developed for European lakes (Table 3.4) with concentrations defined for complying (excellent and good status) and non-complying waters (fair, poor, bad status) (Premazzi and Chiaudani 1992).

Table 3.4 A common classification of European lakes (Cardoso, 2001)

Parameter	**Excellent**	**Good**	**Fair**	**Poor**	**Bad**
TP (mean) (μgl^{-1})	Natural levels	<125% of excellent	125-150% of excellent	150-200% of excellent	>200% of excellent
Chl. *a* (mean) (μgl^{-1})	<2	<5	<10	<25	>25
Chl. *a* (max.) (μgl^{-1})	<5	<10	<20	<50	>50
Secchi depth (mean) (m)	>5	2-5	1.5-2	1-1.5	<1
Secchi depth (min.) (m)	>3	1-3	0.7-1	<0.7	<0.7

Threshold chlorophyll *a* concentrations and secchi depths for lakes subject to eutrophication (less than good status?) have also been set across Europe in response to the UWWTD (Table 3.5)

Table 3.5 Threshold criteria used in European member states to designate lakes and reservoirs subject to eutrophication (Cardoso, 2001)

Parameter	**UK**	**Ireland**	**France**	**Spain**	**JRC Ispra**
TP (μgl^{-1})	>50	>25		>30	reference conditions
Chl. *a* (mean) (μgl^{-1})	-		>60	>8	>10
Chl. *a* (max.) (μgl^{-1})	>30	>35	-	>25	>20
Secchi depth (m)	<3	-	-	<3	<3

As with any indicator, there are, however, problems. Table 3.3 does not reveal the variability that was actually present in the original OECD scheme. Variability can reflect different opinions of the lake status of the people contributing the data (OECD scheme) as well as different sensitivities (susceptibility) of lakes to increased nutrient loading. The latter is fairly critical to the establishment of ecotype-specific reference conditions and boundary values. Variability in how effectively nutrients are transformed into phytoplankton biomass will be significantly affected by non-ecotype specific factors, such as flushing rate (Bailey-Watts *et al.* 1990) or macrophyte buffering in shallow lakes (Jeppesen *et al.* 1997).

The greatest practical problem with using the phytoplankton community as an indicator of ecological status is its dynamic nature. Changes in community composition and abundance associated with water quality changes may require frequent monitoring over several years to be accurately interpreted. The 6-monthly monitoring frequency outlined in the WFD is inadequate and is unlikely to be representative of annual dynamics. At a minimum, chlorophyll *a* should be monitored at the same 3-monthly frequency as is outlined in the WFD for physico-chemical elements, although monitoring at a monthly frequency would be more satisfactory to provide more accurate mean or maximum values, as recommended in the US EPA scheme detailed below (US EPA, 1998).

In terms of representing typical phytoplankton composition, one way of overcoming the problem of inadequate sampling frequency is to use the diatom assemblages in surface sediment samples. These provide a more integrative picture of community change, integrating phytoplankton and phytobenthos communities over space (from the whole of the lake basin) and over time (previous year(s) represented) (Charles, Smol and Engstrom 1984). Another potential approach is to base status classes on “risk of cyanobacteria blooms”. This would fit with Annex V of the WFD which specifically characterises moderate status lakes as those in which persistent phytoplankton blooms may occur during summer months. Risk for a site could be assessed through the use of models, such as PROTECH or by analysis of historical phytoplankton compositional and abundance data. Phytoplankton species commonly associated with cyanobateria bloom-supporting waters could be established and used to overcome the problem associated with any risk analysis based on infrequently monitored data. For example the diatoms *Stephanodiscus rotula* and *Aulacoseira granulata* and the dinoflagellates *Ceratium hirundinella* are all alternative dominant species from cyanobacteria under varying seasonal (temperature) or mixing regimes (Reynolds 1984; Reynolds and Petersen 2000) and could potentially be used as phytoplankton composition indicators of higher risk (poorer quality classes) in terms of potential occurrence of nuisance blooms. Knowledge of contemporary associations between diatom species and bloom-forming cyanobacteria species could also be applied to fossil diatom assemblages to infer reference conditions for particular sites.

An alternative to the risk assessment approach based on models or phytoplankton data is to use a classification scheme of threshold densities of cyanobacteria based on their impact on health and lake usage, such as the scheme developed by the World Health Organisation (WHO, 1998). They have established high, moderate and low exposure levels of cyanobateria that could be associated with different ecological status classes.

The most developed lake monitoring and classification scheme is that of the US EPA (1998). It is a multimetrics approach using up to seven biological assemblages, including chlorophyll *a*, secchi depth, phytoplankton, phytobenthos and sediment diatoms. The approach taken attempts to overcome some of the practical difficulties associated with the use of phytoplankton in biological monitoring programmes.

In the US EPA scheme, assessment is based on a tiered system with each tier requiring increased sampling effort. This allows the assessment to be customised to the users needs, questions and the available resources. The first tier (1A) involves a desktop questionnaire and single mid to late summer survey and is appropriate for regional assessments of lake “trophic state” (Carlson, 1977). More frequent summer sampling (tier 1B) or more detailed surveys of a range of biological groups (tier 2A), or both (tier

2B) are required for more precise characterisation of individual lakes for site-specific management.

Chlorophyll *a* concentration and secchi depth are two parameters used in initial assessment of lake trophic status (alongside submerged macrophytes) based on either the single summer survey (Tier 1A) or, if a more precise seasonal mean is required, monthly sampling during summer (Tier 1B). Phytoplankton composition or surface sediment diatom assemblages are two of the seven biological components recommended for more detailed studies at priority sites in Tier 2. Sampling monthly during the growing season is recommended for phytoplankton composition, with practical measures, such as % cyanobacteria and greens and % *Anabaena*, *Aphanizomenon* and *Microcystis* used to assess status in terms of eutrophication or other water quality pressures. Surface sediment diatom assemblages only require a single visit, but require more intensive analysis and are less associated with threats to water quality.

3.2.6 Summary

In general, phytoplankton composition and abundance are good indicators of a lake's response to changes in nutrient status and could be used, in these terms, as a component of a site's ecological quality status. Chlorophyll *a* concentrations represent a very simple and effective measure of phytoplankton abundance, a key ecological response to nutrient conditions. Current classification schemes do not distinguish disturbed from undisturbed sites and would require development in terms of identification of reference conditions. It could, however, be relatively easy and cheap to produce regional and ecotype-specific classifications. An additional advantage of using chlorophyll *a* is that it integrates the phytoplankton community response to all potentially limiting resources, irrespective of whether it is phosphorus, nitrogen, silicon or light. The disadvantage is that it is highly dynamic and monthly sampling during summer is recommended. It also provides no information on phytoplankton composition. In terms of impacts on ecological structure and the socio-economic value of lake ecosystems, phytoplankton abundance data needs to be complimented with compositional data. At the very least threshold densities or biovolumes of bloom-forming cyanobacteria, such as those developed under WHO guidelines, could be used alongside chlorophyll *a* concentrations to provide the most practical and informative measure of the impact of nutrient enrichment on lake ecological status. Densities below thresholds do not necessarily imply good ecological status, but exceedance of thresholds could be used to indicate less than good status. Monthly sampling during summer is again recommended.

3.2.7 Recommendations for Phase II

The phytoplankton community is ideal for indicating ecological status explicitly in terms of nutrient pollution pressures. There are, however, a number of significant research requirements before it can be utilised in the WFD:

Standard sampling and analysis methods require acceptance at UK and European level.
Ecotype-specific classification schemes based on phytoplankton composition, abundance and bloom frequency/intensity need to be developed.

In terms of composition, one great advantage of all algal communities (phytoplankton, phytobenthos, surface sediment diatoms) is their spatial scale and species richness. This enables the determination of ecotype-specific, nutrient optima and tolerance ranges, with associated confidence limits, for numerous species through fairly limited field survey. Alternatively, classification could be based on indicator groups, but would require the development of a ‘phytoplankton trophic index’. At a minimum, threshold densities of cyanobacteria should be established.

In terms of abundance, the development of ecotype-specific classification based on chlorophyll *a* concentrations should be relatively straightforward. Existing long-term datasets could be used to identify or validate reference values and the boundaries between status classes and also assess the effect of sampling frequency on precision/accuracy of annual mean/maximum chlorophyll *a* concentrations in different lake ecotypes.

In terms of bloom frequency/intensity, the WFD monitoring frequency is rather inadequate. Research should be carried out to establish what densities constitute a bloom and to identify threshold cyanobacteria densities for the full range of ecological status classes. The use of phytoplankton species associations with bloom-forming cyanobacteria, could be investigated to overcome the problem of low sampling frequency, and identify what frequency of sampling is necessary.

3.3 Phytobenthos

The phytobenthos community in lakes is very similar to that found in rivers (Section 2.4). Like phytoplankton, it forms a key component of primary production in lakes and can be the dominant component in shallow (Wetzel 2001) or oligotrophic (Loeb *et al.*, 1983) waters. An increasing abundance of epiphyton, in response to nutrient enrichment, has long been considered an important factor in the loss of submerged macrophytes from shallow lakes (Phillips, Eminson and Moss 1978; Sand-Jensen and Søndergaard 1981). As in rivers, the phytobenthos can be functionally important in sequestering and transforming many inorganic nutrients into organic forms (Stevenson, 1996) and the phytobenthos itself constitutes a significant source of energy for higher trophic levels with most littoral invertebrate grazers feeding on benthic algae, rather than macrophytes.

Despite its significance, the phytobenthos has received relatively little attention in terms of its use as an indicator of lake quality. This may be due to the recognition that the linkage between water column nutrient concentrations and algal biomass tends to be stronger for the phytoplankton (Cattaneo, 1987).

The fact, however, that the phytobenthos does respond to both water column nutrient concentrations and habitat quality, is accessible from the lake shore, and is less dynamic than the phytoplankton community has led to increasing interest in its use as a monitoring tool for lakes and other standing waters (Biggs *et al.* 2000). Annex V of the Water Framework Directive specifically outlines phytobenthos composition and abundance as two criteria that need defining for type-specific, undisturbed conditions in lakes. The response of the phytobenthos to nutrient conditions will, therefore, be considered in these terms, followed by consideration of other factors affecting the community and finally by discussion of the potential application of established phytobenthos-based monitoring and classification schemes to the WFD.

3.3.1 Composition

Phytobenthos communities in lakes are usually dominated by diatoms (Bacillariophyta), green algae (Chlorophyta), blue-green algae (cyanobacteria), or red algae (Rhodophyta), although most other algal groups can occur in benthic habitats. Many species are characteristic of the phytobenthos and absent from or rare in the phytoplankton, including many pennate diatoms (e.g. *Cocconeis*, *Achnanthes*, *Gomphonema* and *Navicula*).

If a lack of grazing or disturbance permits, benthic algae can develop mixed communities, or algal mats. Initially, colonisation by tightly bound adnate algae occurs, composed mainly of diatoms such as *Cocconeis*. If disturbance is low, and light and nutrients sufficient, these are then overgrown by apically attached algae, such as the diatom *Synedra*. Further community development can proceed to stalked diatoms, such as *Gomphonema*, and filamentous algae.

Even though the physiological requirements and responses of attached algae are similar to those of the phytoplankton, the relationship between nutrient conditions and phytobenthos community composition is much more complex. Nutrients diffuse much more slowly into attached communities, with strong gradients through boundary layers and the attached communtiy. The thicker the community the more isolated the algae are from water column nutrient conditions. Benthic algae can also obtain nutrients from the substrates that they are attached to. Clear differences in community composition have been observed between natural and artificial substrates (Cattaneo, 1978). Nutrient enrichment in the overlying water does, however, result in major shifts in community composition. Species associated with low phosphorus (the diatoms *Achnanthidium minutissimum* and *Gomphonema tenellum*) and low nitrogen (nitrogen-fixing species, such as the diatoms *Epithemia adnata* and *Rhopalodia gibba* and cyanobacteria such as *Anabaena* spp.) waters have been shown to disappear following nutrient enrichment (Fairchild *et al*., 1985). If nutrient enrichment proceeds further, phytoplankton can shade the phytobenthos, and light-limitation becomes more significant; community composition then becomes dominated by species tolerant of low light levels.

Based on a number of studies, Lowe (1996) summarises the dominant species according to lake trophic classes (oligotrophic and eutrophic) and depth. The community includes many benthic species found in both lakes and rivers.

Only recently have attempts been made to develop quantitative relationships between the phytobenthos community and nutrient conditions. Danilov and Ekelund (2000) analysed epiphyton and epilithon species diversity from seven Swedish lakes. They concluded that epiphyton diversity showed little relationship with nutrient concentrations, but epilithon diversity was consistently related and could be used as an indicator of nutrient status. Further research would be required to assess whether the relationships shown held for a wider range of lakes. King *et al.* (2000) examined distributions of 138 epilithic diatom species from 17 lakes in the English Lake District and showed that total phosphorus and calcium concentrations were the most important variables explaining species distributions.

3.3.2 Abundance

In detailed investigations of the epipelic community in several lakes of the Cumbrian Lake District, Round (1957; 1960; 1961) showed that annual and seasonal cycles of phytobenthos abundance was similar to that of the phytoplankton, with spring and autumn maxima. Epiphytic biomass, on the other hand, tends to be more constant throughout the year (Wetzel 2001). In general terms, nutrient enrichment in the water column usually results in enhanced growth of attached algae. When nutrient enrichment proceeds further, however, dense phytoplankton blooms can shade phytobenthos development severely (Hansson 1992). The biomass of epipelic algae has also been shown to increase as sediment concentrations of nutrients increase (Wetzel 2001).

Phytobenthos abundance is generally measured as mass per unit area of substrate. The most ideal assessment of phytobenthos abundance is a direct biovolume estimate. This is, however, time-consuming to obtain. Chlorophyll *a* and other pigment concentrations are more widely used as a rough measure of abundance in benthic habitats, with pigment ratios distinguishing between different algal groups. Wet or dry weights are difficult to apply to the phytobenthos, as weights are frequently affected by inorganic matter or non-algal organic matter (detritus, bacteria, fungi).

3.3.3 Influence of factors other than nutrients

Substrate
Substrate has a major effect on species composition. Epilithic and epiphytic communities have many species in common, but tend to have a very different composition from communities found on epipelic habitats, or on other loose substrates (Wetzel 2001). Epipelic communities on fine, organic sediments largely comprise motile algae, as mobility is necessary to move to the surface following disturbance of the sediments. Episammic communtities tend to be dominated by algae attached firmly to crevices in sand-grains.

Light availability
Light-limitation of phytobenthos in lakes can be due to shading by bankside vegetation, but is more often studied in terms of attenuation along a depth gradient. Light attenuation with depth is affected by the colour of the water (dystrophic lakes) and the amount of suspended material (shallow or enriched lakes). In very clear lakes, light can penetrate below the lower limit of vascular macrophyte growth (about 10 m) and support significant phytobenthos biomass.

Grazing
Epiphyte communities in particular are affected by grazers (mainly snails and insect larvae). Moderate grazing can enhance algal growth, as it can lead to improved access to nutrients and light (Wetzel 2001), but heavy grazing pressure usually results in reduced phytobenthos biomass. In heavily grazed areas, nutrient-enhanced algal production that is not expressed in phytobenthos biomass is often instead reflected in increased grazer abundance. Intense grazing pressure has also been shown to affect species composition and reduce algal diversity (Lowe and Hunter, 1988).

Disturbance
In lakes with relatively stable water levels, the phytobenthos community is well adapted to turbulence from wave action, although storm events can cause disruption to loosely attached species. In reservoirs with strongly fluctuating water levels, dessication can severely impact on the phytobenthos community. Many diatoms and cyanobacteria

species are, however, able to survive long periods of drought. In general, disturbance effects on lake phytobenthos are much less important than in river communities.

3.3.4 Classification and monitoring schemes

In terms of the phytobenthos community as a whole, no lake classification schemes have been developed. Monitoring of epilithic diatom communities is carried out at a few lake sites as part of the UK Environmental Change and Acid Waters Monitoring Networks. Standard sampling protocols have been developed for this work (Sykes *et al.* 1999). Standard sampling and analysis protocols also exist in other countries, notably those developed in the U.S.A. (Clesceri *et al.* 1998; US EPA 1998). Phytobenthos is recommended as one of seven potential biological parameters in Tier 2 of the US EPA lake monitoring and classification scheme (US EPA, 1998). The scheme highlights the potential of phytobenthos, but does point out that responses to pollution or disturbance are not adequately known and require further development.

In terms of surface sediment diatom assemblages (phytobenthos and phytoplankton), direct, quantitative relationships with total phosphorus concentrations have been developed, as described in Section 3.2 (Bennion 1994; Wunsam and Schmidt 1995), and could be used to establish a classification scheme. This combined response of phytoplankton and phytobenthos communities may provide the most effective and complete representation of lake ecosystem response to eutrophication and would be highly compatible with palaeolimnological methods for the setting of reference conditions (Section 6.1).

Hughes (2000) examined the response of diatom communities from the epiphyton and epipelon of shallow lakes in Norfolk, with the specific intention of developing their use as a monitoring tool. She identified species that decrease in abundance with increasing TP (e.g. *Achnanthidium minutissimum* and *Gomphonema angustatum*) and species that increase in abundance with increasing TP (e.g. *Achnanthes hungarica* and *Gomphonema truncatum*). Communities on submerged and emergent plants were the most floristically varied and were recommended as having the most potential for development as a monitoring tool.

As in rivers, quantitative analyses of phytobenthos abundance are much more difficult than qualitative analyses of species composition. No standardised sampling methods or established classification schemes exist for phytobenthos abundance in lakes. Lake communities are, however, much less dynamic than those in rivers, and it may be possible to set typical values for different nutrient conditions, using chlorophyll *a* concentrations per unit area as a practical measure. One major problem with the WFD monitoring protocols is the sampling frequency outlined for the phytobenthos ("other aquatic flora") as every 3 years. This is certainly inadequate for this community. At a minimum, the 6-monthly frequency stated for phytoplankton should be adopted for phytobenthos too, although monthly sampling would be more appropriate (see recommendations for phytoplankton monitoring in Section 3.2).

3.3.5 Summary

The phytobenthos community is an important source of primary production in many lake systems. It is widely used to indicate river water quality in terms of nutrient conditions (Section 2.3), and could equally be developed for lakes. The development of more established approaches using surface sediment diatom assemblages (see also

Section 3.2) may be more appropriate than developing new methods based on specific benthic habitats.

3.3.6 Recommendations for Phase II

There are a number of essential requirements if the living phytobenthos community is to be developed for the WFD :

- Research into which benthic community is most sensitive to nutrient conditions (epiphyton, epilithon, etc.) and the development of an appropriate index in terms of nutrients.
- Research into the most effective sampling methodology (frequency, taxonomic level, etc.).

3.4 Macrophytes

Aquatic macrophytes are intrinsically linked to lake water and sediments through their roots and leaves. Individual species are sensitive to physical and chemical changes in these media. Aquatic macrophytes are therefore useful indicators of environmental conditions and environmental change in aquatic ecosystems. They also have considerable ecological importance as they provide refuges, substrates and a source of food for many other aquatic organisms. The ability to quantitatively relate species composition and abundance to nutrient concentrations is important within the Water Framework Directive as well as in national conservation and management programmes.

Enhanced concentrations of nutrients (notably N and P) or eutrophication, result in an increase in the net primary productivity of ecosystems and typically an associated decline in water quality. Phosphorus is widely recognised as the nutrient limiting primary productivity in most freshwaters, and thus, emphasis has been placed upon the reduction of its supply to lakes in restoration programmes. The influence of eutrophication on submerged macrophyte composition and abundance is widely acknowledged, although, as the following review will illustrate, specific controls are less well understood. On one hand, nutrients directly influence species growth biomass and inter-specific competition; on the other, nutrients have indirect effects, and particularly by influencing phytoplankton productivity, which in turn, affects light availability and hence growth rates for macrophytes, and eventually species composition, depth, distribution and surface area of macrophyte beds (Lehmann *et al.* 1999).

This review focuses primarily on the extensive literature on the relationships which exist between aquatic macrophyte species composition and water quality, and on the classification schemes which are based on such information (Section 3.4.1). Nutrient-abundance relationships and particularly the influence of eutrophication and trophic structure are considered briefly in Section 3.4.2.

3.4.1 Composition

In the following section we summarise the existing national and international literature which examines the relationship between lacustrine aquatic macrophyte communities and nutrient concentrations, and the classification schemes which have been adopted. The review focuses firstly on UK-based work, before broadening to cover studies from elsewhere in Europe, and finally, north America.

Relationship with water quality

UK

Some of the earliest studies relating British aquatic plant community composition to their environment were by West (1910) in Scotland and Pearsall (1918, 1920) in the Lake District. Pearsall arranged lakes in the English Lake District into a series according to their productivity, determined by the percentage of cultivated land in the catchment, the rockiness of the littoral zone and water transparency. The series ranged from "rocky" unproductive lakes occupying steep-sided, uncultivated valleys such as Wastwater, to "silted" relatively productive lakes adjacent to gentler, soil-covered slopes supporting woodland and improved pasture such as Coniston Water. Pearsall (1932) developed this further by comparing quantities of dissolved substances in the lake waters and related these to differences in the abundance and composition of planktonic algal assemblages. Spence (1964) described Scottish aquatic and swamp communities by classifying 18 lentic communities dominated by submerged or floating species (Spence, 1964). Spence (1967) and then Seddon (1972) highlighted the likely influence of alkalinity, pH and conductivity, and the importance of the ratio of Ca+Mg to Na+K for plant community composition. Seddon (1972) carried out an extensive Welsh lake survey and used Principal Components Analysis to examine species occurrence in relation to water chemistry. He estimated individual species tolerances, and divided the gradient into groups of species characteristic of dystrophic, oligotrophic, mesotrophic, and eutrophic water and those that were generalists.

The NCC scheme

The work of Palmer *et al.* (1992) resulted in the most extensive aquatic macrophyte classification scheme for UK lakes to date and this is described further in Section 3.4.1. TWINSPAN groupings of macrophyte composition of 1124 freshwater and brackish lakes across Britain correlated strongly with alkalinity, pH and conductivity. Oxidised nitrogen and phosphorus were only measured at a small subset of sites but it was argued that since high alkalinity is generally associated with high concentrations of nutrients, the three correlating variables were indicators of nutrient status.

Integrated Classification Schemes for Welsh Lakes

Classification aspects of this scheme are considered in further detail later in Section 3.4.1. The study (see Allott and Monteith, 1999) involved thorough biological (epilithic diatoms, surface sediment diatoms, aquatic macrophytes, littoral cladocera, zooplankton, open-water zooplankton and littoral benthic invertebrates) physical and chemical surveys of 31 lakes in Wales. Chemical measurements included N and P (involving filtered samples for soluble reactive P (SRP) and nitrate, which were frozen on site).

Physio-chemical analysis of the lakes revealed a single, dominant and wide environmental gradient from low to high pH, alkalinity, conductivity, major ions and phosphorus conditions. Indirect ordination techniques and TWINSPAN classification techniques on individual biological groups indicated that biological variation was strongly related to this gradient. A TWINSPAN classification applied to an integrated biological dataset (taxa from all groups combined in one pseudo-assemblage) resulted in seven distinct site groups which could be discriminated (using Canonical Variates Analysis) by just three variables: pH, SRP and chlorophyll *a*. However, it should be noted that Canonical Correspondence Analysis on the aquatic macrophyte data in isolation found that alkalinity, conductivity and chlorophyll *a* effectively explained the main components of variation, and the direct relationship between plants and nutrients was not explored further.

The Northern Ireland Lake Survey Dataset

The Northern Ireland Lake Survey (NILS) (see Gibson, *et al.* 1995) involved a survey of the floristic composition and water chemistry of 608 lakes (surface area < 100ha) in Northern Ireland. Assessments of macrophyte abundance, using a DAFOR equivalent 1-5 scale (1 = rare, 2 = occasional, 3 = frequent, 4 = abundant, 5 = dominant), were made using a grab from a boat and by walking around the shoreline. A wide range of physical and chemical variables (including ammonium, total Kjeldahl nitrogen, and total phosphorus) were recorded.

Heegaard *et al.* (2001) analysed the NILS macrophyte dataset to examine how individual macrophytes respond to a variety of environmental factors. Predictive models (Generalised Additive Models or GAMS) were used to describe the probability of occurrence of the more common species along individual environmental gradients as response curves. The shape of response was classified into one of eight types, which summarise how tightly a species is constrained and the position of most likely occurrence along the gradient. Conductivity, alkalinity, ionic strength and nutrient (total Kjeldal nitrogen, and total phosphorus) concentrations were found to be the most influential variables. These are strongly correlated with altitude as hardwater nutrient-rich are restricted to lowland areas. Two main species groups characterised sites of high and low ionic concentration.

1. Those constrained to low values of conductivity (optima 38-218 μS/cm), alkalinity (0-1.4 meq/l), Ca (0.05-24mg/l), Mg (0.4-1.34 log (mg/l)), K, (0-0.64 log (mg/l)), and sulphate (0.26-1.56 log (mg/l)) consistently included *Isoetes lacustris*, *Lobelia dortmanna*, *Myriophyllum alterniflorum*, *Sparganium angustifolium* and *Potamogeton polygonifolius*, and often included *P.alpinus, P. gramineus*, and *Littorella uniflora.* These species have been associated with dystrophic and oligotrophic conditions (Spence 1967, Seddon, 1977, Palmer *et al.* 1992).

2. Most of the species constrained to high values of water-chemistry variables are relatively weakly correlated, although many are strongly constrained by high pH. They consistently included *Myriophyllum spicatum*, *Hippuris vulgaris*, *Zannichellia palustris*, *Potamogeton pectinatus*, *Utricularia vulgaris*, and *Lemna trisulca,* and may also include *P. lucens*, *P.crispus*, *P. pusillus*, *L. minor*, *Elodea canadensis*, and *Ranunculus peltatus*. Many of these species are classified as mesotrophic or eutrophic by various authors (e.g. Seddon 1977, Palmer *et al.*1992).

Total Kjeldahl nitrogen, and total phosphorus concentrations were strongly correlated. Seven species, *Lobelia dortmanna, Isoetes lacustris, Myriophyllum alterniflorum, Sparganium angustifolium, Potomogeton polygonifolius, Callitriche stagnalis*, and *Potamogeton praelongus*, were shown to be tightly constrained to the low end of the total Kjeldahl nitrogen gradient. All would be expected to occur well within 50% of the total gradient length. The first five of these were similarly constrained by total P. All of these species are considered oligotrophic by Palmer *et al.* (1992). In addition, *Littorella uniflora* was mildly constrained across the centre of the total P gradient, while

Spirodela polyrrhiza and *Zannichelia palustris* were mildly constrained toward the top end.

This work has potential to provide a framework for defining nutrient "restoration targets" for enriched but naturally oligotrophic lakes, at least within the Northern Ireland region. Importantly however, there is little evidence from Heegaard *et al.* (2001) of direct nutrient relationships with the majority of species, and little evidence of species showing tight dependence on higher concentrations of N or P in isolation. This has important implications for the definition of targets for more naturally alkaline lakes, suggesting that greater physio-chemical complexity has to be taken into account when considering nutrient pollution impacts in such circumstances.

The NILS dataset is the largest and most comprehensive dataset currently available for UK lakes that enables the relationship between macrophyte distribution and nutrients to be assessed. Although the dataset is regionally restricted it could, with contributions from the CCW project, form the foundations of a UK-wide resource. We believe that the approach of Heegaard *et al.* (2001) is probably the most important recent development in this area and provides a potentially powerful predictive tool for the WFD.

Wider Europe

Finland

Toivonen *et al.* (1995) studied species composition and changes over time in aquatic macrophytes in 57 small Finnish lakes, with the aim of: 1) finding the main environmental factors determining the species composition of the lake floras; 2) estimating the relative positions and ranges of individual species along the oligo-eutrophy gradient; and 3) evaluating two a priori classifications with multivariate methods.

On the grounds that conductivity was highly correlated with nutrient concentration and algal productivity the former was used as an indicator of trophic state. The study suggested that trophic state and water transparency were the important determinants of aquatic macrophyte composition. Multivariate analyses indicated an ecological continuum rather than distinct lake groups. Correspondence between the multivariate analysis results and the *a priori* classifications of lakes was good, particularly for nutrient-poor and nutrient-rich waters, as well as for some clear-water lakes. However types representing mesotrophic and eutrophic lakes did not fit into one distinct class, possibly because the botanical lake types were developed for larger lakes where vegetation is more heterogeneous.

This study does not therefore directly relate macrophyte composition to nutrient concentrations, although the inferred relationship with trophic state is strong. The study recognises the concept of an ecological continuum of lake types rather than distinct groups.

Denmark

Jeppesen *et al.* (2000) carried out studies on 71 predominantly shallow Danish lakes covering a long TP gradient (summer mean 0.02 - 1.0 mg P L^{-1}). The study examined zooplankton, fish, phytoplankton and macrophytes and aimed to determine how species richness, biodiversity and trophic structure change along a TP gradient. This was divided into 5 separate classes with the following concentrations: <0.05, 0.05-0.1, 0.1-0.2, 0.2-0.4, >0.4mg P L^{-1}. The species richness of both submerged and floating-leaved vegetation showed marked changes along the TP gradient. The submerged macrophyte species revealed a monotonical relationship with TP. The maximum depth at which submerged macrophytes were recorded also significantly declined with increasing TP (< 0.001 mg TP l^{-1}) (Jeppesen *et al.* 2000). The species richness of floating-leaved macrophytes however, behaved differently to submerged macrophytes demonstrating a unimodal relationship to TP concentrations.

Vestergaard *et al.* (2000a) studied the species richness and distribution of submerged macrophytes in 73 Danish lakes in relation to lake size, depth, water chemistry, trophic level and Secchi disc transparency. The objectives were to identify the individual variables that could best explain the species richness variation among the lakes and to determine the best multiple predictors of species richness. Submerged vascular plants were separated into 54 elodeid (submersed, partly emergent or free-floating perennial herbs) species and 12 isoetid species (quillworts). Species richness, representing the number of observed species in each lake, was calculated for elodeids, isoetids, bryophytes (liverworts and mosses), characeans (stoneworts), and the total number of submerged species was recorded.

The data included location, surface area, mean and max water depth, alkalinity, pH, total phosphorus, total nitrogen, chlorophyll *a* and Secchi depth. The lakes ranged from mesotrophic to hyper-eutrophic (summer concentrations ranging from 0.01 – 0.90 mg total P.L^{-1} and chlorophyll *a* 1.2 –200 μgl^{-1}). This study concluded that alkalinity and transparency were the main predictors of the species richness and distribution of aquatic macrophytes in Danish lakes. Higher alkalinity resulted in a rise in species richness due to the preference of species-rich elodeids for alkaline conditions. This is likely to reflect the importance of bicarbonate concentration on growth, development and long term survival of submerged elodeids (Madsen and Sand-Jensen 1991). In addition, alkalinity is positively related to concentrations of the major cations, total ionic content and pH (Leuven *et al.* 1992), and these may also contribute to increased diversity.

The relationship between lake trophy and species richness was examined using Secchi Disc transparency as a measure of productivity. Maximum species richness was observed in mesotrophic lakes, which exhibited diverse assemblages of small isoetids, mosses and characeans beneath a canopy of elodeids.

Switzerland

The effects of nutrient enrichment on aquatic macrophytes was investigated by Lachavanne *et al.* (1985) between 1977 – 87 through a large-scale survey of Swiss lakes. They described three different phases.

1. Colonisation of deeper areas (ultra-oligotrophic to oligotrophic)
2. Profuse growth (mesotrophic to eutrophic)
3. Regression (highly eutrophic to hypereutrophic)

Lehmann *et al.* (1999) furthered this work by studying the effect of the reversal of nutrient enrichment on aquatic macrophytes at a local scale. The study aimed to compare the distribution of submerged macrophytes along 20km of the lake shore of Lake Geneva between 1972, 1984 and 1995. Lake Geneva was subject to rapid eutrophication until 1980, followed by a reversal, during which time the nutrient concentration of the lake changed dramatically. Light penetration and phytoplankton biomass, however, remained relatively stable. Notable changes in the distribution of some species were observed between 1972 - 95. These included an almost complete loss of Charophytes and *Myriophyllum spicatum* (canopy-producing species) in 1984 and an increase in *Potamogeton crispus* (an erect species). The relationship between growth form and light attentuation was too general to explain these changes and the authors recognised that complex interactions were occurring at the species level, such as interspecific competition, competition with epiphytes, dispersal strategies and physiological and life cycle differences.

USA and Canada

Srivastava *et al.* (1995) examined the relationship between nutrients and macrophytes in 21 lakes in Nova Scotia covering a broad range of water chemistry (TP 0.003 – 6.0 mgl^{-1}; pH 3.7-8.3). Ordination techniques revealed three main groups representing heavily enriched, moderately enriched and non-enriched lakes (Table 3.6). Alkalinity, total phosphorus and total nitrogen were the environmental variables most strongly correlated with species distribution.

Table 3.6 Macrophyte composition in the different trophic state classes

Trophic Status	Macrophyte Composition
Heavily Enriched Lakes (pH ≥ 6.9, TP ≥0.5mg l$^{-1)}$	• Characterised by a high abundance of *Lemna minor*, *Sparganium eurycarpum*, *Carex pseudocyperus*, *Elodea canadensis*, *Typha latifolia* and *Potamogeton foliosus*.
Moderately-enriched lakes (TP 0.025 –0.32 mg l^{-1})	• Characterised by species such as *Potamogeton oakesianus*, *Utricularia intermedia*, *Utricularia purpurea*, and *Scirpus cyperinus*. • Species characteristic of acidic/oligotrophic waters, such as *Dulichium arundinaceum* and *Sparganium americanum*, and those characteristic of more alkaline / eutrophic waters, such as *Elodea canadensis*, *Typha latifolia* and *Potamogeton gramineus* often co-existed in this intermediate group of lakes.
Non-enriched lakes (P < 0.018 mg l^{-1}, pH 3.7-6.3)	• Characterised by species such as *Eleocharis robbinsii*, *Utricularia vulgaris*, and *Potamogeton confervoides*. • Isoetid dominated lakes were separated from *Sphagnum*-dominated lakes.

The authors suggested that whilst water column TP, TN, and alkalinity best explain the plant distributions, they do not necessarily influence plant composition directly but may act as indicators for unmeasured parameters. For example, TP and TN represent the available phosphorus and nitrogen in the sediments, which are the most important source of nutrients for many macrophytes.

3.4.2 Abundance

Recently it has become clear that the functioning of shallow lakes is particularly complex and involves a combination of “top down” and “bottom up” factors, comprising a series of internal interactions with the fish, zooplankton, phytoplankton and macrophyte communities (Moss *et al.* 1994). Scheffer *et al.* (1993) have described shallow lake function with the alternative stable state model. This suggests that over a wide range of nutrient loadings (typically 30 – 1000 μg TP l^{-1}), both clear water plant-dominated and turbid algal-dominated lakes can exist as alternatives, each stabilised by buffer mechanisms. Below this range submerged macrophytes tend to be abundant, while above it phytoplankton dominance is the only stable state. Ultimately, the existence of the two alternative states may be nutrient dependent but ecological feedbacks may reverse or maintain the state. In these systems, therefore, a simple reduction in the nutrient load may often have little ecological relevance and lake restoration may fail.

Extensive work on the ecology of Danish shallow lakes has been carried out by Jeppesen *et al.* (1990, 2000). The interactions between ecosystem components, as well as the top down and bottom up factors influencing lake equilibria, have been rigorously studied. The relationship between macrophytes and nutrient concentration constitutes one of these key inter-relationships.

Jeppesen *et al.* (1990) evaluated the effects of fish manipulation in shallow, eutrophic lakes by studying the relationship between TP and the occurrence of phytoplankton, submerged macrophytes and fish. The results support the view that an increase in nutrient load can increase the dominance of phytoplankton at the expense of submerged macrophytes in larger lakes (>10 ha). Submerged macrophytes were absent in lakes >10 ha and with P concentrations above 250-300 μg P L^{-1}. The study however revealed a different response to nutrient concentrations and macrophytes in smaller lakes (<10ha). The study supports the alternative sTable states model (Scheffer 1990). In shallow Danish lakes, > 3ha the shift from level 1 -2 occurs at ca. 50μg P L^{-1} and the shift from level 2-3 occurs at ca. 125 μg P L^{-1}. In smaller lakes (<3ha), however alternative sTable states were present at TP concentrations of 650μg P L^{-1}. The data were limited so these thresholds must be interpreted cautiously. The differences in threshold concentrations between lakes of various sizes are thought to be a result of the strength of the buffering mechanisms maintaining the macrophyte stage.

3.4.3 Classification and Monitoring Schemes

UK

The NCC Scheme

Palmer *et al.* (1992) introduced the most extensive aquatic macrophyte classification scheme for UK lakes to date. This scheme often forms the basis of site selection for conservation purposes in the UK today. Based on a TWINSPAN analysis of the macrophyte composition of 1124 freshwater and brackish lakes across Britain, the scheme classifies standing waters into one of 10 types depending on their submerged and floating vegetation.

TWINSPAN analysis of a dataset comprising the relative abundance of floating leaved and submerged plants in 1124 lakes, generated 10 vegetation groups which were related to lake alkalinity, pH and conductivity. They were allocated site type descriptions, with an emphasis on lake trophy and substrate characteristics, as shown in Table 3.7. The findings supports relationships described in earlier studies (e.g., Spence 1964).

Table 3.7 Palmer Site Types (Palmer *et al.* 1992)

Type	Site Type
1	Dystrophic
2 and 3	Oligotrophic/base-poor
4	Mixture of influences
5	Mesotrophic
6	Brackish
7,8,9 and 10	Eutrophic/base rich

The scheme also allocated each species with a Trophic Ranking Score (TRS) based on the range of site types within which it is found. The average TRS can be used to represent a whole or part of a site and, when historical data are available, can infer whether water quality change (e.g. acidification or eutrophication) has occurred. It can also be used to indicate differences in trophic state within a single water body and attempt to identify pollution sources.

Despite its considerable merits the NCC classification scheme has limitations with respect to its potential application to the WFD. Northern England and Scotland generally contain more freshwaters with high conservation value, so this scheme, which was devised to identify SSSI's, is biased towards sites in these areas. There are, therefore, concerns as to how applicable this classification scheme is to sites in southern, lowland Britain. Importantly, the classification scheme does not directly relate macrophyte species to nutrient loading. The NCC standing waters database had very limited data on nitrogen and phosphorus concentrations. As a result the trophic status of standing waters was loosely defined according to pH, alkalinity and conductivity measurements. This is acceptable over broad chemical gradients, as high alkalinity is generally associated with high concentrations of nitrogen and phosphorus. However, there is a possibility that the nutrient status of some sites will have been mis-represented using these criteria.

Integrated Classification Schemes for Welsh Lakes

Recently, a change in conservation ethic has seen a move away from classifications based on analysis of single biological groups and toward wider ecosystem properties. In response to this, Allott and Monteith (1999), in collaboration with the Countryside Council for Wales (CCW), explored a variety of classification techniques based on a number of biological and environmental attributes. Using data collated from intensive surveys of 31 Welsh lakes, their aim was to 'develop a spatial-state classification for the training set lakes using biological data, and to evaluate the scheme with specific reference to biological variation within the individual species groups, environmental influences on this biological variation, and the stability of the classification structure'.

The CCW study was limited in terms of the relatively small dataset size (31 lakes), a bias towards larger standing waters and thus inadequate representation of the smaller water bodies (<2ha) which are common in Wales. However, it has great potential, methodologically, analytically and in terms of the collated data, to play a role in the development of a scheme for the WFD. Macrophyte and chemistry (including nutrient) data are largely compatible with data collated on the Northern Ireland Lake Survey (NILS) (see below). These two sources could therefore form the basis of a larger, and eventually UK-wide, dataset on which more rigorous analyses could be performed.

In a comparison of TWINSPAN classifications based on individual biological groups and on a dataset comprising all groups (the integrated group), it was shown that the aquatic macrophyte TWINSPAN scheme most closely resembled the integrated biological classification, thus highlighting the value of aquatic macrophytes in representing wider ecosystem variation. However, the aquatic macrophyte classification was relatively poor at resolving site groups amongst lower alkalinity sites compared, for example, to epilithic diatoms**.** Interestingly, the Palmer scheme showed relatively poor correspondence to both the integrated TWINSPAN scheme and the macrophyte TWINSPAN scheme. This perhaps stems from the Scottish bias of the former, with relatively few Welsh lakes being included. However it is also likely that the smaller number of lakes used in the Welsh study compared to Palmer *et al.* (1992) is an important factor.

The Norfolk Broads Scheme

Over the last forty years, many Norfolk broads have undergone a switch from a clear water state, dominated by a diverse submerged macrophyte flora, to a turbid, phytoplankton-dominated condition. These changes have been attributed to eutrophication of the waterways (Kennison *et al.* 1998).

To enable appropriate restoration measures to be taken, knowledge of the dynamics of macrophyte populations is required. The Broads are unusual in that macrophyte abundance have been regularly surveyed in a number of sites since 1993. These extensive data sets have enabled the development of a useful local classification scheme. Vegetation has been classified into communities or assemblages to assess a site's importance on a regional, national and international scale and to define target assemblages for use in habitat restoration programmes.

Kennison *et al.* (1998) selected 13 broads covering ranges of size, depth, chemistry (including nutrient concentrations (TP, and nitrate), riverine connectivity and restorative management. Each broad was visited at the same time each year, between 1983-1995. The submerged and floating vegetation was monitored along permanent transects using a grapnel. Weighted mean abundance scores per species per lake were clustered using TWINSPAN into three main assemblages, which were further divided into sub-types, described as Broad Macrophyte Types (BMTs). Ordination analysis was used to assess how macrophyte species composition and abundances changed through time. Examples of both dynamic and stable macrophyte assemblages were observed, some highlighting the success of recent restoration work.

Comparisons with the schemes of Palmer *et al.* (1992) and Rodwell (1995) proved difficult because they exclude key and dominant macrophytes found in the Broads. The BMT can however fit into the existing NVC framework.

This is only a small local dataset, and the scheme makes no attempt to directly relate the macrophyte assemblage to physio-chemical variables such as nutrient concentrations. Despite this, the Broads dataset is valuable as it incorporates both a spatial and a temporal dimension. The existence of long term datasets of biological and nutrient data provides great potential for the relationship between macrophytes and nutrients to be more fully explored. Lake managers in the Broads can use BMTs to assess the relative stability of macrophyte assemblages in individual lakes on a year to year basis.

The NVC Classification scheme

Aquatic macrophyte communities have also been defined within the National Vegetation Classification (NVC) (Rodwell, 1995). This covers a wide geographical area and describes aquatic, swamp, fen and mire vegetation across Great Britain (Rodwell, 1995). It describes 24 aquatic communities, some of which have sub-communities totalling 38 community types, from both standing and running water. Although the data used by the NVC was collected from sites across Britain, it is based on the NCC data set in which 50% of the data are from Scottish sites.

The United Kingdom Acid Waters Monitoring Network (UKAWMN)

The UKAWMN was established by the UK Department of the Environment in 1988 to determine the chemical and biological effects of decreasing acidic emissions on 22 acid sensitive lakes and streams freshwaters. In addition to chemical monitoring, annual surveys are conducted for salmonids, aquatic benthic invertebrates, epilithic diatoms and aquatic macrophytes (Patrick, 1991). Although only designed to detect the effects of atmospheric deposition – the sites tend to be in small upland catchments with minimal local anthropogenic disturbance - the lacustrine macrophyte work is a rare example of long-term monitoring for this biological type. The lacustrine macrophyte sampling protocols consist of bi-annual:

- Ekmann grab sampling from a boat on fixed transects, running perpendicular to the shore-line;
- Grapnel trawls across the lake basin
- Shoreline inspection

The data is sensitive to temporal changes in the relative abundance of taxa and changes with water depth in absolute cover of individual species. There is little evidence of change in UKAWMN lakes to date, but the dataset now provides a valuable record of oligotrophic macrophytes communities over the past decade.

The Environmental Change Network (ECN)

Partly drawing from UKAWMN protocols, the ECN lacustrine macrophyte protocol (Sykes *et al.* 1999) also involves depth-transects and shoreline inspection. In addition, emphasis is placed on fixed shoreline transects, i.e. species cover estimates across the shallow littoral zone. Two to three yearly monitoring is now underway at a number of ECN freshwater lakes.

Wider Europe

Finland

Traditionally Finnish aquatic macrophyte classifications have been based on two a priori schemes: (1) limnological, (2) the national botanical lake system (Maristo 1941). Lakes have been classified into 6 groups based on the physico-chemical parameters of the water as well as the physiognomy of the vegetation as shown in Table 3.8.

Table 3.8 The Finnish aquatic macrophyte classification schemes

Lake Type	Botanical lake type
Hypertrophic	*Lemna-Nuphar*
Eutrophic	*Typha-Alisma*
Clear-water-meso-eutrophic	Mesotrophic elodeid
Mesotrophic	*Equisetum-Phragmites*
Clear-water oligotrophic	Myriophyllum
Brown-water oligotrophic	*Equisetum-Nuphar*
	Nuphar
	Nuphar (clear water variant)

Sweden

In 1994, the Swedish Environmental Protection Agency set up a comprehensive evaluating system for a variety of ecosystems, termed 'Environmental Quality Criteria' (Swedish Environment Protection Agency 2000). The system aimed to provide a way of interpreting and evaluating scientific environmental data, which could be easily understood, while criteria could be harmonised with those from similar schemes worldwide.

There were two aspects to the assessment; an appraisal of the state of the environment per se in terms of the quality of the ecosystem, and an appraisal of the extent to which the recorded state deviates from a 'comparative' value'. The comparative value represents an estimate of a 'natural' state. (equating to a 'reference condition' in the WFD). The results of both appraisals are expressed on a scale of 1-5. Aquatic environments were to be assessed in terms of a variety of physical and chemical factors including nutrients/eutrophication, and by biological condition - including aquatic plants.

Current trophic status was determined using total phosphorus, total nitrogen data and the phosphorus/nitrogen ratio (Table 3.9 and 3.10). The classifications of these parameters are based on even percentile distributions and are derived independently from each other.

Table 3.9 Current condition assessment for concentration of total phosphorus and total nitrogen in lakes (μg/l)

Class	Description	TP Concentration May-October	TN Concentration May-October
1	Low concentrations (oligotrophic)	≤ 12.5	≤ 300
2	Moderately high concentrations (mesotrophic)	12.5 – 25	300-625
3	High concentrations (eutrophic)	25 – 50	625-1.250
4	Very high concentrations (eutrophic)	50 – 100	1.250-5.000
5	Extremely high concentrations (hyper-eutrophic)	>100	>5.000

Table 3.10 Current condition assessment for ratio of total nitrogen/total phosphorus in lakes

Class	Description	Ratio June - September
1	Nitrogen surplus	≥ 30
2	Nitrogen – phosphorus balance	15 – 30
3	Moderate nitrogen deficit	10 – 15
4	Large nitrogen deficit	5 - 10
5	Extreme nitrogen deficit	< 5

Two methods of assessment of floating-leaved and submergent plants were used; the TRS (according to Palmer *et al.* 1992) and one based on the number of species present (Table 3.11).

Table 3.11 Current conditions: number of species of submerged and floating-leaved plants

Class	Description	Total number of species
1	Very high numbers of species	> 18
2	High numbers of species	14 – 18
3	Moderate number of species	9 – 14
4	Relatively few species	4 – 9
5	Very few species	≤ 4

The boundaries for classes 1 – 5 comprised the 75th, 50th, 25th and 10th percentiles, respectively, in the background data. Further to this classification the predominant type of vegetation were defined as follows: A – Isoetid, B – Elodeid, C – Floating-leaved, D – Emergent.

The current chemical and biological state was then related to reference values, based on historical studies or unaffected sites in the same geographical region. For nutrients, deviation from reference value was derived by placing lakes into one of five classes based on the ratio of current to reference value TP concentrations (Table 3.12).

Table 3.12 Deviation from reference value, concentration of TP in lakes

Class	Description	Recorded concentration/reference value
1	No or insignificant deviation	≤ 1.5
2	Significant deviation	1.5 – 2.0
3	Large deviation	2.0 –3.0
4	Very large deviation	3.0 – 6.0
5	Extreme deviation	> 6.0

Reference conditions for macrophytes were stratified according to latitude, lake area, and altitude (Table 3.13).

Table 3.13 Reference values for number of species and indicator ratios for submerged and floating-leaved plants in lakes of various sizes and locations

Lake area km^2	Northern Sweden		Southern Sweden	
	No of species	Indicator ratio	No of species	Indicator ratio
> 60 m above sea level				
> 0.1	3 – 5	5.5	4 – 12	7.4
0.1 – 1	9 – 14	6.5	11 – 16	8.1
1 – 10	10 – 18	6.3	15 – 23	8.0
≥10	17 – 21	6.5	17 – 25	8.0
60 – 199m above sea level				
> 0.1	-	-	5 – 11	6.9
0.1 – 1	5 – 13	6.3	10 – 17	7.0
1 – 10	10 – 16	5.8	17 – 25	6.5
≥10	13 – 20	6.3	>17	6.6
>200 m above sea level				
0.1 – 1	4 – 11	6.3	8 – 16	7.2
1 – 10	8 – 15	6.2	15 – 25	6.2
≥10	13 – 17	5.9	-	-

For aquatic plants, deviation from reference condition was determined by comparing the current condition with the reference condition as shown in Table 3.14.

Table 3.14 Deviation from reference values using indicator ratio and number of species for submerged and floating-leaved plants

Class	Description	Number of species and indicator ratio
1	No or insignificant deviation	The number of species and indicator ratio are equal to the reference value
2	Slight deviation	The number of species or indicator ratio deviate from the reference value
3	Significant deviation	The number of species and indicator ratio deviate from the reference value
4	Large deviation	The number of species and indicator ratio deviate from the reference value; one of the measures deviates greatly
5	Very large deviation	Mass presence of 1 – 3 species of elodeids/free floating or emergent plants.

This scheme incorporates key features of relevance to the WFD as it defines conditions for both nutrient loadings and macrophytes at high to low ecological status, and it recognises and defines deviations from the high reference state. Furthermore, the scheme allows the growth forms of macrophytes to be incorporated which is important given that these can be influenced by changes in trophic state. The scheme is thorough and is based on empirical evidence, which is then transformed into a simple scale for application and understanding. The scheme does not, however, directly relate the aquatic plant communities to nutrient concentration data.

USA and Canada

Historically, lakes in north America have been classified using a host of classical indicators including open-water nutrient concentrations, algal biomass expressed as chlorophyll *a* concentration, and water transparency as measured by using a secchi disc (e.g. Likens, 1975, Carlson, 1977, Forsberg and Ryding, 1980, Kratzer and Brezonik, 1981).

Recently, attempts have been made to integrate nutrient concentrations in the water column with those in the plants themselves. Canfield *et al.* (1984) used this “Potential water column nutrient concentration” (WCP) in conjunction with existing classification schemes (e.g. Carlson 1977) to classify lakes. They concluded that the WCP values were substantially higher than measured open water concentrations with 20 – 96 % of the phosphorus associated with submersed macrophytes. The extent of the effect of the macrophytes on the WCP values depended on the quantity of macrophytes relative to the total lake volume. One study lake had widespread submerged macrophyte growth and an open water TP concentration of 10 mg m^{-3}, which, based on traditional classification schemes (e.g. Likens 1975, Forsberg and Ryding 1980), would be classified as oligotrophic. However, using the WCP, TP was estimated to be 80 mg m^{-3}, a level associated with eutrophic conditions and much closer to most other lakes in the same physiographic region. Macrophytes did not have a significant effect on the assessment of trophic state when macrophytes contributed less than 25% of the phosphorus in the water column, and the mean macrophyte concentration in the water is less than 1g wt m^{-3}. WCP estimates are therefore probably only useful in the differentiation of shallow macrophyte-dominated lakes. Unfortunately, the estimation of WCP values is a labour intensive process and requires much greater effort than current approaches for classifying lakes.

Lake classification is an important part of the overall U.S. strategy for developing lake management programs and is seen as a vehicle for rapid prioritisation of lakes for protection and restoration. USEPA have developed a multiple attribute (or multimetric) approach to take account of pollution tolerance, diversity and ecological functions. This approach includes a broad range of measurements which, in combination, form an index to represent overall biological condition.

The current USEPA lake biological multimetric assessment includes up to seven biological assemblages: planktonic algae, attached algae, sediment diatoms, aquatic plants, bottom-dwelling invertebrates, fish and planktonic animals. Habitat scores are also incorporated and include watershed, nearshore zone, water chemistry and sediment characteristics. Lake condition is assessed using additive indices that integrate both the habitat and the biological scores. Lake complexity is ultimately reduced to three habitat and three biological scores, providing a simple scheme with which to guide management options.

Biological assessment is based on a tiered system with each tier involving increasing sampling effort. This allows the bio-assessment to be customised to the users needs, questions and available resources. The tiers, ranging from a simple trophic state assessment to a detailed biosurvey are shown in Table 3.15.

Table 3.15 The tier system in the biological assessment (USEPA 1998)

Tier 1A	Trophic State Indices and macrophyte cover. Sampled once during index period. Inference limited to regional assessment.
Tier 1B	Trophic State indices and macrophyte cover. Sampled repeatedly during growing season.
Tier 2A	Tier (1A or 1B) plus two or more integrating biological assemblages: macrophytes, macro-invertebrates, sedimented diatoms, fish. Sampled once during index period.
Tier 2B	Tier 1B plus two or more short-term biological assemblages: phytoplankton, zooplankton, periphyton. Sampled repeatedly during growing season.

Importantly, macrophytes are the selected biological group for trophic assessment in the early tiers. Quantification is based on cover estimates and the identification of dominant species (Tier 1), while later Tiers require rake sampling with the identification of species and estimates of biomass.

The USEPA's multimetric index involves a comparison of a metric with a reference distribution of values followed by a decision as to whether the value is within the expected range. For macrophytes this is based on the criteria in Table 3.16.

Table 3.16 Macrophyte metrics and their response to stress

Metric	**Response to Stress**
Total vegetated area (% of littoral)	Substantially more or less than reference
% exotic or weedy species	More than reference
No. of exotic species	High
Density/biomass in vegetated areas.	Substantially more or less than reference
No. of taxa	Low
% dominant species (by weight).	High
Maximum depth of plant growth	Reduced under enrichment, deeper under acidification

Each metric is then given an ordinal score of 5 (good), 3 (fair) or 1(poor) based on its similarity to the reference conditions. The index includes the sum of all metric scores and the total index value for a site is compared to the distribution of the index values in reference conditions. The habitat component scores may give important clues to the causes of impairments reflected in biological indices rated fair or poor. Those habitat variables that are significantly different from the reference conditions are identified as possible causes of the degradation and may require further investigation.

This multimetric method is valuable in that sensitivity to pollutant stress is likely to vary between aquatic communities. Targeting multiple species and assemblages enables the detection of a broad range of stresses and reflects the condition of the whole ecosystem. The USEPA approach addresses many issues of relevance to the WFD and provides useful guidelines in setting lake reference conditions. However, this scheme does not directly relate environmental variables to macrophytes. The multimetric index functions as an overall indicator of biological condition, but whilst the habitat scores may, for example, suggest that enrichment is the possible cause of change, the actual cause of degradation cannot be quantified.

3.4.4 Summary and Recommendations for Phase II

- The general relationship between “trophic” status and macrophyte community structure has been recognised for decades.

- As far as we are aware, all of the major studies concerning the influence of water chemistry on aquatic macrophyte composition are based on spatial state relationships.

- Traditional lake classification approaches compartmentalise lake types according to one or a combination of physical, chemical and biological characteristics. In many of these, the relationship of resulting groups is related to a trophic scale, ranging from low to high alkalinity and nutrients. However, recent statistical studies demonstrate that rather than forming discreet clusters, lakes types tend to occupy the continuum of a “trophic” gradient. This suggests that the boundaries within any classification scheme will, to some extent, be arbitrary.

- Recently, several ordination-based studies have attempted to directly relate aquatic macrophyte assemblages to specific physical and chemical factors. In some cases total phosphorus and nitrogen species have not been measured and variables such as conductivity have been used as a surrogate for nutrient availability. Generally, these studies demonstrate strong co-variance in explanatory variables (including alkalinity, conductivity, pH, and phosphorus and nitrate when available,) along the principle axis of variation in species composition. Species composition of other biological groups is also particularly sensitive to this gradient.

- Although much variation is species assemblages is attributed to “trophic state” the specific influence of N or P is rarely assessed. To some extent this results from the “Forward Selection” approach often adopted in multivariate analysis. Variables are usually chosen for explanatory models in order of their explanatory power, and subsequent variables are chosen to explain residual variation. Dissolved inorganic carbon availability, as indicated by alkalinity, is physiologically crucial and it is of no surprise that this variable is often the first to be selected. Since alkalinity is often closely correlated with nutrient concentration, the explanatory power of the latter in subsequent analysis is diminished and in some cases is not considered further. Similarly, chlorophyll *a* concentration, which can be considered a summary variable for nutrient concentration, and in addition may represent important controls on the aquatic light climate, is also often selected in preference to N or P.

- Using Generalised Additive Models, Heegard *et al.* (2001) have shown very clear negative responses by a number of species to increasing total P and N concentration in relatively oligotrophic situations. This approach has considerable potential for defining nutrient “restoration targets” for enriched, naturally oligotrophic lakes. However species optima and tolerances for the majority of species were poorly defined along nutrient gradients. This suggests that simple predictive models may be of limited use in the assessment of more naturally alkaline or nutrient rich lakes by the WFD.

- Recent work has also highlighted the ecological complexity of aquatic systems, particularly as regards shallow lakes. Rather than exhibiting linear responses to changing chemistry, macrophyte floras may undergo abrupt transition (i.e threshold responses), as a result, for example, of increased competition for light by phytoplankton, triggered by a decline in grazing.

- Most studies rely on spot data for a single site visit. However nutrient concentrations often show significant seasonal variability.

- Few studies take into account the potentially large reservoir of nutrients locked within lake sediments and within the aquatic plant biomass itself (see Canfield *et al.*, 1984).

- Spatial state classifications have underpinned most attempts to understand or predict the effect of changing nutrient concentrations at specific sites. However, we can find few examples of temporal studies in which these models have been thoroughly tested. Given points made above there are two clear weaknesses in the application of existing studies to the specific quantification of macrophyte-nutrient relationships: First, it is often unclear to what extent species responses are directly driven by nutrients, rather than by other co-varying chemical, physical or biological parameters. Secondly, and particularly regarding shallow lakes, it is unclear how appropriate linear approaches are for modelling temporal change at specific sites.

- Sweden and the USEPA provide the best examples of schemes for defining reference conditions for nutrients and also for macrophyte communities. However, there is little attempt to directly relate the two lake properties.

- Given the paucity of historical data in most circumstances, we accept that a spatial approach in the attempt to quantify macrophyte nutrient relationships is necessary. However it would appear that there is currently insufficient UK data to allow a suitably rigorous assessment of nutrient effects for the full region. Only the Northern Ireland lake survey provides data on aquatic macrophyte populations and high quality nutrient chemistry at a sufficient number of sites to allow the assessment of nutrient effects within a particular “lake type” or the statistical “partialling out” of co-variables. There is also insufficient methodological compatibility to allow amalgamation of data from individual projects. We are unclear about the methodological compatibility of most data derived from other studies in Europe and Scandinavia but in most cases the value of such studies will also be limited in terms of compatibility of eco-regions.

- It is clear that further work is required in order to more clearly elucidate the importance of nutrient chemistry to aquatic macrophyte communities within the UK and to better understand the extent to which temporal change is likely to follow spatial change. To these ends we advocate a substantial geographical expansion of lake surveys, compatible with the Northern Ireland Lake Survey and the CCW study. Ideally these would include thorough assessment of macrophyte populations, employing a boat and grab/grapnel techniques and shoreline surveys. Water chemistry should be assessed on a quarterly basis over at least one year and should include on-site freezing of samples for nutrients and chlorophyll *a*.

- We also recommend that wherever high quality historical records for macrophyte assemblages are available at specific sites, an attempt is made to relate these to historical nutrient concentrations. In some cases in should be possible to use palaeo-diatom techniques to infer historical change in TP. We believe this approach is crucial for the validation of any spatially derived model for use in the assessment of temporal change.

3.5 Benthic Invertebrates

Benthic invertebrates are a diverse and generally abundant group with a wide range of environmental tolerances and preferences (Pinder 1999; Rosenberg and Resh 1993) which can act as long-term indicators of environmental quality (Cairns and Pratt 1993). In the context of lakes benthic invertebrates can be defined as largely sedentary freshwater organisms which live in or on the bottom substrates for at least part of their relatively long life cycles (Rosenberg and Resh 1993). They play an essential role in key processes of lakes particularly in terms of the food chain, productivity, nutrient cycling and decomposition (Reice and Wohlenberg 1993). Therefore, in principle any environmental changes in lakes, for example, in nutrient concentrations, should be reflected by changes in the structure of the benthic invertebrate community thereby indicating the ecological health and functionality of the ecosystem. However, methods to monitor water quality in lakes using benthic invertebrates are much less established compared to rivers (Pinder 1999); also see section 2.5. This disparity in sampling effort is particularly marked in the UK where there is no national programme set up for monitoring lake water quality using benthic invertebrates apart from a limited number of sites which are sampled under the auspices of the Acid Waters Monitoring and Environmental Change Networks. However, under Annex V of the WFD the benthic invertebrate fauna of lakes will, in future, be an important biological quality element in the assessment of the ecological status of lakes. In particular the WFD outlines the following criteria which need to be related to type-specific, undisturbed conditions in lakes:

- Composition and abundance of benthic invertebrate fauna
- Ratio of sensitive invertebrate taxa to insensitive invertebrate taxa
- Diversity of invertebrate taxa

3.5.1 Composition and Abundance

Influence of factors other than nutients

Historically approaches to establishing relationships between benthic invertebrates and nutrient concentrations have concentrated on studying the profundal fauna of deep stratifying lakes (>3m depth). In these deeper waters, below the photic and wave action zones, environmental conditions tend to be relatively uniform and predictable. Characteristically, the substrate is dominated by silt, with reduced physical fluctuations, predation pressure and biological diversity (Brodersen and Lindegaard 1999), limiting the benthos to relatively few animals, mainly dipteran (fly) larvae of the family Chironomidae (midges), oligochaetes (worms) and sphaeriid mussels, although the numbers of individuals and production may be depth limited (Brinkhurst 1974). In contrast to the deep profundal, a much greater variety of invertebrates are found in the more complex and diverse habitat conditions of smaller shallow (<3m depth), non-stratified lakes or littoral areas of deeper lakes (Brodersen and Lindegaard 1999). This habitat complexity makes it difficult to find a single pattern of distribution of total numbers of benthic organisms which can truly characterise the condition of a lake (Brinkhurst 1974). The littoral benthic invertebrate fauna may be influenced by a whole range of abiotic and biotic factors such as the re-suspension of substrate, decomposition rates, wind exposure, ambient temperatures, and variations on oxygen regime as well as by lake morphometry (Brinkhurst 1974). As is the case in some deep waters it may often be that local events at substrate/water interface are more important than conditions in the water column (Brinkhurst 1974; Little *et al.* 2000).

Classification and monitoring schemes

The Chironomidae are probably the best known taxonomic group in the profundal benthos for being useful trophic state indicators. Chironomidae larvae are well suited for this role as they have a high species richness compared to other benthic invertebrate groups, they occur over the whole spectrum of nutrient conditions and, because individual species have highly specific environmental tolerances, they change species composition in tandem with changing lake trophic status (Rosenberg 1992).

Early lake trophic classification systems, focussed primarily on profundal chironomid taxa that indicated an oligotrophic to eutrophic/dystrophic gradient in the hypolimnion (Naumann 1929; Thienemann 1921; Thienemann 1922). The concept of these schemes was similar to the Saprobien system in rivers in which the occurrence of indicator species were related to the degree of organic pollution (Cairns and Pratt 1993; Kolkwitz and Marsson 1909). These lake classification schemes were based on the premise that organic enrichment or eutrophication usually results in changes in the substratum (through increased sedimentation of organic matter) and low dissolved oxygen concentrations (at high concentrations of enrichment) leading to the disappearance of intolerant taxa. For example, Thienemann (1921) showed that in oligotrophic waters (with an oxygenated hypolimnion) the profundal chironomid fauna was dominated by *Tanytarsus* species while in eutrophic lakes (with a deoxygenated hypolimnion) the chironomid community was dominated by *Chironomus* species which could tolerate anoxic conditions. These original lake classification schemes were subsequently modified by (Brundin 1958; Brundin 1949; Saether 1979; Saether 1975; Wiederholm 1980) to produce a series of lake types based on the relationship of the profundal chironomid assemblages to the productivity of the overlying water column (Table 3.17).

Table 3.17 Classification of northern temperate lakes based on profundal chironomid fauna (modified from Lindegaard 1995)

Lake type/trophic level	**Indicator chironomids**
Ultraoligotrophic	*Heterotrissocladius subpilosus*
Oligotrophic	*Tanytarsus lugens* or *Heterotrissocladius grimshawi* or *H. scutellatus*
Mesotrophic	*Stictochironomus rosenchoeldi* or *Sergentina coracina*
Moderately eutrophic	*Chironomus anthracinus*
Strongly eutrophic	*Chironomus plumosus*
Dystrophic	*Chironomus tenuistylus* or *Zalutschia zalutschicola*

Saether's (1979) lake trophic classification scheme identified 15 lake types (six oligotrophic, three mesotrophic and 6 eutrophic) based on differing profundal chironomid communities. These communities were shown to be fairly well correlated with trophic conditions as measured by either total phosphorus/mean lake depth or by chlorophyll *a*/mean depth in many northern temperate waters (Saether 1979; Wiederholm 1980). The use of the phosphorus and chlorophyll *a* divided by lake mean depth helped to avoid problems in some deep lakes where the correlation between trophic state and the profundal communities was found to be low. In these deep lakes, with a large hypolimnion, it was found that oligotrophic conditions could be maintained in the profundal zone even though the phytoplankton production was sufficient to cause eutrophic conditions in more shallow systems(Lindegaard 1995). This lake trophic classification work was utilised to develop a Benthic Quality Index (BQI) for assessing the water quality status of Palaearctic lakes with a mixture of indicator species(Wiederholm 1976; Wiederholm 1980). The BQI was based on linking 5 groups of indicator species of chironomid with a range of different trophic conditions. BQI values ranged from 1 in eutrophic lakes which were dominated by *Chironomus plumsosus* to 5 in oligotrophic lakes where *Heterotrissocladius subpilosus* larvae predominated. If there were no indicator species present in the profundal then a value of 0 was scored, i.e. would represent a hypereutrophic lake (Wiederholm 1980). Later studies have shown the usefulness of these methods for lake classification based on the profundal chironomid fauna (Gerstmeier 1989; Johnson 1989; Kansanen, Aho and Paasivirta 1984; Kansanen, Paasivirta and Vayrynen 1990; Merilainen 1987).

In these lake trophic classification models oxygen concentrations and food availability (as indicated by total phosphorus, chlorophyll *a* and algal biovolume) were considered to be the most important community structuring factors linking chironomid communities with lake trophic state (e.g Brodin 1982; Heinis and Davids 1993; Saether 1979). In northern temperate waters these models have proved to be useful (e.g. (Kansanen *et al.* 1984; Lindegaard 1995)) as long as oxygen concentrations in the profundal zone remained high enough to support the chironomid communities (Brodersen and Lindegaard 1999). As for food availability chironomids are totally reliant on food derived from precipitating organic matter produced in the photic zone except in relatively shallow oligotrophic lakes where there may be some phytobenthos production (Lindegaard 1995). The chironomids typical of oligotrophic lakes are often free-living and thus are able to move around to forage for scare food resources (Wiederholm 1984). In eutrophic lakes the chironomids tend to be more sedentary generally occupying fixed tubes from which they can feed by browsing and/or by filtration exploiting the more abundant food resources (Lindegaard 1995). As well as behavioural adaptations these eutrophic species may also have the physiological adaptations, such as possession of haemoglobin (helping to maintain their oxygen uptake despite decreasing ambient oxygen concentrations), to allow them to inhabit more nutrient-rich waters (Heinis and Davids 1993; Pinder 1999; Walker 1993).

The other major component of the profundal benthic community, the oligochaetes, has also been investigated as indicators of trophic state of deep lakes (e.g. Millbrink 1983; Millbrink 1979) indicated that oligochaete communities react to increasing eutrophication in a predictable way by increasing in total abundance, by some species declining in abundance while other species become very numerous. A simplified indicator system similar to the BQI used for chironomids was developed for oligochaetes (Ahl and Wiederholm 1977). The oligochaete index was based on five oligochaete species typical of lakes of varying trophic status as represented by total phosphorus divided by mean depth. Although Brinkhurst (1974) suggested that there was no general relationship between oligochaete abundance and trophic status, Wiederholm (1980) did demonstrate a strong relationship between oligochaete abundance (adjusted for depth) and trophic state as expressed as chlorophyll *a* for the Swedish Great Lakes – the relationship between chironomid abundance and trophic state was not so clear-cut. The eutrophication of Lake Geneva was described by the calculation of three indices of trophy based on the structure of the tubificid and lumbriculid worm communities(Lang and Lang-Dobler 1980). With increased organic sedimentation certain species increased or decreased such that the oligochaete community could be classified into three different species groups, i.e. species which were respectively dominant in oligotrophic, mesotrophic and eutrophic conditions. Thus the relative proportion of these three oligochaete species groups could used to indicate to accurately describe the lake trophic state (Lang and Lang-Dobler 1980). Lang, in further studies on the recovery of Lake Geneva from eutrophication, showed that the mean relative abundance of oligochaete species characteristic of oligotrophic lakes could be related to lake trophic state as measured by total phosphorus and/or primary production (Lang 1985; Lang and Reymond 1992). However, this predicted relationship may be obscured at sample locations where there as is excessive organic deposition or persistence of low oxygen concentrations (Lang 1998; Lang and Reymond 1996).

The lake classification and monitoring schemes outlined above were designed for the more uniform and predictable conditions of deep lakes and are not suitable for the more common shallow non-stratified lakes (as well as the littoral areas of deeper water bodies). Because of the more complex and diverse habitat conditions existing in these systems there have been only a relatively few studies which have tried to link the composition and abundance of the littoral benthic invertebrate communities with lake trophic status (e.g. Devai and Moldovan 1983; Kansanen *et al.* 1984). Chironomid species considered to be characteristic of littoral and sub-littoral areas of Nearctic and Palaearctic lakes have been listed (Saether 1979; Saether 1975). However, the littoral species Saether identified as being typical of meso- and eutrophic waters are not universally regarded as being particularly good indicators as they are known to occur over a broad range of trophic conditions (Lindegaard 1995).The fauna of the upper stony littoral of 39 lowland Danish lakes were studied by using multi-variate methods (Brodersen, Dall and Lindegaard 1998). This Danish study clearly demonstrated the difficulties in classifying lakes by their littoral invertebrates because of the highly complex factors affecting their distribution. Mean lake depth was found to be a more important variable than the variables used to indicate trophic state, i.e. chlorophyll *a* and total phosphorus concentrations. However, the use of weighted averaging models of estimated species optima and tolerances to chlorophyll *a* for the 126 invertebrate taxa was found to work reasonably well in indicating the trophic status of these Danish lakes most of which were either eutrophic or hypereutrophic.

One additional lake classification and monitoring method (first suggested by Thienemann 1910) that has attempted to utilise the composition and abundance of benthic invertebrates as an biological indicator of trophic status has been to try and use chironomid pupal exuviae (or skins), which collect on the leeward shore of lakes after pupae come to the surface of a lake to emerge (Ruse, 2000). Chironomid pupal exuviae have also been used successfully to monitor the organic enrichment in streams (e.g. (Wilson and McGill 1977)). The theory is that these pupal exuviae, if collected, represent a passively accumulated sample (integrating emergence variation in time and space), of species present in the lake which in turn reflect the conditions in the water column and sediments. However, this technique has the problem of needing a high degree of taxonomic expertise to analyse exuviae samples plus there are sampling difficulties associated with the narrow time period during which chironomid species emerge although Ruse (2000) suggests that four samples collected between April and October is sufficient to provide sufficient coverage of species emergence in temperate lakes.

3.5.2 Ratio of sensitive to insensitive invertebrate taxa

Linking the profundal benthic structure with lake trophic state has been investigated by looking at the proportion of oligochaetes and sedentary chironomids (i.e. those species living in tubes in sediment or at the sediment surface) in the benthos (Wiederholm 1980). The development of a ratio of oligochaetes to oligochaetes plus sedentary chironomids based on numbers of individuals is based on the fact that tolerant oligochaetes (e.g. *Limnodrilus hoffmeisteri*) increase in abundance relative to sediment-dwelling chironomids with increasing organic enrichment. Using this measure a strong relationship between oligochaete to chironomid ratio (adjusted for depth) and lake trophic state as measured by chlorophyll *a* concentration was demonstrated although this linear correlation didn't work for deep eutrophic lakes (Wiederholm 1980). Although the applicability of the ratio is still unclear it is still considered to have potential to be utilised as an early warning system for impending eutrophication (Lindegaard 1995).

3.5.3 Diversity of invertebrate taxa

Measures of diversity have been little used in studies of the profundal benthos primarily because of difficulty in identifying taxa and because profundal communities are difficult to characterise using diversity indices as they tend to be dominated by comparatively few species (Wiederholm 1980). Wiederholm (1980) suggested that species richness or the total number of taxa is a much better measure of the diversity of the benthos by demonstrating that a strong correlation could be made between species richness (once adjusted for sample depth) and the trophic state (as indicated by chlorophyll *a* concentrations) of the Swedish Great Lakes. However, Wiederholm (1980) considered that this relationship would not work for either deep highly eutrophic lakes or for very shallow oligotrophic lakes. Nevertheless, Wiederholm (1980) maintained that it should be theoretically possible to produce a general relationship between species richness and lake fertility.

3.5.4 Summary

Despite the promise of the above biological indicator approaches in linking changes in the profundal benthos (in particular the chironomid assemblages) to changes in the lake trophic state it is unlikely that they will be used widely in the UK for monitoring ecological quality of deeper stratifying waters. Potential difficulties, associated with the widespread adoption of the above approaches, include that the indicator species concept which underpins these schemes and which were primarily developed for northern Europe, may not be appropriate for species occurring in the UK. There also may be problems of in distinguishing natural variability in species composition from changes caused by anthropogenic nutrient enrichment. For example, in many Scottish lakes natural allochthonous matter may supplement that derived from phytoplankton productivity thereby confusing the relationship between open water nutrient concentrations and the profundal benthos (Fozzard *et al.* 1999). In addition the distinctions between different lakes, as indicated by the composition and abundance of chironomid and oligochaetes, are relatively subtle and requires a greater degree of taxonomic sophistication than is usually needed for comparable river invertebrate surveys . Sampling would also require the use of a boat and the process to sort samples and identify chironomid and oligochaete samples to species level would be very time-consuming (Moss, Johnes and Phillips 1996).

Although there are practical advantages in sampling the littoral benthic communties, in contrast to the profundal benthos, it is recognised that before they can be utilised to indicate ecological status it is imperative to firstly develop lake typologies based on the littoral benthos (Lindegaard 1995). However, as the United States Environment Protection Agency (USEPA) has concluded, because the littoral benthic community structure is so influenced by so many factors any changes in the structure of the benthos can not be correlated with changes in nutrient concentrations unless a great deal is known about the dynamics of the biota in relation to eutrophication (USEPA 2000). Hence, the USEPA, in its review of nutrient criteria for lakes and reservoirs, decided that open-water measures, i.e. total phosphorus, total nitrogen, chlorophyll *a* and secchi disc depth, were much better indicators of trophic change in lakes rather than littoral benthic invertebrates (USEPA 2000).

Given the above disadvantages in using profundal benthos and the lack of progress in linking lake typologies to the littoral benthic communties it would appear that the chironomid pupal exuviae scheme, although it has itself some disadvantages, offers the most potential, both in terms of its practicality and sensitivity to changes in nutrient status, for determining biology nutrient relationships in UK lakes using benthic invertebrates.

3.5.5 Recommendations for Phase II

In the UK the American multimetric approach to ecological assessment (Barbour and Yoder 2000) has been combined with the site reference approach (as in RIVPACS) to develop the Predictive System for Multimetrics (PSYM) biological methodology for assessing ponds and canals (Biggs *et al.* 2000). The multimetric approach defines an array of measurements (or metrics), each of which represents a measurable characteristic of the biological assemblage (e.g. benthic invertebrates) that changes in a predictable way with increased or decreased environmental factors. The individual metric values are integrated into a single measure or multimetric index which summarises the overall ecological condition of the waterbody, i.e. it reduces the complexity of a lake. In the case of UK ponds the most effective invertebrate metrics for measuring environmental degradation were found to be Average Score per Taxon (ASPT), number of dragonfly and alderfly families, and number of beetle families. Although there is not a complete understanding of how the metrics respond, quantitatively or qualitatively to perturbation in general or to particular stressors, examination of individual metrics can help to indicate the observed degradation in water quality from, for example, eutrophication (Biggs *et al.* 2000). The development of a similar PSYM methodology for lakes is an obvious area for future research but which so far been hampered by lack of agreement on what standard sampling technique to adopt for this type of waterbody (Biggs *et al.* 2000). In the context of developing a multi-metric index to indicate nutrient pollution in lakes future research should probably concentrate on modifying or calibrating the existing chironomid pupal exuviae classification and monitoring scheme. On the basis of this review chironomid pupal exuviae appear to be the most sensitive and practical benthic invertebrate indicator of nutrient status in lakes currently available for potential usage in the UK.

3.6 Fish

As with fish in running waters (Section 2.6), in developing techniques for assessing reference conditions for standing waters in Great Britain it is impossible to ignore the effects of history and geomorphology (Maitland 1990). The impact of the last ice age can still be felt and there is still somewhat of a north/south divide in the distribution of species in Britain - fish communities in the north and on islands having many fewer species than comparable systems in the south. Thus, for example, typical northern standing waters, such as Lochs Stack, Shin and Calder have only six or seven native species whilst Lochs Ken, Lomond and St Mary's Lochs in the south have nine (Ken) to fifteen (Lomond). In the south of England, several additional native species (e.g. Silver Bream *Abramis bjoerkna*) are available to increase the community complexity. Most of the northern communities have remained stable for thousands of years, whereas those in the south have changed substantially over the last two centuries. Each new introduction can produce instability in its new community, as studies of Loch Lomond have shown in recent years (Maitland 1972, Adams and Maitland 2000).

Consequently, as discussed above , in the south the high range of species available allows changes in fish communities from, say salmond to cyprinid, as nutrients increase (see below). In the north, however, where there are virtually no cyprinids, such a succession is impossible and so sensitive species are gradually eliminated, with no replacement by others. An example may be Loch Leven where, even after decades of eutrophic conditions (Bailey-Watts and Maitland 1984) Brown Trout *Salmo trutta* and Perch *Perca fluviatilis* persisted, in spite of some kills due to deoxygenation, because there were no large cyprinid species present to occupy the habitat.

Secondly, the nature of the lake habitat concerned and the ability of different species to survive there is an essential first consideration. Thus, in rich lowland lakes as eutrophication increases, the succession from salmonids to percids to cyprinids is perfectly possible (Leach *et al.* 1977). However, in rocky, cold upland lakes, few percids of cyprinids can maintain themselves and so again sensitive species may be gradually eliminated, with no replacement by others. Finally, conditions in some upland rivers and lakes (e.g. in the Grampians) are so extreme that no fish are found there.

3.6.1 Composition and abundance

The fish community is thought to have a significant structuring role in lake ecosystems (Northcote 1979, Carpenter and Kitchell 1993, Lamarra 1995, Jeppesen *et al.* 2000). In general, fish yields increase as the productivity of a lake increases (Carline 1986). It is, however, the changes in species composition that cause the greatest concern, with the loss of species of economic (salmonids) and conservation (coregonids) value following enrichment (Maitland and Lyle 1991).

In general, the composition of fish communities in Great Britain is, as indicated above, related more to historical events and a complex range of environmental factors than concentrations of nutrients per se. Nevertheless, nutrient concentrations do play a part in determining community composition, usually indirectly through food chains or changing of water quality (e.g. lowering of oxygen content because of excessive algal or macrophyte growths). Oxygen depletion as dead algae and macrophytes decompose has resulted in substantial fish kills in some eutrophic lakes and the fish communities of these waters may now be extinct or limited to species characteristic of richer systems, such as Bream *Abramis brama* and Roach *Rutilus rutilus*. Because of the more obvious problems there, emphasis was given to the study of lakes during the intensive research on eutrophication during the 1970s and 1980s (Maitland and Hudspith 1974).

Case studies: composition

Ryder (1972) has described the limnology and fish composition of 14 large North American lakes as they were about 1800, prior to the adverse effects of man-made eutrophication, exploitation and introduction of alien species. For several lakes there was relatively little early information 'but this circumstance is not so serious, since an adequate description of the limnology and ichthyofauna as it was about 1800 A.D. probably can be provided by an interpretation of present knowledge.' (Ryder 1972). A similar review, of large oligotrophic lakes in Europe, was attempted by Toivonen (1972) based on old literature and fish catch information. In the UK, similar estimates can be made of status of fish populations in fresh waters prior to the three main activities of man which have had serious impacts on fresh water - the industrial revolution, the extensive use of fertilisers and the introduction and translocation of alien species.

Jeppesen *et al.* (2000) studied the changes in trophic structure, species richness and biodiversity along a phosphorus gradient in 71 mainly shallow lakes in Denmark. Fish species richness was unimodally related to total phosphorus, peaking at 0.1-0.4 gm PL^{-1}. At low nutrient concentrations, piscivorous fish (particularly perch, Perca fluviatilis) were abundant and the biomass ratio of piscivores to cyprinids was high. With increasing total phosphorus, the numbers of cyprinids increased and the ratio of piscivores to planktivorous and benthivorous fish was low, and cyprinid densities were low. The mean body weight of the dominant cyprinids, roach *Rutilus rutilus* and bream *Abramis brama*, however, decreased Fish are believed to have a significant structuring role in eutrophic lakes by controlling large planktivores. When this is not done there is an increase in cladoceran mean size and a decline in chlorophyll *a*. Experimental work by Cottingham and Schindler (2000) confirmed that large zooplankton which thrive in the absence of fish can buffer lakes against small nutrient inputs.

Thus, although changes in the nutrient input to lake systems usually has an effect on primary production the way in which changes are expressed at higher trophic levels depends very much on the nature of the invertebrate and fish communities present (Moss 1976, Andersson *et al.* 1988, Meijer *et al.* 1990a, 199b, Wright and Phillips 1992, Daldorph and Thomas 1995).

McComb and Davis (1993) showed that, in southwest Australia, nutrients in water bodies in coastal areas are derived mainly from fertiliser applications in catchments and rural industries as well as sewage. Consequences included increased macroalgal growth and phytoplankton blooms and subsequent changes to invertebrate and fish populations from both increased algal production and low oxygen concentrations.

Comparative analyses of fish populations in naturally acidic and circumneutral lakes in northern Wisconsin (Wiener 1983) revealed that water chemistry factors, believed to be pH and waterborne calcium concentration, strongly influenced the species composition and richness of fish communities in these lakes. Fish communities in the naturally acidic lakes contained significantly fewer species than those in the circumneutral lakes. Common as well as rare species occurred with lower frequency in the acidic lakes than in the circumneutral lakes. The differences in species composition and richness of fish communities between the two groups of lakes did not appear to be related to differences in physical habitat characteristics, past fish migrations, or productivity.

Feldberger Haussee in Germany is regarded as a naturally slightly eutrophic water body, but from 1950-70 extensive anthropogenic eutrophication took place resulting in massive algal blooms (Krienitz *et al.* 1996). A restoration programme in the 1980s diverted sewage and manipulated the fish community. This resulted in an increase in herbivorous zooplankton, a decrease in phytoplankton biomass and an increase in water clarity.

Case studies: abundance
The effects of nutrients on the biological structure of brackish and freshwater lakes in Denmark were compared by Jeppesen *et al.* (1994). Whereas the fish biomass rose with increasing phosphorus concentration in freshwater lakes, that of brackish lakes was markedly reduced at phosphorus concentrations above ca 0.4 mg P L^{-1}. The conclusion was that predation pressure on zooplankton is higher and algal grazing capacity lower in eutrophic brackish lakes than in comparable freshwater lakes and that these differences in trophic structure have major implications for any measures available to reduce the recovery period following a reduction in nutrient loading.

In British Columbia, over a period of 20 years, 20 lakes were fertilised weekly through the growing season with liquid fertilisers (Hyatt and Stockner 1985, Stockner and MacIsaac 1996). The lakes showed a positive response at all trophic levels with increased production of bacteria, phytoplankton and zooplankton, resulting in increased growth and survival of juvenile Sockeye Salmon *Oncorhynchus nerka*, resulting in larger adult returns worth 10-20 million dollars per year. It is concluded that 'The benefit:cost ratio of lake fertilisation is the best of currently available salmonid enhancement techniques.' (Stockner and MacIsaac 1996).

Stockner *et al.* (2000) have reviewed the causes and consequences for fisheries resources of cultural oligotrophication. They recommend all means of recycling phosphorus and a consideration of ways to reintroduce recycled nutrients in a balanced N:P ratio to some aquatic ecosystems, in a carefully controlled and ecologically sensitive way to restore sufficient fisheries production levels. They point out that 'If we continue to mismanage phosphorus sources and ignore the importance of nutrient balances for the maintenance of productive fisheries, then choices soon will have to be made between having aesthetically clear fresh waters but unproductive fisheries, or productive fisheries in " greener" lakes and streams'.

Kootenay Lake in British Columbia was significantly affected by nutrients entering from a fertiliser plant constructed in the early 1950s (Northcote 1972). Large loadings of phosphorus entered the Kootenay River drainage causing massive blue green algal blooms in the 1950s, 1960s and early 1970s. Pollution abatement measures and subsequent closure of the plant reduced phosphorus loadings to the lake to less than historic concentrations by 1988, resulting in oligotrophication (Edmonson and Lehman 1981, Lang and Reymond 1992). Algal productivity declined sharply and this coincided with a major decline in stocks of Kokanee *Oncorhynchus nerka*. Since 1986 there have been no runs of this species into several major tributaries formerly used for spawning and in addition the the average size of adult Kokanee has decreased with a subsequent reduction in fecundity. Reduced nutrient concentrations are the major factor implicated in these declines (Crozier 1994).

In experiments with high and with low fertility, Haines (1973) showed that the growth rate of bass (*Micropterus*) was lower in high fertility ponds whereas that of carp (*Cyprinus*) was higher. This was explained by the possible effect of lower oxygen, poor visibility and increased ammonia in the high fertility ponds, which affected bass but not carp.

In New Zealand, Fish (1963) examined the performance of introduced Brown Trout *Salmo trutta* in three lakes. He found that in two lakes which had high primary production and low dissolved oxygen concentrations fish grew slower and were in poorer condition than fish in the third lake which grew faster and were in better condition because of better water quality. The main cause of eutrophication in the two richer lakes was the development of agriculture in their catchments.

Jenkins (1982) showed that the Morphoedaphic Index (MEI) (total dissolved solids in mg/L divided by mean lake depth in m) - originally devised by Ryder (1965) - can be used to predict both fish harvest and standing crop in US reservoirs. However, Schlesinger and Regier (1982) found that in intensively fished lakes, stepwise regression analysis showed that the climatic index (mean annual air temperature) accounts for 74% of the variability of maximum sustainable yield (MSY). The MEI accounted for only 7-9% additional variability. Youngs and Heimbuch (1982) suggested that mean depth is a surrogate for area, which gives a better correlation (94% of variation) with fish yield.

Fish production was shown by Downing and Plante (1993) to be positively correlated with temperature, lake phosphorus concentration, chlorophyll *a* concentration, primary productivity and pH. In the set of lakes they studied, the correlation with Morphoedaphic Index was not good.

3.6.2 Influence of factors other than nutrients

As with rivers and explained above, many factors other than nutrient input affect the composition and abundance of freshwater fish in lakes and these include pollution, acidification, physical barriers, habitat destruction, land use, alien introductions, inappropriate fishery management, etc. Often, individually, these may be much more important in determining the nature of a fish community or its production, and frequently one or more of them may act together to produce significant change. For example, natural salmon and trout populations may be absent from many lakes because of barriers to migration, i.e. either natural physical barriers (isolated from rivers) or chemical barriers further downstream in estuaries (deoxygenation or toxic pollution). The following are a few examples from the many thousands of studies available.

Case studies: factors other than nutrients

Ranta and Lindstrom (1993), in a study using principal component analysis and involving 390 lakes in Finland, found that water quality explained no more than 15% of the variation in fish yield in contrast to fishing effort which explained 50%. A simple theoretical model for fish yield against water quality indicated little linear relationship.

Breukelaar *et al.* (1994) found that suspended sediment concentrations increased linearly with the biomass of benthivorous fish. Bream *Abramis brama* caused an increase of 46 g sediment/ m^2/ day/ 100 kg Bream/ ha and a reduction of 0.38 m^{-1} in reciprocal Secchi depth. There was no relationship between the size of fish and the amount of resuspension, but the effect of Bream was twice as great as Carp *Cyprinus carpio*. Benthivorous feeding was reduced in May when zooplankton was available. Assuming a linear relationship, chlorophyll *a* increased by 9.0 ug L^{-1}, total P by 0.03 mg L^{-1} and Kjedahl-N by 0.48 mg L^{-1} per 100 kg Bream. Silicate, chlorophyll *a*, total P and total N were all positively correlated with fish biomass.

Lake 226 in the Experimental Lakes Area of Canada had fertiliser (N, P and C) applied to its northeast basin (Mills 1985) but only N and C to its southwest basin. The responses of lake whitefish (*Coregonus clupeaformis*) were measured over four years. Fish in the northeast basin grew faster, had higher coefficients of condition, greater recruitment and production than those in the southwest basin. Survival of 0+ fish was better in the northeast basin, but not that of older year classes.

3.6.3 Classification and monitoring schemes

Various schemes for the classification of lakes have been suggested and, though even the best of these is open to criticism, most of the systems so far presented have been more successful than similar schemes for running waters. This is mainly because each body of standing water is an entity with characteristic and reasonably uniform physico-chemical and biological components. Running waters, on the other hand, can rarely be precisely defined and often exhibit a wide range of conditions and communities within a single system. The classification schemes proposed for standing waters so far have been based on a variety of parameters including type of origin, physical (especially thermal), chemical and biological characteristics, including fish.

Classification according to origin has been discussed, and three main types of basin distinguished: rock, barrier and organic. Within each of these are found further subdivisions. Though this classification is a useful one and extremely practical in that most lakes fall distinctly into one class or another, it has two main disadvantages. Firstly, many lakes have changed dramatically since they were first formed, and are still doing so. This difficulty is solved to some extent in the classification suggested by Pearsall (1921) by arranging lakes in an evolutionary sequence. Secondly, lakes with entirely different origins can be similar from an ecological point of view and vice versa.

Lakes can be arranged according to their superficial areas, the volumes of water they contain, their mean or sometimes maximum depths, their latitude, altitude or salinity. All these classifications must be regarded as more or less artificial and, while useful in comparing a large number of lakes, are not of great value from the ecological point of view, mainly because the lakes form a complete series in each case. Any divisions, therefore, tend to be arbitrary ones.

Whipple (1898) classified lakes into three types according to their surface temperatures - polar, temperate and tropical. Forel (1904) divided lakes into the same three types, but based on their bottom temperatures. Each of these three types was subdivided into two categories - shallow and deep. Shallow lakes are defined as those which have a variable bottom temperature, and deep lakes as those with a constant bottom temperature. However, the most satisfactory classification according to thermal characteristics is the system developed by Hutchinson and Lofler (1956) where there are five major classes of water: amictic, cold monomictic, dimictic, warm monomictic and oligomictic.

Because of the importance of temperatures in lakes, especially as far as stratification is concerned, systems of classification involving thermal characteristics are obviously potentially useful ones. In addition, the subdivisions are real ones with only a few intermediate cases, and this makes such systems even more valuable. The disadvantage of classifications relying solely on thermal characteristics is that no account is taken of the nutrient content and consequent productivity of such waters, and it is quite possible for two lakes which are very similar thermally to differ chemically point of view, and consequently to be dissimilar ecologically.

Several workers have attempted to relate the distribution and abundance of plants and animals to one or more chemicals, and to classify waters in terms of their chemistry. Such systems are often useful but usually rather arbitrary. Total hardness or alkalinity is frequently used for classifying into nutrient types. Spence (1967) defined as nutrient-poor waters which have up to 15 mg/L of calcium carbonate, as moderately rich with 15-60 mg/L, and as rich with more than 60 mg/L.

One of the most useful biological classifications of standing waters was originally suggested by Thienemann (1925) and later elaborated by others. This scheme suggests four major types of open water: dystrophic, oligotrophic, mesotrophic and eutrophic. Oligotrophic lakes are nutrient-poor, usually deep, clear lakes which never have oxygen deficiency; the chironomid midge *Tanytarsus* is often dominant and the culicid midge *Chaoborus* absent. Eutrophic lakes are nutrient-rich, usually shallow, turbid lakes which may have an oxygen deficiency in deeper water. The chironomid *Chironomus* is normally dominant and *Chaoborus* is present. Dystrophic lakes have variable amounts of nutrients but high amounts of humus, making the water brown; they are usually shallow or only moderately deep, and may show oxygen deficiencies in deeper water. *Chironomus* and *Chaoborus* may be present, but in low numbers. Mesotrophic lakes are intermediate in character between oligotrophic and eutrophic ones. Though this classification is extremely general and in some ways arbitrary, it has proved its value over a long period, and the types described are widely used.

The fish communities of these lake types in Europe may broadly be described as follows:

Dystrophic	Sometimes no species, always very few
Oligotrophic	Few species, dominated by salmonids and sometimes coregonids
Mesotrophic	Fewer species, sometimes dominated by percids and cyprinids
Eutrophic	usually a wide range of species, often dominated by cyprinids

No system of classification is ideal. Nevertheless many are of value, and if the systems themselves are defined and understood correctly, they provide useful methods of categorising and comparing lakes. It is probable that some combination of the systems discussed above is the most acceptable way of defining a lake quickly, for example some lakes can be edaphically oligotrophic but others only morphologically so. To describe two lakes as eutrophic may be insufficient when one is deepish, dimictic with a heavy precipitation of marl, while the other is shallow and oligomictic. In spite of complications, however, the development of schemes of classification is much more likely to be successful for standing than for running waters and the development of more sophisticated schemes is likely to be useful.

In Belgium, Belpaire *et al.* (2000) used an Index of Biotic Integrity (IBI) to assess the status of fish communities in 104 standing waters and 757 sites on running waters. Scores for standing waters were substantially different from running waters and only 18.5% of waters were considered to satisfy the basic ecological demands. It was concluded that the IBI is a valuable tool to assess the ecological quality of water bodies for the Water Framework Directive.

Regier and Henderson (1973) proposed a model which inter-relates previous conceptions. It is directed at events and processes at a fish community level.

Of major relevance in a European context is the EEC Directive 659 (1978) 'on the quality of waters needing protection or improvement in order to support fish life' which is discussed more fully in Section 2.6. There is a specific observation in this Directive concerning phosphorus in lakes which indicates a formula (involving loading, mean depth and renewal time) to be applied to lakes of average depth between 18 and 300 m.

3.6.4 Type-specific sensitive species

Several fish in Great Britain can be regarded as disturbance-sensitive species and these include Atlantic Salmon *Salmo salar*, Brown Trout *Salmo trutta*, Arctic Charr *Salvelinus alpinus*, Powan *Coregonus lavaretus* and Vendace *Coregonus albula*. All of these species have undergone decline over the last century in Great Britain and elsewhere in Europe. Several of the studies of these declines have implicated nutrients in the lakes concerned, largely following excessive algal and macrophyte growths which have lead to low oxygen concentrations and smothering of spawning grounds, with resultant problems for sensitive species. Declines of migratory species, such as Atlantic salmon and sea trout have largely been associated with water quality problems further downstream in estuaries.

Case studies: type-specific sensitive species

In Windermere, there has been a decline in the catches of Arctic Charr *Salvelinus alpinus* by anglers and, since 1984, an increase in the numbers of Brown Trout *Salmo trutta* taken in the South Basin (Le Cren *et al.* 1974, Mills *et al.* 1990). This has been associated with increasing eutrophication of the lake. In the early 1990s, echosounding data indicated that pelagic salmonid density in the North Basin was some 2-5 times greater than that in the more eutrophic South Basin. However, with a significant reduction of phosphorus from sewage works this ratio has reduced and there are now increased densities of fish (mainly small fish and probably Arctic Charr) in both the upper and deeper layers of water in the South Basin (Elliott *et al.* 1996).

Both coregonids and salmonids require relatively well oxygenated, cooler, deeper waters (Maitland 1992). When primary productivity increases to the point that the hypolimnion becomes anoxic they will disappear and be replaced by percids; further enrichment then leads to dominance by cyprinids such as Carp *Cyprinus carpio* and Bream *Abramis brama* (Oglesby *et al.* 1987). However, as emphasised above, this cannot happen in lakes in northern Scotland because these cyprinids do not occur there.

Whitefish (*Coregonus*) eggs were sampled by Muller (1992) in thirteen Swiss lakes just prior to hatching. The proportion of viable eggs was taken as a sign of success. Successful incubation was not linked to hypolimnetic oxygen concentrations (usually high during the winter egg incubation period) but was governed by trophic state in terms of total phosphorus concentration in spring. This was explained by the production-related uptake of oxygen by sediments.

At the end of the 19th century, Arctic Charr *Salvelinus alpinus* dominated the fishery in Lake Zug in Switzerland, forming 85% of the total harvest (Ruhle 1984). Due to increasing eutrophication during the 20th century, the annual catch had dropped from 100,000 fish to less than 5,000 by 1980, and the proportion of the catch was only 2-3 % of its former level. The main impact on the Arctic Charr seems to have been through deoxygenation of the hypolimnion and sedimentation of the traditional spawning grounds. A similar situation took place with Arctic Charr in Lake Constance over approximately the same period (Hartmann 1984).

Water quality in Lake Simcoe in Canada has been affected by an excessive supply of phosphorus which has stimulated an excessive growth of algae and macrophytes in parts of the lake (Overton *et al.* 1994). This resulted in critically low dissolved oxygen concentrations in the deeper water in the lake which in turn has been linked to a decline in whitefish and lake Trout populations.

Influence of factors other than nutrients

As with running waters, many factors other than nutrients have lead to the decline and extinction of sensitive fish species in rivers and there are numerous studies describing such events.

Case studies: influence of factors other than nutrients

Bronmark and Weisner (1992) argue that, in eutrophication, change from macrophyte domination to phytoplankton domination can be due to changes in fish community structure. A catastrophic disturbance event (e.g. winter kill) acting selectively on piscivores, cascades down food chains, eventually reducing macrophyte growth through shading by epiphyton, an effect that is reinforced by increasing phytoplankton biomass. The transition back from the phytoplankton to the macrophyte state depends on an increase in piscivore standing stock and a reduction in planktivores.

An experiment by Holopainen *et al.* (1992) with Crucian Carp *Carassius carassius* in a small pond confirmed the earlier work of Cahn (1929), Crivellie (1983) and others, and showed that the mean zooplankton biomass was lower and phytoplankton higher at high fish density. Water transparency was negatively correlated with chlorophyll *a* at low fish density, but turbidity appeared to reduce transparency at high fish density. Similar results were obtained by Roberts *et al.* (1995).

Classification and monitoring schemes

Disturbance-sensitive species are included in various classification schemes for lakes but there are no schemes dealing specifically with such fish. Some lake species have received considerable attention in recent years, in terms of both survey and conservation management, but as yet there are no fully adequate monitoring schemes in Great Britain.

3.6.5 Age structure

Though there are many studies indicating a positive relationship between increased growth of fish and increasing nutrients in lakes, there is little evidence of any significant changes to age structure. Theoretically, it is likely that age structure could be affected where (a) sudden increases in food supply initiated a rapid increase in juvenile recruitment, thus skewing the age structure towards young fish, or (b) deoxygenation, resulting from increased plant growths eliminated all or part of one or more age classes, but not others.

Case studies: age structure

The Bodensee is one of the best studied examples of the eutrophication process. Based on the phosphate content only, the lake probably changed from oligotrophic to eutrophic between 1935 (no measurable phosphate present) to 1959 (7-9.5 mg/m^3) (Kliffmuller 1962). Numann (1972) showed that, as a consequence of enrichment, the growth of whitefish (*Coregonus*) had increased so much that they were being caught in nets prior to maturity, resulting eventually in a decrease in the fishery. In addition to the accelerated growth, various other changes in the lake caused changes in the breeding areas of fish present, which meant that four previously isolated populations of whitefish were able to inter-breed. Isolation mechanisms before enrichment had been minimised.

Using biomanipulation, Perrow (1989) has shown that there is a positive relationship between young roach and algal densities in Alderfern Broad, caused by the feeding of roach on zooplankton there, thus diminishing their grazing effect on algae. Population cycles may result from such interactions (Townsend and Perrow 1989) which can thus result in different age structures in different years.

Influence of factors other than nutrients

Many factors other than nutrients are known to affect the age structure of fish in lakes (see summary list of anthropogenic factors affecting fish above). Notable among the impacts which are likely to affect fish population age structure are pollution, acidification, fluctuating water levels and fisheries. Any of these which impact on one age group more than another or which affect recruitment by stopping access to spawning grounds or damaging spawning grounds are likely to result in significant changes in age structure of the fish populations concerned.

It must not be forgotten that many natural factors affect the age structure of fish in lakes, notable among which is climate. It is well established that favourable climatic conditions during spawning, egg incubation, hatching and early feeding can producestrong year classes which may dominate a population for many years. The growth and survival of Perch *Perca fluviatilis* is much affected by temperature. Long warm summers produce very strong year classes whilst the opposite is the case with cold summers (Le Cren *et al.* 1967, Craig 1980). Thus there may be great variations in age classes within Perch populations, but with the same good (and poor) age classes appearing in many populations across Great Britain.

Classification and monitoring schemes

Though there are considerable data in the literature which would provide the basis for schemes of classification of fish populations on the basis of age structure, no comprehensive scheme has yet been attempted for the freshwater fish species occurring in lakes in Great Britain. Age structure is examined during most ecological studies of fish in lakes but there is as yet no monitoring programme looking at this parameter regularly.

3.6.6 Summary

The main points emerging from this review are as follows:

(a) The composition and abundance of fish populations in lakes can be affected by increased (and decreased) nutrients, usually indirectly through ecological pathways.

(b) Some fish species are particularly sensitive to disturbance, and a number of populations or rare species in lakes in Great Britain and elsewhere have become extinct as a result of increased nutrient concentrations, often through decreased oxygen concentrations or smothering of spawning grounds.

(c) Though growth rates are frequently increased by higher nutrient concentrations, these rarely affect age class structure unless change in concentrations (up or down) occurs rapidly or age classes are reduced or eliminated as aconsequence (e.g. through reduced oxygen concentrations).

(d) In the majority of cases involving damage to fish populations in lakes, factors other than nutrients are more important (e.g. acidification, level fluctuations, alien fish species).

(e) Present schemes of classification for lakes are more satisfactory than for rivers, though further research is required and there is little monitoring of fish populations in lakes in relation to reference conditions.

(f) Considerable research is still required in order to provide satisfactory ecological classification and referencing systems for fish in lakes if the requirements of the Water Framework Directive are to be met.

3.6.7 Recommendations for Phase II

The following are recommendations for Phase II research which would significantly increase the ability to define, classify and assess reference conditions for fish populations in lakes in Great Britain.

(a) Develop a more sophisticated version of the SERCON assessment for fish, adapted for standing waters, which will operate effectively for lakes across the whole of Great Britain.

(b) Initiate a comparative study of fish populations in selected lakes, comparing stocks (i) within lakes before and after significant anthropogenic nutrient inputs, and (ii) between comparable lakes which have either high or low anthropogenic nutrient concentrations.

(c) Carry out an analysis of available data on fish communities in lakes in Great Britain which will provide a basis for their classification in relation to physicochemical parameters.

(d) Review existing methods of assessing and monitoring methods for fish populations in lakes, using data from Great Britain.

(e) Carry out an analysis of available data on age structure in natural populations of fish species in lakes in Great Britain which which will provide a basis for their classification in relation to habitat and geography.

(f) Review the status and impact of alien species in standing water systems in Great Britain, with due regard to alien species from abroad and 'alien' species outwith their natural distribution here.

3.7 Summary

Of all surface waters, the influence of nutrient conditions on biological quality is probably best understood for lakes. In general, greatest ecological change occurs at low nutrient concentrations (sites where TP <50 µg/l) with the loss of less competitive macrophyte species and their associated fauna. The influence of nutrients is, however, more predictable in deep lakes where primary production is dominated by the phytoplankton community. In shallow lakes, the greater significance of macrophytes and top-down control of phytoplankton means that water-column nutrient influences are less strong, although not necessarily insignificant. Greatest ecological change occurs in shallow lakes when submerged macrophyte-dominance is replaced by phytoplankton-dominance. This can occur over a wide range of nutrient concentrations (30–1000 µg TP l^{-1}). Below 30 µg TP l^{-1} submerged macrophytes tend to be abundant, while above 100-150 µg TP l^{-1} phytoplankton dominance is more typical (Scheffer *et al.*, 1993; Jeppesen *et al.*, 1997).

3.7.1 How sensitive are different groups of organisms to increased nutrient concentrations?

No integrated analysis has been carried out on lakes to assess which biological element is most sensitive to nutrient conditions. All biological elements contain taxa that are potentially sensitive to changing nutrient status, providing nutrients are limiting productivity e.g. specific algae, macrophytes, benthic invertebrates and fish. Primary producers, particularly phytoplankton, represent the most direct, and therefore immediate, response. Other biological groups may, however, be extremely sensitive to indirect effects, such as reduced oxygen conditions.

There is evidence from CCW's Integrated Lake Classification Scheme that macrophytes are the most representative biological group of the 'ecosystem' as a whole (Allott and Monteith, 1999). This may be because macrophytes are not only sensitive to environmental conditions (alkalinity and nutrient concentrations were the main environmental gradients) but also because they underpin the other communities through the physical habitat structure they provide. This analysis covered 31 lakes spanning a range of sizes (area and depth). In shallow lakes specifically, numerous studies highlight the key structuring role of both macrophytes and fish (Carpenter and Kitchell, 1993; Jeppesen *et al.*, 1998). Any effects of nutrient conditions on their community structure and abundance will, therefore, have major ramifications throughout shallow lake ecosystems.

3.7.2 Which existing techniques hold the most potential for further investigation and development, or are new techniques required?

The most established UK schemes and their advantages and disadvantages are listed below (Table 3.18):

Table 3.18 Monitoring and classification schemes applicable to the assessment of biology-nutrient relationships in UK lakes

Lake Schemes	Advantages	Disadvantages
Phytoplankton: Chlorophyll *a* (OECD)	Highly sensitive to nutrients Simple, rapid response Integrates limiting factors Present in all lakes	Highly dynamic
Planktonic and benthic diatoms: DI-TP (Bennion *et al.*)	Highly sensitive to nutrients Integrates over space/time Sediment records for defining reference conditions Present in all lakes	Specialist EA training requirement
Macrophytes (CCW, NILS)	Sensitive to nutrients Practical relevance Data rich Historical records for defining reference conditions	Not consistently present Species poor
Chironomid pupal exuviae (Ruse)	Integrates over space/time Sediment records for defining reference conditions Present in all lakes	Nutrient response difficult to interpret Specialist EA training requirement
Fish None established		Nutrient response difficult to interpret Species poor

Ideally the optimal classification scheme would incorporate the most sensitive indicators from all biological elements. This would have the advantages of indicating a response over a range of temporal and spatial scales. For example, phytoplankton composition and abundance (chlorophyll *a*) may provide the most direct and rapid responses to nutrient enrichment compared with macrophytes, but they are also more dynamic and may indicate change when nutrient concentrations are not changing. Both could be used in combination and if changes associated with nutrient enrichment occur in both then more confidence could be attributed to a ‘eutrophication-response’ interpretation. A multi-phyletic scheme would also better represent an ‘ecosystem’ response. Community indices, such as DI-TP, could be used in combination with more specific ecological quality indicators e.g. threshold densities of bloom-forming cyanobacteria or presence/absence of coregonid fish species.

3.7.3 Is ecological status influenced more by factors other than nutrients?

Alkalinity/pH has more direct biological significance than nutrients in all systems (lakes, rivers, coastal). This was clearly illustrated for freshwater lakes in the CCW lake classification study (Allott and Monteith, 1999), the Northern Ireland Lake Survey

(Heegaard *et al*., 2001) and a survey of chironomid distributions in lakes (Ruse, 2000). The generally observed positive correlation between alkalinity and nutrient concentrations can make it difficult to assess the effect of nutrients alone, particularly in low alkalinity/pH environments.The influential effect of alkalinity can, however, be addressed in the ecotyping process, through initial classification using the obligatory and optional factors of geology and ANC.

Nutrients tend to be a secondary and indirect driver of community composition but are clearly a significant driver of productivity. For this reason phytoplankton abundance is probably the most effective biological measure of nutrient conditions in lakes.

3.7.4 To what extent can current EA monitoring and assessment practices fulfil the requirements of the WFD with respect to nutrients (e.g. differentiating between ecological status classes)?

In England and Wales lake monitoring is generally irregular and inconsistent. A few sites are regularly monitored by the EA (reservoirs, Norfolk Broads and Cumbria), the AWMN and the ECN. A routine national monitoring programme needs to be established for qualifying/priority waters. Similarly no standardised assessment practices have been developed, although guideline sampling methodologies exist (PSYM, ECN). With respect to nutrients, phytoplankton, phytobenthos, macrophytes and chironomid pupal exuviae offer the most potential for development.

A national loch monitoring programme was introduced in Scotland in 1995. Lochs greater than 1 km^2 are surveyed and classified every five years according to three environmental pressures: eutrophication, acidification and toxic pollution (Fozzard *et al*., 1999). This scheme incorporates routine monitoring of total phosphorus concentrations but does not involve any biological monitoring or classification schemes.

In terms of monitoring nutrient conditions, measures are most comparable between sites and year-to-year in the form of annual means, even though seasonal measures (winter loading, growth season mean) may be more ecologically relevant. The 3-monthly monitoring frequency outlined in the WFD is generally inadequate for accurately representing annual means. Monthly monitoring is undoubtedly preferable. The WFD will require confidence limits to be assessed for the 3-monthly monitoring frequency. Analysis of existing frequently monitored, long-term datasets could be carried out to assess confidence limits associated with various measures (annual mean and maxiumum) and sampling frequencies (monthly, 3-monthly, 6-monthly) for nutrients and chlorophyll *a*.

3.8 Recommendations for Phase II

Although the influence of nutrient conditions on biological quality is relatively well understood for lakes, there has been very limited development of quantitative biological monitoring and classification methods. There is a real need for developing and validating potential schemes across the full spectrum of lake types.

Practically the EA should focus R&D on those indicators that are already being used as, or are widely recognised as, sensitive indicators of nutrient status, such as chlorophyll *a*, phytoplankton (particularly cyanobacteria and diatoms) and macrophyte assemblages. All require development for WFD purposes and the possibility exists to combine them in a form of multi-metric index to nutrient pollution.

There is, however, also great scope to collate and analyse existing long-term lake datasets, such as those held for nutrients, chlorophyll *a* and phytoplankton composition for several of the Cumbrian Lake District lakes, Norfolk Broads and Cheshire meres (and Loch Leven and Lough Neagh). High quality historical datasets for macrophyte and fish assemblages also exist for several sites, and could be related to diatom-inferred changes in TP. These analyses could be used to explore community changes in response to changing nutrient conditions. Expert judgement of the site's history could then be used to identify characteristic biology associated with high (reference), good and moderate status classes, particularly of more impacted lowland lakes, where reference sites no longer exist.

Routine EA monitoring will need to be greatly developed for lakes as a whole. To achieve a consistent approach to monitoring and assessment for the Great Britain Ecoregion, the EA needs to progress future research developments with SEPA and SNIFFER, preferably through co-ordinated European programmes, such as the WFD Common Implementation Strategy. Particular EC projects related to lake monitoring and classification include ECOFRAME, EUROLAKES and REFCOND.

4 TRANSITIONAL WATERS

4.1 Introduction

Over the past few decades there has been increasing concern over the polluted status of transitional waters (estuaries and coastal lagoons) and coastal seas, particularly with regard to the increased loads of nitrogen (N) and phosphorous (P) based nutrients. Fluxes of N have been of particular concern, as primary production in estuaries and coastal seas is held to be limited by N availability (Ryther and Dunstan 1971, Boynton *et al.* 1982, Codispoti 1989). There is no doubt that there have been significant increases in concentrations of nitrate (NO_3^-) and dissolved inorganic phosphorus (DIP) in many estuaries (DOE/CDEP 1986, Vannekom and Salomons 1980). Estimates of present-day rates of fixed-N inputs from rivers to the world's oceans range from 1.5-5 T_{mol} y^{-1} (Duce *et al.* 1991, Galloway *et al.* 1995) and fairly robust log-log relationships have been shown to hold for nitrate and phosphate export per unit area of catchment and the population density per unit area of catchment for many of the world's major rivers, i.e. where populations are high, nutrient loads to estuaries and coastal seas are high (Wollast 1983, Peierls *et al.* 1991, Howarth *et al.* 1996). Of the estimated 1.5-5 T_{mol} N y^{-1} entering the world's oceans from rivers, approximately 70% can be attributed to anthropogenic activities (Wollast, 1983, Duce *et al.* 1991, Galloway *et al.* 1995). In addition, nutrient loads to estuaries and coastal seas are set to increase as population densities in coastal regions continue to increase throughout the world. For example, it is predicted that 75% of the US population will live within 75 km of the coastline by 2010 (Williams *et al.* 1991).

Nutrient loads in estuaries are affected by their catchment geology and the anthropogenic activities within those catchments (Balls 1994, Hessen 1999). Nedwell *et al.* (1999) compiled nutrient concentration and load data for the 155 major UK estuaries using data from the Harmonised Monitoring Scheme (Davidson *et al.* 1991). Regional geographic differences are clearly reflected in the loads of nitrate, ammonia, phosphate and silicate transported through each estuarine system. Oxidised N (NO_3^- + NO_2^- or TO_xN) was by far the greatest fluvial input of N, with the greatest loads in both TOxN and DIP being carried by the rivers that drain the intensive agricultural catchments; the Severn, Humber, other east coast estuaries including the Thames and those discharging into the Solent on the south coast (Nedwell 1999).

There is evidence that as a result of these historical increases in riverine and estuarine nutrient loads that winter nutrient concentrations are elevated in both UK coastal and southern North Sea waters (Hickel *et al.* 1993, Allen *et al.* 1998). However, discernible increases in coastal nutrient concentrations are often localised to regions subject to large nutrient inputs e.g. German Bight by the Rhine and Scheldt plumes (Gieskes and Kraay 1977, Jickells 1998). Allen *et al.* (1998) argue that long-term data for the western Irish Sea indicates that winter concentrations of NO_3^- have doubled from 4 μM in the 1960s to 8 μM in the 1990s. In addition, winter NO_3^- concentrations on the western side of the Irish Sea, where the fluvial inputs are much smaller, are usually 8-9 μM, whilst those in Liverpool Bay may reach 20-25 μM as a result of discharges form the Mersey, Dee and Ribble (Gowen *et al.* 2000).

4.2 Phytoplankton

Phytoplankton can grow in artificial media, which contain remarkably few elements, (Harrison, 1980). The elements are divided between macro elements, which they require in high concentrations and microelements, which they require in low concentrations. Nitrogen and phosphorus are macro elements and for routine laboratory culture to obtain high growth rates and biomass 500 mol m^{-3} nitrate and 50 mol m^{-3} phosphate are used. In the natural environment the supply of nutrients is extremely variable. It is still a matter of considerable debate as to which elements are the most important in limiting phytoplankton growth. There are very few areas were phytoplankton growth could be defined as nutrient replete.

Analysis of annual fluvial loads entering UK estuaries (Nedwell, 2001) showed that in general TON loads are at least an order of magnitude greater than loads of ammonium and phosphate. The data for silicate was less complete but annual loads are comparable to TON loads. The greatest loads are found in the Severn, Mersey, Clyde, Humber and Thames estuaries. In contrast, estuaries that drain poor agricultural areas with low population densities have lower loads, i.e., west Wales, north Scotland. Normalising loads on a catchment area reveals that loads to most UK estuaries are relatively constant, 1.1 x 105 moles N km^{-2} y^{-1}. P loads were very much lower, 4.85 x 103 moles P km^{-2} y^{-1} and showed much greater geographical variation. Normalised for estuary area the loads reveal a variation of 2 orders of magnitude. Nedwell (2001) concluded that the annual nutrient loads to UK estuaries was small compared to those reported for the much larger European; the Severn 2280 Mmoles N y^{-1}, the Humber 3223 Mmoles N y^{-1}, the Seine 6641 Mmoles N y^{-1}, the Rhine / Meuse 29,000 Mmoles N y^{-1}. The average P load for the UK estuaries was about 5 x 103 moles km^{-2} y^{-1} which is above the European average and based on Harmonised Monitoring Scheme (HMS) data which includes only dissolved P so this is likely to underestimate the total P flux.

The concentrations of nitrate entering an estuary will be dependent on the flow, such that the nitrate concentration of the River Ythan can range from 370 to 520 mmol m^{-3} while the close in proximity River Dee exhibits concentrations of 28 to 57 mmol m^{-3} (Macdonald, 1994) even though they have TON loads of about 100 Mmoles N y^{-1}, (Nedwell, 2001). In most estuaries the increasing salinity is matched by declines in nitrate, phosphate and silicate as the freshwaters are mixed with the low nutrient marine water. If the relationship between salinity and nutrient concentration is linear then it shows that the system is conservatively mixed. Deviations from linearity indicate that some process has altered the nutrient concentration, for example, nitrogen assimilation by growing phytoplankton (Sanders, 1997). This type of chemical analysis can be undermined by STWs located at the lower reaches of an estuary, which impact on the ammonium concentration, such that the highest concentrations are at the mouth of the estuary.

4.2.1 Composition

There is a scarcity of reports, which directly show that the species composition of an estuary has been altered by changes in the nutrient load. There are a number of reasons for this. It is only relatively recently that species composition has started to be considered as part of quantifying water quality. Most methods of estimating water quality have been based on chemical composition. Chlorophyll *a* and primary productivity measurements were usually the only biological parameters considered. These chemical and biological methods are relatively easy, most are automated and require little training or expert knowledge. Species composition analysis is still a manual process requiring a high degree of expert knowledge and tends to take longer than the other techniques previously mentioned. To some extent taxonomic studies have been stamp-collecting exercises with little regard to the chemical analysis. This is understandable because they are often intended to be the basis of an inventory of species distribution and presenting the data as Floras. For this reason this report will concentrate on the measurement of chlorophyll *a* as a proxy for phytoplankton biomass and the particular characteristics of the estuarine system that influence phytoplankton productivity.

The response of phytoplankton species composition to changes in nutrient status has been demonstrated by Pinckney (1999) using mesocosm scale bioassays measuring growth rates, primary productivity and group specific biomass. They found that the degree of mixing (turbidity) and nitrate enrichment determined whether diatoms or crytomonad species would flourish. These seasonal changes in nutrient status and the corresponding phytoplankton response have been modelled (Roelke, 1999). They considered competition between three groups of algae, P specialists, N specialists and intermediates under continuous and pulsed nutrient inputs over a range of loading ratios. They suggest that pulsing nutrients will favour smaller faster growing species.

It has been suggested (Howarth, 1999) that N fixing cyanobacteria are poorly represented in estuarine systems because of the low concentrations of molybdenum (Mo) which is required for the synthesis of the dinitrogen fixing enzyme, nitrogenase. Cole (1993) demonstrated that the assimilation of Mo by cyanobacteria is inhibited by the concentrations of sulphate found in estuarine systems.

Influence of factors other than nutrients

The composition of an assemblage is determined by a number of factors either autogenic or allogenic. Autogenic factors are those which the phytoplankton can influence, such as life cycle, nutrients, water quality and predation. Allogenic factors are those which the phytoplankton had no control over, such as salinity, temperature, light and turbulence.

The most important factor determining species composition with in an estuarine system is the ability of the algae to tolerate the wide range of salinities found in these waters. There are few species that are able to grow in fresh water as well as marine water. The species assemblage at a specific point in an estuary will be a reflection of the ability of the phytoplankton to acclimate to the local salinity conditions. Marshall (1990) separated the phytoplankton of three estuarine rivers of the Chesapeake system in the US into assemblages based on their salinity tolerance, oligohaline (freshwater), mesohaline (intermediate) and polyhaline (high salinity). They showed how some algae are unable to tolerate the increase in salinity gradient and are only found in the oligohaline zone (*Skeletonema potamos*) while others show a more gradual decline in representation (*Cyclotella striata* and *Cyclotella meneghiania*). Species assemblages were closer to each from corresponding locations in another river rather than from points relatively close to each other within the same river. Investigating similar relationships in the Delaware and Chesapeake Bays, Marshall (1993) identified 263 and 354 species respectively, separated into two groups, tidal fresh-oligohaline and meso-polyhaline regions based on diatom species. No attempt was made in this study to relate the differences in species composition with nutrient load. There was some consideration of light availability and this will be discussed later in this report.

Classification and monitoring schemes

Monitoring for species composition can be achieved at two levels. At the species level individual cells have to be identified. This can be done by microscopic examination or the recently developing field of flow cytometry. Either way a high degree of technical expertise is required. A less specific technique is to determine the major photosynthetic pigments, particularly the accessory pigments, such as chlorophyll c and carotenoids, which are characteristic of algal groups. An important consideration for monitoring is the fact that some species bloom for very short periods of time and are likely to be missed if the sampling frequency is greater than one to two weeks.

4.2.2 Abundance

Dong et al. (2000) undertook a full year study of the nutrient load and environmental parameters which determine phytoplankton production in two contrasting UK estuaries, the oligotrophic River Conwy and the nutrient enriched River Deben. The study demonstrates well the response of phytoplankton in estuaries to increased nutrient status on a seasonal basis. In the Conwy estuary blooms were observed in May and October 1998. The highest chlorophyll concentrations during May 1998 were found towards the lower end of the estuary where as in October 1998 the chlorophyll maximum, which was lower than the spring bloom, was found at the head of the estuary. This study demonstrates a number of characteristics of phytoplankton blooms. The spring bloom tends to be the most intense as it is based on high nutrient loads that often enter the system with the winter freshwater inputs. The spring bloom is terminated by the consumption of nutrients and the summer months are often characterised by reduced chlorophyll concentrations. The autumn bloom is frequently less intense than the spring bloom and is dependent on nutrient recycling of the previous bloom rather than fresh inputs of nutrients from riverine inputs. This study demonstrates the patchiness of blooms within an estuary.

In contrast to the Conwy estaury, the River Deben estuary shows little spatial variation in the spring and autumn blooms for most of the estuary; there was no difference in chlorophyll concentrations between the spring and autumn blooms for 3 of the 4 major sampling sites (Dong et a., 2000). The exception was at the head of the estuary where the increase in chlorophyll was gradual from February to July 1999 (2 to 19 mg Chl m^{-3}) with a large increase in August 1999 (48 mg Chl m^{-3}). Species analysis of the diatoms revealed that at this site the major diatom present in May 1999 was *Navicula gregaria* (69%) a benthic species, whereas in August 1999 it had been reduced to 26% by the appearance of the planktonic *Thalassiosira eccentrica* (56%).

The major findings of Dong et al. (2000) demonstrate that despite the relatively high nutrient concentrations in the River Deben estuary light was the primary limiting factor of algal production in both estuaries. They made the point that chlorophyll blooms locally could exceed the 10 mg Chl m^{-3} spring summer average but when the chlorophyll concentrations were calculated on a whole estuary basis most UK estuaries would not exceed the 10 mg chl m^{-3} limit. From nutrient bioassay enrichment experiments they demonstrated that the potential for nutrient limitation was greater in the Conwy, probably due to the lower concentrations, but that because light played such an important role in limiting phytoplankton productivity any changes to nutrient input management were unlikely to be measured in phytoplankton biomass. It is particularly noticeable that in May 1998 the maximum chlorophyll concentration was found in the upper estuary and that two (*Achnanthidium minutissimum* and *Navicula minuta*) of the major five dominant diatom species were benthic (32%). This was also the case in the August 1999 chlorophyll maximum for the Deben estuary, where *Gyrosigma attenuatum*, *Navicual gregaria* and *Nitzchia frustumum* (38%) made a substantial contribution to the diatom community.

Ythan estuary. This estuary, in the North East of Scotland, has received considerable attention because of the macroalgal *Enteromorpha* blooms and their effect on the local bird populations (Raffaelli, 1999). Between the late 1960's and early 1990's the DIN input to the estuary increased from 100 to 500 mmol m^{-3} strongly linked to changes in farming practices (Domburg, 1998). The trend in the reactive soluble phosphate is less clear with a mean value of 1.6 mmol m^{-3}. There was no change in the silicate concentration, 200 mmol m^{-3} (Balls, 1995). The changes in *Enteromorpha* spp. biomass have not been directly linked to the increase in nitrogen concentrations. There was no change in chlorophyll concentrations from 1967 and 1993, with mean annual concentrations of ~3 mg m^{-3} (Balls, 1995). In addition there was no spatial variation within the estuary of chlorophyll concentrations. The Ythan estuary is macrotidal and appears to be, in terms of phytoplankton biomass, insensitive to the five-fold increase in DIN. This estuary has been nutrient enriched but the consequences do not include eutrophication as indicated by phytoplankton biomass.

The Forth estuary. The summer average chlorophyll concentrations in the Forth estuary have shown a substantial change in intensity and location (SEPA, 2000). High concentrations of chlorophyll (+110 mg m^{-3}) were measured in the upper estuary at salinities of 2‰. These high concentrations were associated with a region of high turbidity and low oxygen concentrations. Following the introduction of secondary treatment plants these summer chlorophyll maximums have not been observed and there has been a gradual increase in the dissolved oxygen concentrations. From 1986 two areas of increased chlorophyll concentration (70-80 mg m^{-3}) were observed at more saline locations, 19 and 24‰.

The Chesapeake Bay. Harding (1997) concluded that between 1950 and 1994 there was a 5 to 10 fold increase in chlorophyll concentrations in the Chesapeake Bay system at the seaward region of the estuary and a 1.5 to 2 fold increase elsewhere which paralleled increases in nutrient loading over the same period. They were able to separate out the seasonal effects of varied freshwater inputs to show an increase in the baseline concentrations of chlorophyll *a*.

Our understanding of what nutrients limit phytoplankton comes from two sources. Firstly is the comparison of measured inorganic concentrations of N:P:Si. The mean ratio of these elements in living material is 16:1:16, the Redfield ratio. If the ratio of the dissolved inorganic nutrient differs from this, say 32:1:16 twice as much N and P, the system would be limited by P. Secondly, bioassays or enrichment studies. Water samples from the study site are enriched with added nutrient and the response of the phytoplankton is measured by either 14C or 15N assimilation of chlorophyll increase and compared to control bottles. Stimulation resulting from increased N or P (or both), or as a result of increased light is an indication of nutrient or light limitation. Using bioassays on a large scale, 13 m^{-3}Doering (1995) demonstrated the transition of P to N limitation down a salinity gradient; between 0 and 10‰ P was limiting and at 25‰ N. They emphasized the importance of external nutrient supplies in determining the limiting nutrient in an estuary, predicting higher P inputs than N inputs in low salinity zones and higher N inputs than P inputs in high salinity zones.

There is evidence that there are seasonal shifts from P to N limitation in the accumulation of phytoplankton biomass. Fisher (1992) by measuring the ratio of dissolved inorganic nitrogen to soluble phosphate, phosphate and ammonium turnover times showed that in periods of high freshwater runoff, winter and spring, P limited accumulation of biomass, whereas in the periods of low freshwater runoff N limited phytoplankton growth rates. They concluded that growth rates exerted primary control over biomass accumulation. In a conceptual model the diatoms were able to dominate in a P limited estuary with Si controlling the taxonomy during the spring period. This was associated with a biomass maximum, which led to high sedimentation rates and on to summer anoxia from organic decomposition. In the summer during N limited conditions, Si is no longer important and the biomass contains a higher proportion of flagellates.

An interesting variation on the transition from P to N limitation on a seasonal basis comes from Pennocks (1994) study of the Delaware estuary. Using small-scale bioassays and analysis of nutrient concentrations they found that P limited growth in the spring based on elevated DIN inputs, accumulation of P by phytoplankton and low rates of P regeneration. In the summer P no longer limited growth, whereasDIN:DIP ratios and bioassays indicated the potential for N limitation of growth. This was not supported by particle composition analysis and nutrient flux estimates, which suggested steady state growth. They concluded that the growth rates were supported by a constant N flux from upstream and rapid N regeneration in the lower estuary. This finding that bioassay data conflicted with the analysis of molar ratios of DIN and DIP was also experienced by Twomey (2001) who concluded that the discrepancy was due to problems with measuring low concentrations of DIP or that the phytoplankton were using an alternative form of P.

The transition from P to N limited along the gradient of an estuary can occur within a small range of salinities. Jordan (1991) reported that the ratio of N:P at the entry to the upper Rhode River estuary (salinity 0.2‰) was 26 but had changed to 2.7 at the exit of the upper estuary (salinity 10‰). These ratio changes were due to consumption of nitrate for phytoplankton production and release of phosphate from particulate P after sediment deposition. Using longer-term (2 d) nutrient addition bioassays, Holmboe (1999) also demonstrated the seasonal transition from P limited in the spring to early summer, to N limited increases in chlorophyll in the eutrophic Hjarbaek Fjord, Denmark. They concluded that both short and long term nutrient addition bioassays corresponded well with the type of limitation which could be predicted by analysis of seasonal N:P loadings and N and P concentrations of surface waters.

Influence of factors other than nutrients

Light limitation

Though most discussions on nutrient effects are concerned with N and P, phytoplankton have an absolute requirement for light to drive the photosynthetic process. The availability of light is determined by time of years (hours of daylight) and the sediment load, which can absorb light, reducing the amount available for phytoplankton photosynthesis. High productivities can be found in highly turbid estuaries because the balance between the euphotic zone and the mixed layer ensures that the algae are exposed to sufficient light. More often upper reaches of estuaries are light limited because of the high sediment load entering the estuary from the freshwater system.

The differences in the species assemblages found in the lower parts of the Delaware and Chesapeake systems by Marshall (1993) were attributed to differences in the turbidity. The lower turbidity of the lower Chesapeake, which led to a higher proportion of neritic (oceanic) species, was due to reduced rainfall and flow rates during the study period.

Silica limiation

Diatoms have an absolute Si requirement for the construction of frustules, silica impregnated cell walls. The ratio of N:Si and P:Si can determine whether the conditions locally will favour diatoms or allow alternative algal groups to bloom. Malone (1996) and Conle (1992) demonstrated how Si limitation can control the size of diatom production during a spring bloom and that once the Si has been depleted the diatom bloom will crash resulting in a rapid decline in chlorophyll concentrations. They also suggested that a bloom that is limited by Si will have greater sinking rates of diatoms than those blooms limited by N or P.

An example of how climate can affect the rates of productivity is from the Hudson River estuary in the US. This estuary has received high concentrations of nutrient inputs but has generally exhibited relatively low rates of productivity. Howarth (2000) showed that during dry summer periods of 1995 and 1997 the rates of primary productivity were greatly higher than those years when freshwater discharge was high.

Classification and monitoring schemes

The relationship between the changes in species composition and changes in nutrient status are not well understood for estuarine phytoplankton. The main approach to monitoring changes in biomass and species composition is to investigate the relationship between the load or concentration of nutrient and the amount of chlorophyll *a*. While these types of relationships have been documented for lakes for a number of years it is only relatively recently that they have been investigated in estuaries. The relationship has to be measured over a period of time because as one of the parameters declines, the inorganic nutrient, the product increases. It is necessary to relate the initial concentration with the final product. This requires a complete sampling survey.

In an important study Monbet (1992) studied the relationship between the log annual mean chlorophyll concentration (mg m^{-3}) and (i) the log mean annual loading of dissolved inorganic nitrogen (DIN g m^{-2} y^{-1}) and (ii) the log annual mean concentration of DIN (mmol m^{-3}). While there was a weak relationship between DIN loading and chlorophyll concentration the relationship between DIN concentration and chlorophyll concentration was much stronger. Monbet made a distinction between microtidal (tidal range < 2m) and macrotidal (tidal range > 2m) estuaries. For a given DIN concentration the chlorophyll concentration was always greater in the microtidal estuary. This suggests that although nutrient loadings and concentrations are related, the tidal range and the processes associated with it, such as mixing, current velocity, turbidity and light attenuation, greatly influence how phytoplankton are able to utilise the nutrients in some estuaries. In the macrotidal estuaries there appears to be a threshold 8-9 mmol DIN m^{-3}

below which there are no reports of chlorophyll concentration. It is interesting to note that although some of these concentrations are very high (800 mmol m^{-3} DIN), certainly in relation to the uptake kinetics of nitrogen assimilation of phytoplankton, all of the observed estuary DIN concentrations are less than the Nitrate Directive concentration of 50 mg $NO_3 l^{-1}$ (806 mmol m^{-3}). While this study shows a very positive relationship between DIN and chlorophyll concentrations it should be remembered there is no indication of the quality of the data; for example, how many observations were used to calculate mean values, what the seasonal spread of the data was and whether any estuaries were excluded from the analysis.

Flindt (1999) showed that the relationship for log total N concentrations between 10 and 250 mmol m^{-3} and log phytoplankton biomass between 1 and 100 mg Chl m^{-3} was linear in Roskilde Fjord during the summer months. They also reported that the increased shading caused by the high chlorophyll concentrations reduced the depth to which the seagrass Zostera marina could grow and that the depth was inversely related to total N concentration. Boynton (1996) reported that there was a relationship between the annual total nitrogen load (g N m^{-2} yr^{-1}) and (i) the total nitrogen concentration (mmol m^{-3}) $y = 0.53 x + 39.4$, $r^2 = 0.84$ (ii) the chlorophyll *a* concentration (mg m^{-3}) $y = 0.7 x + 16.9$, $r^2 = 0.96$. Meeuwig (1998) has used a similar approach to test for an empirical relationship between chlorophyll and N and P loads and the effect mussels have in clarifying turbid estuaries. In a recent study on the Conwy and Deben estuaries, Dong (2000) have reported correlations between the peak spring chlorophyll *a* concentration and the log value of the nutrient loads to the estuaries and the log nutrient load per unit of catchment for total oxidised nitrogen, ammonium, phosphate and silicate. This is particularly interesting because most data points were between 1 and 11 mg Chl m^{-3}, i.e. relatively low chlorophyll concentrations.

4.2.3 Bloom frequency and intensity

In a study of interannual variability in the spring bloom in the Rhode River, Maryland US, between 1987 and 1995, Gallegos (1997) classified the years as extraordinary (+ 120 mg Chl m^{-3}), average (70-110 mg Chl m^{-3}) and no bloom (< 60 mg Chl m^{-3}). The processes most responsible for determining the size of the spring bloom were: the rate of phosphorus release from the sediment; the timing of nitrate depletion; the maximum concentration of nitrate; and the capacity of the phytoplankton to accumulate phosphorus.

The seasonal influence on the size distribution of phytoplankton in the York River estuar (Virginia, US) has been shown by Sin (2000). For example, they found that the nano (3-20 µm) and pico (< 3 µm) plankton dominated in the mesohaline region of the estuary in the summer bloom where as the microplankton (> 20 µm) dominated the winter bloom. They suggest that as the nutrient and light requirements of phytoplankton are greatly determined by cell size (Raven, 1986; Raven, 1996) analysis of phytoplankton structure will be needed to understand the relationships between community structure and nutrient status and history.

Influence of factors other than nutrients

The seasonality of nutrient availability in an estuary is greatly influenced by the climate. There are clear links between the timing and size of freshwater inputs into estuaries and the consequent productivity of the phytoplankton. The responses of algae to pulses of inorganic nutrients can determine which species is able to become dominant. Gallegos

(1992) described how the size and the timing of an estuarine bloom could be influenced by the timing and intensity of freshwater inputs. A bloom that was triggered by a local input of freshwater had a shorter lag between the rainfall and bloom and was of a smaller scale than a bloom triggered by freshwater input from a more remote region.

Classification and monitoring schemes

Measuring chlorophyll *a* is likely to remain the primary method for determining the abundance of phytoplankton for the foreseeable future. The method is robust, relatively cheap and reliable. For abundance and bloom frequency and intensity studies snap shots of chlorophyll measurements, for example every three months, will miss a considerable amount phytoplankton activity. The use of continuous monitoring by fluorometry could reveal considerable information about the size and frequency of phytoplankton blooms, especially in locations that are sensitive or susceptible to change. Continuous monitoring does generate a lot of data and this aspect would need to be addressed.

United States Environmental Protection Agency. Estuarine and Coastal Marine Waters: Bioassessment and Biocriteria Technical Guidance

http://www.epa.gov/ost/biocriteria/States/estuaries/estbiogd.html

The EPA in its technical guidance for Estuaries and Coastal waters presently has three levels (tiers) of phytoplankton analysis. The sampling procedure is the same for all three tiers, the intensity of sampling and degree of analysis increases with each tier.

Tier One

Phytoplankton standing stock is estimated by chlorophyll *a* measurements. A sample is collected at each station at one-half the Secchi depth using a Kemmerer or Van Dorn sampler. Chlorophyll *a* is determined using a fluorometer or spectrophotometer as discussed in APHA (1992). The presence of any phytoplankton blooms observed during the cruise should be noted. Dominant phytoplankton species should be identified.

Tier Two

In addition to chlorophyll *a* measurements, samples from each station should be preserved for subsequent analysis to identify the dominant taxa and those taxa that might be responsible for observed blooms (USEPA 1992). Phytoplankton populations can vary rapidly over space in response to tides and currents, and over time in response to ambient temperature and nutrient inputs. For Tier 2, phytoplankton should be sampled at least once during an index period (usually summer) and at least once outside that index period.

Tier Three

Phytoplankton should be sampled at least once during an appropriate index period and a minimum of three other times per year to capture seasonal changes in the composition and abundance of the assemblage. Following a review of data collected from historical data or through any of the assessment tiers described here, the resource management agency may determine that a higher frequency of sampling is needed to characterize the phytoplankton assemblage based on its potential for rapid spatial and temporal variation.

The WFD recommended frequency of surveillance monitoring for physico–chemical elements is every three months. For biological monitoring it is recommended that it should be carried out at least once during the surveillance-monitoring period. This is at odds with the objective of this type of monitoring, namely long-term assessment of change. For operational monitoring, it is suggested that monitoring should take place at intervals not exceeding six months for phytoplankton and three months for physico – chemical analysis. As a qualification to the above recommendations Annex V suggests that monitoring should take into account the variability in parameters resulting from both natural and anthropogenic conditions. The frequency should also minimise the impact of seasonal variation and that the results reflect changes in the water body as a result of changes due to anthropogenic pressure. Additional monitoring during different seasons of the same year shall be carried out, where necessary, to achieve this objective.

The ability of phytoplankton to respond, and be affected by, changes in nutrient concentration, light intensity and tidal mixing complicate the requirement to monitor changes in phytoplankton composition, biomass and blooms. Variability within a single estuary, in a single year or over a number of years can be considerable. Data from Dong (2000) of monthly sampling of chlorophyll from the head and mouth of the Deben estuary shows mean values of 14.5 and 3.9 mg m^{-3}. The former is above the 10 mg m^{-3} limit for the UK indicator value for spring-summer eutrophication. None of the data points for the Felixstowferry site were above 10 mg m^{-3}. The data averaged for the whole estuary was reported as less than 10 mg m^{-3}. On the Conwy at Deganwy sampling station, which showed the greatest seasonal variation in chlorophyll concentrations, the annual mean was similar to that at Felixstowferry. Yet the spring bloom exceeded the 10 mg m^{-3} indicator limit. Given the spatial and temporal variation of chlorophyll within an estuary and between estuaries it is difficult to avoid seasonal effects and to separate them from changes caused by anthropogenic pressure.

Table 4.1 Monthly chlorophyll *a* concentrations from the Deben and Conway estuaries (Dong, 2000)

Month	Deben Melton	Deben Felixstowferry	Conwy Deganwy
		Chl *a* (mg m^{-3})	
Feb	3.2	3.2	0
Mar	8.2	1.9	0
Apr	13.9	0.6	0
May	17.1	8.2	13.4
Jun	14.5	2.5	6.4
Jul	17.7	5.1	1.1
Aug	48.6	3.8	
Sept	10.7	9.5	1.3
Oct	17.1	4.4	5.7
Nov	5.7	2.5	
Dec	2.5	1.3	1.1
Mean	14.5	3.9	3.2
Min	2.5	0.6	0.0
Max	48.6	9.5	13.4

To be able to evaluate water quality using phytoplankton, as a biological indicator, decisions have to be made as to how fine an analysis is to be undertaken. Biannual measurements will over the long-term reveal how the system is changing but the natural variation will make short-term analysis extremely difficult. It is unlikely that it will be able to separate natural variation from anthropogenic pressures

Glasgow (2000) concluded that weather patterns ultimately control when/whether the elevated inorganic nitrogen supply will support increased algal production, so that estuarine algal blooms, hypoxia, and fish kills will remain difficult, at best, to predict in modelling efforts. They recommended that decadal data sets, with sufficient sampling frequency to capture nutrient loadings from major storm events, be used to assess fluctuations in algal production of lower rivers and estuaries, and relationships with changing nutrient inputs.

4.2.4 Summary

Phytoplankton are responsible for a high proportion of the primary production in transitional waters and as such are an attractive group to study as indicators of biological change in response to nutrient change. This is due in most part because they have low generation times and can display rapid phenotypic responses to environmental change. There are three major characteristics, which can be used to quantify phytoplankton responses. These are species composition, phytoplankton abundance and the frequency and intensity of blooms. Transitional waters can exhibit the greatest range of nutrient concentrations of any surface water type. At the headwaters of an estuary the salinity approaches freshwater, <1‰ but increases to +30‰ at the mouth of the estuary. This range of salinity applies the primary pressure on species composition; very few species can tolerate such a wide range of salinities. The salinity at a fixed point in an estuary will vary according to the tide and also seasonally in response to variations in freshwater inputs. This means that the species composition at a fixed point is likely to change in response to variables other than nutrients. A major drawback in the use of species composition as a characteristic of phytoplankton response is the high degree of expert knowledge and skill required to obtain useful data. This is not such a problem when considering phytoplankton abundance. This is normally determined by measuring the chlorophyll *a* content of the water body. This is probably the best indicator of phytoplankton abundance as it is relatively straightforward, requires little expert knowledge and is rapid. A number of studies have demonstrated a relationship between the nutrient load of an estuary and the mean chlorophyll concentration, suggesting a clear link between increased inputs and increased phytoplankton abundance. That a number of estuaries do not show such a clear response suggests that not all estuaries can be treated the same. It appears that those estuaries with a macrotidal range are less sensitive to increased nutrient inputs and do not exhibit increased chlorophyll concentrations. The conditions that are thought to be favourable for phytoplankton growth are rarely found along the gradient of an estuary system at the same time. The availability of nitrogen and phosphorus can differ along an estuary leading to nutrient limitation of phytoplankton growth. When these nutrients are replete growth can be limited by additional factors, such as light, silica for diatoms and temperature. The location, timing and intensity of blooms frequently show a high degree of variability, spatially, seasonally and inter-annually. The interactions between nutrient availability, the climatic influences of rainfall, light and temperature and phytoplankton growth are complex. We have a very poor capacity to predict the spatial and temporal growth of phytoplankton in an estuarine system. We have very limited data that combines well-documented biological data with chemical data for transitional waters. It is imperative that long-term databases are built up so that we can properly quantify the natural range of variability that phytoplankton in transitional waters exhibit.

4.2.5 Recommendations for Phase II

It is difficult to be precise about recommendations for Phase II research because to be able to fulfil the requirements of the WFD with respect to phytoplankton our understanding of how phytoplankton respond to changes in their environment needs to be greatly expanded. The limitations of laboratory investigations need to be fully comprehended. For example, it is very difficult to reproduce growth rates observed under field conditions in the laboratory with the same physical and chemical environment. Of the three areas highlighted in this survey, species composition, abundance, intensity and frequency of blooms, it is algal abundance that would be the most rewarding in the short term.

As stated previously species composition analysis requires a high degree of specialist training. There are a number of new techniques that may expand this field.

Photosynthetic pigment analysis. Phytoplankton biomass is difficult to measure accurately, as filtering water samples is non-specific and detrital material is often co-recovered, hence the chlorophyll concentration of water is often used as a proxy for biomass. Phytoplankton cells can be very plastic in their responses to environmental change and this can be reflected in the ratio of chlorophyll *a* : Carbon. These problems are some what amplified when pigment analysis is used to determine the species composition in a particular locality. Goericke (1998) demonstrated that using analysis of chlorophyll *a* to chlorophylls *b* and *c* ratios led to considerable errors due to the variation between species and within species due to light limitation. They suggested using the ratio of chlorophyll *a* to carotenoid analysis as a method for determining species composition.

Flow cytometry. The application of flow cytometry to phytoplankton biology is a rapidly expanding field. There appear to numerous applications but in this context the ability to determine the species composition from natural water samples (Reckermann, 2000; Jonker, 2000) must help overcome the problems associated with light microscopic analysis for species composition.

The trophic diatom index has been developed to biologically monitor eutrophication in rivers (Kelly, 1995). The index appears to be site specific and concentrates on benthic species (Kelly, 1998). This approach has been adopted by a number of monitoring authorities but does not appear to have much support in the scientific literature. It is doubtful whether it can be applied to the pelagic estuarine diatoms but a study of its suitability for benthic diatom species is soon to commence (Underwood G, personal communication).

Considerable effort has been invested in understanding what limits the growth of phytoplankton during blooms. This has resulted in variations on the theme of enrichment studies to characterise which species of nutrient is the limiting concentration. These techniques, while revealing useful information, have sometimes shown ambiguous results. To be able to quantify the degree of nutrient limitation during growth under the prevailing physical and chemical environment would advance our understanding of phytoplankton nutrient acquisition. The various merits and limitations of the approaches used to identify limiting resources have recently been reviewed by Beardall (2001). While the nutrient addition bioassay will continue to be used, new methodologies are being developed, which will broaden the techniques available for assessing nutrient limitation and responses of phytoplankton communities to nutrient change. One such technique is chlorophyll *a* fluorescence. The application of chlorophyll *a* fluorescence to nutrient limitation by measuring nutrient induced fluorescence transients (NIFTS) and Fourier Transform InfraRed (FTIR) spectroscopy reveals the nutrient status of the phytoplankton. Beardall (2001) has shown that phytoplankton samples taken from Lake Lucerne with a total phosphorus concentration of less than 0.13 mmol m^{-3} exhibited NIFT responses while those from Lake Zurich, total phosphorus concentration 0.55 mmol m^{-3} did not. Similar findings have been found for the diatom *Thalassiosira weissflogii* when exposed to N, P or Si limitation, (Lippemeier, 2001).

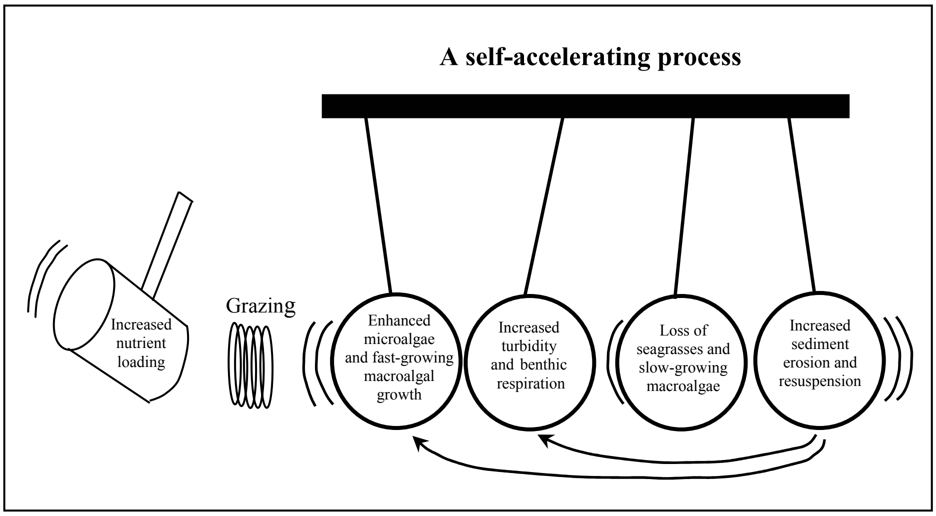
Sampling for any experiment is a compromise between the information needed to meet the objectives and the resources available. Phytoplankton in transitional waters show great natural variability in space, seasonal and interannual dimensions. While it is always tempting to sample at every possible opportunity the resources available are likely to limit the number of sampling events to not more than once a season, possibly once a month. Even with the smaller time scale it is probable that some bloom events will be missed. It is thus imperative that the mid-short term databases available are analysed to determine the level of natural variability.

4.3 Macrophytes

4.3.1 Composition

Increased N and P loading of a body of water leads to an increase in plant growth. It is generally observed, however, that some species thrive only at the expense of others. Eutrophication is often evident by the increased abundance of small, fast-growing ephemeral algal species that capitalise on the increased nutrient concentrations (Schramm 1996). These algae may out-compete the original plant community of large, perennial species, leading to a dominance of faster-growing autotrophs (e.g. Wallentinus 1981, Kemp *et al.* 1983, Cambridge and McComb 1984, Kautsky *et al.* 1986, Shepherd *et al.* 1989, Borum *et al.* 1990, Sand-Jensen and Borum 1991). Increased nutrient loading may ultimately lead to the progressive replacement of sea-grasses and slow-growing macroalgae (including brackish charophytes) by blooming, fast-growing macroalgae. This is shown schematically in Figure 1.

Figure 4.1 Conceptual model of the effect of increased nutrient loading on submerged vegetation (Duarte 1995)



Influence of factors other than nutrients

The effects of elevated nutrient concentrations on plant community structure in nutrient-poor estuaries and lagoons is particularly poorly understood, due to the paucity of case studies concentrating on this aspect of eutrophication (Parr and Wheeler 1996). The expression of increased nutrient concentrations as increased algal biomass at eutrophic sites is, in turn, a highly complex process that is controlled and mediated by many variables. The standing crop of any macroalga can show large heterogeneity within comparatively small areas as well as large differences between species (Parr and Wheeler 1996). Moreover, the mode of action by which increased nutrient concentrations affect plant species distribution and ecosystem composition may be responsible for changes that are not immediately recognised as ‘typical’ of a nutrient-enriched or eutrophic system.

It is not only changes in nutrient regime that dictate changes in plant community, but more often changes in nutrient loading coupled with some other environmental variable. With respect to macroalgal blooms, for example, even with large-scale increases in nutrients, blooms will only occur when other factors are not limiting (Aubert 1990, DeVries *et al.* 1996, Fletcher 1996b). This means, of course, that some transitional areas will be more vulnerable to the effects of eutrophication (in terms of changes in plant communities) than others. Probably one of the most important physical factors determining the response of algal communities to excess nutrient loading is the degree of ‘exposure’ at the locality (Fletcher 1996b). Parr and Wheeler (1996) suggest that changes to shore ecology should be considered against a background in which exposed and sheltered shores may be occupied by different species, and further point out that different macroalgal phenotypes may grow in exposed and sheltered locations.

Other factors that may influence macroalgal composition at a particular site include light, oxygen and substrate availability and grazing pressure. These are discussed in subsequent sections of this review, with some overlap between the transitional and coastal waters sections.

Classification and monitoring schemes

With respect to macroalgae, the monitoring of changes in potentially eutrophic areas began in the 1970's. The following section discusses case-studies from transitional waters within the UK and Europe where nutrient enrichment is thought to have caused significant changes in the composition of macrophyte communities.

Prior to signs of nutrient enrichment, estuaries were ecosystems dominated by seagrasses and slow-growing brown macroalgae that formed dense standing stocks (Flindt *et al*. 1999). These areas also contained a large number of fast-growing, opportunistic macroalgae, but their biomass was kept relatively low since they were more heavily grazed than the rooted vegetation (Geertz-Hansen *et al*. 1993). In an oligotrophic system, the threshold level of nutrient enrichment will be much lower than in a system that is naturally more productive (Fennessy 1993). Thus it will require only a relatively small increase in the nutrient load for the ecosystem to start to change. Moderate increases in nutrient concentration may lead to increased biomass of macrophytes but, initially, at least, little discernible change in species diversity (Irving 1993). Small to moderate increases in the nutrient loading of a body of water may be considered beneficial (Fletcher 1996b), and, indeed, may pass unnoticed at many sites. Edwards (1972, 1973), for example, reported very little difference in the marine benthic flora of the nutrient-polluted Tyne estuary and the relatively unpolluted Wear estuary, England.

When in excess, however, and given the right hydrological and environmental conditions (see later sections), nutrient loadings can cause marked ecological changes. Changes in the marine benthic flora due to changes in nutrient loading are documented for transitional waters in the British Isles and Europe in Table 4.2.

Table 4.2 Transitional waters in the British Isles and Europe where the marine benthic flora has been affected by nutrient enrichment

Location	Available N and P data	Changes in local flora	Reference
British Isles			
Ythan Estuary, Scotland	2 to 3-fold increase in N loading between mid 1960's and mid 1980's. N in river Ythan increased from 3 mgl^{-1} in 1960's to 6 mgl^{-1} in 1980's to > 9 mgl^{-1} in 1990's	Increased growth of green macroalgae forming blooms – Predominantly *Enteromorpha*, but also *Chaetomorpha* and *Ulva* spp.	Hull 1987 Raffaelli *et al*. 1989, 1998, 1999
Firth of Forth, Scotland	Increased sewage discharge since 1800's	41.6% reduction in macroalgal species. Restricted flora of often-stunted growths of *Ulva lactuca, Chondrus crispus, Gigartina stellata, Ceramium rubrum, Polysiphonia nigrescens, Chaetomorpha* sp., *Hildenbrandia rubra, Dumontia incrassata* and *Corralina officinalis*	Traill 1886 Johnston 1971/1972 Wilkinson and Titley 1979
Firth of Clyde, Scotland	-	Reduced flora comprising sepcies of the genera *Dumontia, Gigartina, Hildenbrandia* and *Ralfsia*	Clokie and Boney 1979
Humber-Wash estuarine system, England	Nutrients in water column: 3-10 mgl^{-1} TON 2-10 mgl^{-1} PO_4 (1990 data)	Mass occurrence of *Ulva*, *Enteromorpha* or *Chaetomorpha* since 1989	MacGarvin 1995
Helford Estuary, England	Nutrients in water column: 2 mgl^{-1} TON 20 mgl^{-1} PO_4 (1990 data)	Total loss of *Zostera marina* beds between 1986 and 1988. Luxuriant growth of *Enteromorpha* and *Ulva* in certain years	Tompsett 1994
Deben, Orwell and Stour Estuaries, England	Nutrients in water column: 0.9-2.5 mgl^{-1} TON (annual mean)	Increasing *Enteromorpha* weed mats	MacGarvin 1995

Location	**Available N and P data**	**Changes in local flora**	**Reference**
Plymouth Sound, Tamar and Yealm Estuaries, England	Nutrients in water column: 0.3-2.8 mgl^{-1} TIN (1990-93 data)	Decline in *Zostera* beds	Davies 1991 Parr and Wheeler 1996
Severn Estuary, England	Nutrients in water column: 3.1-7.6 mgl^{-1} TIN (1981-83 data)	Large reduction in abundance of *Fucus serratus* and *Ascophyllum nodosum* since 1976	Little and Smith 1980 Hiscock 1981 Parr and Wheeler 1996
Europe			
Venice Lagoon, Italy	Nutrients in water column: 0.09-0.9 mgl^{-1} NH$_4$ 3.1-4.3 mgl^{-1} NO$_3$ (1985-90 data)	Increase in the green macroalga *Ulva rigida* and corresponding decline of the seagrasses *Cymodocea nodosa* and *Zostera noltii.*	Sfriso 1987
		Decline and / or disappearance of the brown algae *Dictyopteris membranacea, Cladostephus verticillatus, Taonia atomaria* and the red genera *Laurencia, Dasya* and *Polysiphonia*. Replacement by *Dictyota dichotoma, Punctaria latifolia, Codium fragile, Ulva fasciata, Petalonia zosterifolia* and *Corralina officinalis*	Sfriso *et al.* 1989, 1992
		95 species of macrophyte recorded in 1987 compared to 104 recorded in 1960's and 141 recorded in 1930's	Schiffner and Vatova 1938 Pignatti 1962 Sfriso 1987
Orbetello Lagoon, Italy	Nutrients in water column: Up to 0.01 mgl^{-1} NH$_4$ Up to 1.4 mgl^{-1} NO$_3$ Up to 0.01 mgl^{-1} PO$_4$ but large variations in nutrient concentrations reported over recent years	Replacement of seagrasses *Posidonia oceanica* and *Cymodocea nodosa* by *Chaetomorpha linum, Cladophora vagabunda* and *Gracilaria verrucosa* from 1976 onwards	Bombelli and Lenzi 1996

Location	**Available N and P data**	**Changes in local flora**	**Reference**
Sacca di Goro, Italy	Nutrients in water column: 2 mgl^{-1} N 0.2 mgl^{-1} P	*Ulva* blooms and proliferation of *Gracilaria verrucosa*	Viaroli *et al.* 1994
Palmones Estuary, Spain	'high' nutrient concentrations in water column	Proliferation of *Ulva* and corresponding disappearance of *Zostera noltii* and *Gracilaria* mats	Perez-Llorens 1991 Niell e*t al.* 1996
Tancada Lagoon, NE Spain	-	Decrease in the abundance of submerged rooted macrophyte cover, and increase in floating macroalgae – *Chaetomorpha linum* and *Cladophora* sp.	Menendez and Comin 2000
Miro and Modego Estuaries, Portugal	-	Progressive increase in *Enteromorpha* and corresponding decline in seagrasses *Zostera marina* and *Z. noltii*	Oliveira and Cabecadas 1996
Diana and Urbino Lagoons, Corsica	Nutrients in water column: Diana: 0.026 mgl^{-1} NH_4 0.092 mgl^{-1} NO_3 0.019 mgl^{-1} PO_4 Urbino: 0.019 mgl^{-1} NH_4 0.066 mgl^{-1} NO_3 0.011 mgl^{-1} PO_4	Shift from a high diversity of communities and species to a single community with a low species diversity. Invasion of *Cladophora vagabunda, Ulva lactuca, Chaetomorpha linum* and *Enteromorpha intestinalis*	De Cassabianca 1996
Rinkøbing Fjord, Denmark	-	Significant reduction of eelgrass coverage since 1972	North Sea Task Force 1994
Nissum Fjord, Denmark	-	Significant restriction of eelgrass coverage from 1966 to 1988	North Sea Task Force 1994
Isefjord-Roskilde Fjord, Denmark	Increasing nitrate concentrations since 1941	Significant increase in relative abundance of *Cladophora, Chaetomorpha, Enteromorpha* and *Ulva* spp. and significant decrease in relative abundance of large perennial brown algae e.g. *Fucus serratus, Fucus vesiculosus*	Middelboe and Sand-Jensen 2000

Location	Available N and P data	Changes in local flora	Reference
Veerse Meer Lagoon, the Netherlands	Nutrients in water column: 3.0 mgl^{-1} NH_4, NO_3 0.8 mgl^{-1} PO_4	Mass growth of *Ulva*	Hannewijk 1988 Nienhuis 1992 Kamermans *et al.* 1998
Dutch and German estuary areas, Wadden Sea	Considerable increases in N and P inputs – approximately doubling in 20 years in the Dutch sector	Restricted growth and retreat of red seaweeds. Increased growth of *Enteromorpha*, *Chaetomorpha* and *Ulva* weed-mats since 1989. Substantial declines in eel-grass since 1930's	MacGarvin 1995
Oslofjord, Norway	Large increase in sewage loading over the last 20 years	Disappearance or reduction in abundance of *Rhodochorton purpureum, Phyllophora truncata, Spermothamnion repens* and *Ascophyllum nodosum*. Recolonization by *Enteromorpha compressa, Fucus spiralis, Porphyra purpurea, Ulva lactuca* and *Ceramium capillaceum*	Rueness 1973

4.3.2 Abundance

The excessive growth of several genera of brown algae (Phaeophyceae) and red algae (Rhodophyceae) has been linked with elevated nutrient concentrations in the marine environment – see Table 4.3.

Table 4.3 Reported increases in the abundance of macroalgae (Phaeophyceae and Rhodophyceae) linked with elevated nutrient concentrations

Algal taxa	Reference
Phaeophyceae	
Ectocarpus	Wilson *et al.* 1990, Jeffrey 1993, Kiirikki and Blomster 1996
Pilayella	Wilce *et al.* 1982, Lotze *et al.* 1999
Myriotrichia	Wear *et al.* 1999
Rhodophyceae	
Furcellaria	Baden *et al.* 1990
Phycodrys	Baden *et al.* 1990
Acrochaetium	Wear *et al.* 1999

In general, however, most reports of eutrophication refer to significant increases in green algae (Chlorophyceae), which often occur in dense blooms, forming huge macroalgal mats or 'green tides' (Fletcher 1974). This is further discussed in section 4.3.3, which deals with macroalgal blooms.

Influence of factors other than nutrients
The relationship between increased nutrient concentrations in estuaries and changes and/or increases in the abundance of certain algae is an extremely complex issue, and one which can be prevented or mediated by a host of other factors (Crouzet *et al*. 1999). These include the hydrography of the area, flushing rate and bathymetry, substrate sustainability for macroalgal growth, mixing depth (in turn, affected by stratification, temperature and salinity) and dessication of intertidal reaches (Parr and Norton 1994, Parr 1994). Other factors of particular importance are light availability and grazing.

Light availability
Light plays a very important role in the structure of the plant community in estuarine waters. Nienhuis (1992) suggests that at turbid estuarine sites, high N loading may not necessarily give rise to increased algal growth since in such places light availability rather than nutrient supply may limit net primary production. Indeed, at nutrient enriched, eutrophic sites, reduced light penetration due to increased sedimentation or shading by phytoplankton and / or ephemeral algae can prove limiting for some benthic algae, particularly those in the sublittoral zone. In such conditions, competitive advantage would be given to low-light-adapted algae, as suggested, for example, for *Phyllophora truncata, Phycodrys rubens* and *Polysiphonia nigrescens* which have widely replaced *Fucus* spp. communities in Kiel Bight below 2m depths (Vogt and Schramm 1991). Advantage would also be afforded to algae that can make efficient use of variable irradiances as shown for *Ulva rotundata* (Henley and Ramus 1989).

Distinct seasonal patterns in algal blooms are reported for eutrophic sites world-wide (see section on monitoring). Though there may be no significant difference in nutrient loading throughout the year, algal biomass is generally significantly higher throughout the summer months (when there is greater light availability) than it is during the winter. Laboratory and field investigation at the Ythan Estuary, Scotland, for example, have demonstrated that in the control of *Enteromorpha* blooms at the site, nitrogen only becomes a dominant factor when interacting with light (Taylor and Raven 2001).

Grazing
The effects of grazing on macroalgal standing crop are discussed in section 4.3.3.

Classification and monitoring schemes
The presence of large quantities of free-floating macroalgae in shallow waters and/or forming mat-like growths on soft, intertidal sediments has numerous effects on the local ecosystem (see Fletcher 1996a, 1996b, Raffaelli *et al*. 1998 for comprehensive reviews). With respect to plant life, blooms of nuisance species often displace the original slow-growing macrophyte communities, causing significant changes in the local flora. The link between nutrient loading and this replacement sequence has been confirmed by experimental nutrient additions, both to natural ecosystems (Harlin and Thorne-Miller 1981, Kemp *et al*. 1983, Twilley *et al*. 1985) and mesocosms (Neundorfer and Kemp 1993, Pedersen 1995, Short *et al*. 1995, Taylor *et al*. 1995, Short and Burdick 1996, Worm and Sommer 2000). Increased nutrient loading has led to the widespread eutrophication of shallow coastal systems, hence opportunities to document the changes in submerged vegetation have been many (Duarte, 1995). Qualitative changes during the eutrophication process are documented for a range of sites (see section on case-studies), but quantitative data for such localities is somewhat lacking. The data that are available, however, suggest that the change in vegetation is not a continuous or gradual

process parallel to the rate of increased nutrient addition. Rather, changes occur in a more step-wise manner, with sudden shifts in vegetation that may be coupled both directly and indirectly to increased nutrient loading (Kemp *et al.* 1983, Nienhuis 1983, Cambridge and McComb 1984). Direct effects include proliferation of fast-growing macroalgae due to increased nutrient availability, whilst indirect effects primarily involve increased shading of the larger, slow-growing benthic macrophytes by ephemeral, free-floating or bloom-forming macroalgae (e.g. Smith *et al.* 1999) and reduced dissolved oxygen concentrations due to increased plant standing crops (Crouzet *et al.* 1999).

Taxonomic composition and biomass cover, and cover of disturbance-sensitive taxa are the suggested attributes with which the WFD proposes to set reference conditions for transitional and coastal waters repectively (Pollard and Huxham 1998), but as discussed in previous sections, in addition to the nutrient loading of a particular site, many other biotic and abiotic factors influence macroalgal production. These may influence both algal biomass and the structure and composition of algal communities, seasonally and on a year-by-year basis. In order to accurately document changes that occur in specific algal communities, therefore, monitoring programmes would have to be structured in such a way as to take these factors into account.

With respect to the North Sea and Europe's coastal waters in particular, MacGarvin (1995) describes the current monitoring and review of nutrient concentrations and their effects as being in 'a state of absolute chaos'. The author goes on to suggest that, if possible, the situation is even worse with regard to assessing biotic change. It appears that, with remarkably few exceptions, the background data with which to assess possible changes in macroalgal communities simply does not exist. Parr and Wheeler (1996) similarly note that whilst some work has been undertaken on the effect of increasing nutrient concentrations on macroalgal standing crop, especially that of the bloom-forming genera, comparatively little work has been undertaken on other expressions of eutrophication in tidal waters such as changes in species diversity and macroalgal dominance. From the data reviewed here, it appears that this statement holds true some 5 years later.

4.3.3 Bloom frequency and intensity

With respect to macroalgae, algal blooms occurring as a symptom of increased nutrient loading and eutrophication most often involve members of the Chlorophyceae. The appearance of so-called 'green tides' has been reported at numerous localities world-wide. Table 4.4 lists localities relevant to this review and indicates the algal genera associated with floral changes at such sites.

Table 4.4 Examples of green tides reported for the British Isles and Europe – Transitional waters (Adapted from Fletcher 1996a)

Location	Algal taxa	Reference
British Isles		
Medway Estuary, England	*Enteromorpha*	Wharfe 1977
Forfar Loch, Scotland	*Enteromorpha*	Coleman and Stewart 1979, Ho 1979
Clyde Estuary, Scotland	*Enteromorpha*	Perkins and Abbot 1972
Ythan Estuary, Scotland	*Enteromorpha*	Raffaelli *et al.* 1989, 1991

Table 4.4 cont

Location	Algal taxa	Reference
Rogerstown Estuary, Ireland	*Enteromorpha*	Fahy *et al*. 1975
Belfast Lough, Ireland	*Ulva*	Cotton 1910, 1911, Letts and Richards 1911
Europe		
Lagune du Provost, France	*Ulva*	Casabianca-Chassany 1983, 1989
Mar Menor Lagoon, Spain	*Chaetomorpha*	Perez-Ruzafa *et al.* 1991
Venice Lagoon, Italy	*Ulva*	Sfriso 1987, Sfriso *et al*. 1987, 1991
Oslofjord, Norway	*Cladophora*	Klavestad 1978
Oslofjord, Norway	*Enteromorpha*	Grenager 1957, Rueness 1973, Bokn and Lein 1978, Klavestad 1978
Odense Fjord, Norway	*Ulva*	Frederiksen 1987
Roskilde Fjord, Denmark	*Ulva*	Geertz-Hansen and Sand-Jensen 1990
Veerse Meer Lagoon, Netherlands	*Ulva*	Nienhuis 1992, Kamermans *et al*. 1996, 1998

Influence of factors other than nutrients

Grazing

Direct measurements of trophic status often depend on measuring algal standing crop, but this is the balance between productivity and removal (via grazing or death). Consequently, grazing of macroalgae by invertebrates has a profound effect on both measured macroalgal biomass and community species composition (Parr and Wheeler 1996).

Consumers of living algal material include a range of gastropods, amphipods, shrimps, shore crabs, polychaetes and shorebirds (Raffaelli *et al.* 1998). Many of these consumers reach high densities when macroalgal mats are present at eutrophic sites and may have the potential to prevent algal blooms, reduce their intensity or accelerate their decline. Enclosure experiments by Warwick *et al.* (1982) in the Lynher Estuary, England, suggest that small crustaceans can accelerate the decline of Enteromorpha blooms during the autumn and that variations in the interactions between these grazers and their fish predators may account for some of the year-to-year variation in macroalgal growth at this site. Geertz-Hansen *et al.* (1993) similarly suggest that certain consumers may have the potential to actually prevent a bloom developing. The authors report that at the Roskilde Fjord, Denmark, grazing pressure on Ulva lactuca was negligible in the inner part of the estuary, allowing biomass accumulation. In the outer reaches of the estuary, however, grazing by invertebrates matched or exceeded the algal growth rate, and Ulva was scarce.

Grazing represents a significant pressure on algal communities, since changes in the dominant macroalgae may result in localised changes in the associated faunal communities. In direct contrast to the effects of grazing pressure, several reports in the literature document the facilitation of algal growth by invertebrates associated with algal mats at nutrient-enriched sites. Increased abundance of the lugworm Arenicola marina, for example, is often associated with disturbed sediments, Parr and Wheeler (1996) suggesting than an increase in Arenicola abundance at eutrophic sites provides potential anchorage for a range of macroalgal colonisers. Reise (1983), similarly documents the feeding tubes of the worm as a stable substrate for germlings of the opportunistic macroalga Enteromorpha to anchor on to.

Physical factors

Shallow, sheltered, enclosed sites are particularly susceptible to algal blooms, as reported for the Ythan Estuary, Scotland (Taylor *et al.* 1998, Taylor 1999), and the Rogerstown Estuary, Ireland (Fahy *et al.* 1975). Of the Dutch estuaries, Nienhuis (1992) suggests that the Veerse Meer Lagoon is especially vulnerable to eutrophication because of the long residence time of the water mass, the low extinction coefficient and the almost permanent stratification.

In direct contrast to these examples, some regions e.g. the Thames Estuary, England, may have very high nutrient loadings but are not associated with reports of excessive blooms of algae or changes in the local flora (Southgate 1972). It is hence apparent that many factors other than nutrient enrichment may be influential in bringing about changes in plant life in the marine environment. In order to successfully estimate potential changes to floral communities it is first essential to have relevant base-line data pertaining both to nutrient loading and the afore-mentioned environmental and physical factors. Unfortunately, such information is lacking for many sites suspected of being affected by eutrophication (Irving 1993).

The role of sediment in nutrient recycling

Macroalgal biomass has a major impact on the underlying sediment (Raffaelli *et al.* 1998). The redox potential of the sediment under thick algal mats is markedly negative, indicating an environment low in oxidised and high in reduced compounds. Under these highly reduced conditions the sediment supports re-mineralisation and recycling of macroalgal nutrients (McComb *et al.* 1981, Josselyn and West 1985, Jeffrey *et al.* 1992). Owens and Stewart (1983) have shown that ammonium-N release from sediments of the Eden Estuary, Scotland, is greatest at the time when macroalgal biomass declines and inputs of decomposing material and microbial activity are high. Similarly, Lavery and McComb (1991) suggest that decomposing mats of Chaetomorpha linum from the Peel-Harvey estuarine system, Western Australia, promote the release of large amounts of ammonia and phosphate from sediments in a redox-dependent manner and that this may provide a source of nutrients to further promote algal growth. McComb *et al.* (1998) report that at the site, nutrient release is both stimulating algal biomass production and supporting a sizeable fraction of the standing crop. Taylor and Raven (2001) suggest that a similar recycling of nutrients is occurring at the Ythan Estuary, Scotland, and that reducing external inputs of N, for example, may have little or no effect on the biomass of the *Enteromorpha* standing crop at the site.

Classification and monitoring schemes

At high nutrient loadings, general trends depicting a shift from slower growing, large macrophytes to opportunistic, rapidly-growing forms can be seen from the data in Table 4.2. Much of the data available, however, is qualitative rather than quantitative, and this, coupled with so many other variables influencing algal growth, makes it impossible to predict if or when a particular nutrient regime will promote a particular macroalgal bloom. There appears to be a 'threshold' above or below which changes to the local macrophyte community might be expected, however. Certainly, English Nature considers that there is evidence of serious effects on benthic flora at concentrations of 6.5 mgl^{-1} TON and are mindful of evidence of effects on growth or species composition at concentrations lower than between 0.15 and 0.25 mgl^{-1} TON (Burn 1993, cited in MacGarvin, 1995).

As previously discussed, delivery of nutrients from underwater sediments may extend the eutrophication process for many years (Nienhuis 1992), hence quantifying nutrients locked up in sediments may represent a way of measuring the *potential* for algal growth. In discussing the role of sediments in nutrient recycling, Taylor (1993) suggests that it is the nutrient flux per volume / area of water, and not the actual concentration or total input of nutrients, that is crucial to the effects of eutrophication. Monitoring of sediment nutrient concentrations may hence give a 'preview' of nutrient availability for a particular macroalgal standing crop in the future. Sediments could therefore be used to give some indication of trophic status, allowing scientists and managers some foresight into what (if any) 'problems' are likely to occur before changes in algal communities and / or the presence of macroalgal blooms actually become evident. A 'prevention rather than cure' strategy might be employed if there is sufficient knowledge of local sediment movement, residence times of nutrients within that sediment and a comprehensive understanding of sediment-nutrient dynamics at individual sites.

Monitoring frequency

Bloom-forming genera such as *Enteromorpha* and *Ulva* exhibit extremely high growth rates particularly when nutrient and light levels are high (e.g. Taylor *et al*. 2001). For this reason, these opportunistic algae generally exhibit their maximum biomass during the summer months when algal blooms reach a definite 'peak'. Whilst in some regions macroalgal mats can be found throughout most of the year (Montgomery and Soulsby 1980, Nicholls *et al*. 1981), most authors report a seasonal distribution in their occurrence, with maximum cover (and usually biomass) observed during the summer and minimum cover observed during the winter. Given these seasonal trends, Parr *et al*. (1993) suggest that the measurement of seaweed standing crop is most accurately conducted on a monthly basis during the growing season. In the UK, this technique has proved effective in the monitoring of macro-algal blooms at the Ythan Estuary, Scotland, (Taylor and Raven 2001) and at Langstone Harbour, England, (Pye 2000), the two UK sites recognised by OSPAR (1992) as potentially eutrophic and requiring further study.

Parr and Wheeler (1996) point out that monthly sampling would also accurately record any sudden crashes in macroalgal blooms. At certain sites, the persistence of blooms may become halted by nutrient limitation. There follows a sudden and massive mortality producing large quantities of organic matter which are decomposed by bacteria. During this process, large amounts of dissolved oxygen are consumed. Anaerobic conditions may prevail for some time, but eventually oxygen concentrations are restored again through the photosynthetic activity of other plants. Should conditions allow, a second algal bloom may occur (Irving 1993). Dystrophic crises of this kind have ben reported for the Venice Lagoon, Italy (e.g. Sfriso *et al*. 1987) and for the Etang du Provost, France (Castel *et al*. 1996).

Annex V Paragraph 1.3.4 of the WFD suggests that monitoring frequencies should be selected which take account of the variability in parameters resulting from both natural and anthropogenic conditions. The times at which monitoring is undertaken should be selected so as to minimise the impact of seasonal variation on the results and that additional monitoring during different seasons of the same year should be carried out, where necessary, to achieve this objective. With respect to macrophyte communities, such a monitoring programme is necessary in order to accurately reflect seasonal variations in both the biomass and surface cover of plant material. Indeed, monthly sampling over an extended period would be the most accurate way of monitoring floral changes, Taylor and Raven (2001) reporting that in a 3 year sampling programme at the Ythan Estuary, Scotland, there was considerable annual variation in the amount of *Enteromorpha* at the site, with respect to both mean and peak biomass.

Kamermans *et al*. (1996) suggest that on a year-to-year basis, local weather conditions may have a significant effect on algal biomass. In a survey in March 1996, after a calm spring and winter period, quantities of *Ulva* in the sediments of the eutrophic Veerse Meer Lagoon, the Netherlands, reached up to 90 g Ash Free Dry Weight (AFDW) m^{-2}. The previous year, however, after a very stormy and windy spring, there had been significantly lower biomass in the sediment, with a mean 5 g AFDW m^{-2} *Ulva* recorded in March 1995. At the Ythan Estuary, Scotland, a particularly low algal coverage was reported in 1996, Raffaelli (1999) suggesting that there is a direct link between this and a major hydraulic event the previous October (1995) when exceptionally heavy rainfall dramatically increased river flows and caused wide-spread flooding within the catchment. Raffaelli (1999) suggests that low coverage by algal mats in 1996 is due to the removal of much of the over-wintering *Enteromorpha* from within the sediment by the flood event the previous winter. Given that our transitional waters are such dynamic systems, comprehensive monitoring schemes are essential, therefore, in order to accurately reflect both seasonal and annual changes in macrophyte communities.

Aerial photography or Compact Aerial Spectrophotometric Imagery (CASI) are potentially useful tools for mapping macroalgal mats over large areas of inter-tidal mudflat (Perkins M, personal communication). Routine monitoring in these ways would, however, be prohibitively expensive.

4.4 Benthic Invertebrates and Fish

4.4.1 Nature of nutrient and organism relationships

Invertebrate and fish researchers in estuarine and coastal disciplines tend to consider eutrophication more broadly as an increase in the rate of supply of organic matter to estuarine and coastal waters (Nixon 1995). Hence the link is to the supply of organic matter and not the concentration or load of inorganic nutrients (*hypernutrification*) (Nixon 1995). Similarly, in contrast to research work on phytoplankton and macroalgae, it is often assumed in invertebrate research that there is ‘little concern with inorganic nutrients in marine ecosystems except for their potential influence on the rate and form of the supply of organic matter’ (Nixon 1995); although it is recognised that very high concentrations of ammonia may be of ecotoxicological concern (Sullivan and Ritacco 1985).

It is therefore clear from the outset of this study that the direct impact of inorganic nutrients, from an ecotoxicological point of view in estuarine and coastal waters, will have received limited attention. In contrast a great deal of research has focused on the impacts of nutrient enrichment in fresh, transitional and coastal waters from a 'eutrophication' perspective and the subject has been discussed and well reviewed (Nixon 1995). Therefore from the point of view of this report the direct interactions between elevated nutrient concentrations (hypernutrification) and organisms will be covered in the ecotoxicology part, whilst those interactions that may result from eutrophication (e.g. the decay of organic matter and subsequent hypoxia) will be covered in the indirect relationship section. The impact of organic pollution on migratory fish is one such area of great concern.

Relationships between benthic macroinvertebrates and fish and nutrients, in transitional and coastal waters, arise from both direct and indirect interactions. Direct interactions are usually assessed using a single species and form the basis of ecotoxicology, or the toxic effects of chemical and physical agents on living organisms, especially on populations and communities within defined ecosystems (Boudou and Ribeyre 1989). However, even within a single trophic level, responses to a contaminant or 'stressor' can vary amongst species, and other stressors within the environment may influence the elicited response (Breitburg 1999). Hence, how a susceptible trophic level responds to a particular stressor, or in this case nutrient, will depend upon spatial and temporal species composition and the duration and intensity of exposure to the nutrient(s). Ecotoxicolgy is still regarded as a relatively young discipline (Carmago and Ward 1995) and whilst most ecotoxicological studies are simple and direct, they do at least highlight the potential impact of anthropogenic contaminants in the environment and give a logical basis on which to build a multi-dimensional approach.

There are, however, also indirect effects of inorganic nutrients on benthic invertebrates and fish, which may be beneficial e.g. increased biomass (Lin and Hines 1994, Breitburg *et al.* 1999) or detrimental e.g. toxic algal exotoxins (Glasgow *et al.* 1995) or hypoxia and sulphide poisoning from decomposition (Diaz and Rosenberg 1995, Raffaelli *et al.* 1999). The rise and fall of the Potomac River stock of striped sea bass has been attributed to an initial increase in sewage derived nutrients through the 1940s and 60s increasing the fertility of the spawning and nursery grounds, whilst the decline in the 1970s has been attributed to improvements in sewage treatment and reduced nutrient discharge rates (Tsai *et al.* 1991). In a similar way Edgar *et al.* (1999) examined the distributions of 390 taxa of benthic macroinvertebrates and 101 species of fish in Tasmanian estuaries (48 and 75 estuaries each, respectively) and suggested that the low numbers of species recorded along the western coast reflected a low faunal biomass for the area, which may in turn have been related to low nutrient concentrations and low primary production. Some studies have examined benthic invertebrate responses to both hypoxia and elevated sulphide concentrations, and some whole system studies have addressed combinations of direct and indirect responses to anthropogenic perturbations or 'stressors'. For example, Breitburg *et al.* (1999) examined the variability in responses in an estuarine food web, including phytoplankton, copepods, fish and benthic invertebrates to nutrients and trace elements and how these responses were transmitted through the various trophic levels of the estuarine food web.

4.4.2 Direct Relationships: Ecotoxicology

Ammonia ecotoxicology

Aquatic sources and chemistry

Ammonia is a natural and ubiquitous nitrogen compound of aquatic environments, arising directly from organisms (egestion / excretion) and the microbial mineralisation of organic matter (organic ammonification). Although labile and toxic its concentration in pristine waters is usually below 14 µg N l^{-1} (Wollast 1983) and is therefore not usually of concern. However, where there is an anthropogenic source e.g. sewage discharge, industrial effluent or fertiliser run-off concentrations of ammonia can rise by several orders of magnitude (Wollast 1983, Nedwell *et al.* 1999).

Ammonia exists in water in equilibrium between the un-ionised (NH_3) and ionised (ammonium NH_4^+) fractions; the sum of the 2 fractions being referred to as total ammonia nitrogen or TAN. The proportions of the two fractions are governed largely by pH, but are also influenced by the salinity and temperature of the water. The dissociation constant for ammonia can be calculated using Whitfield's theoretical model (Whitfield 1974), which agrees well with the experimentally derived constants of Khoo *et al.* (1977) and (Kohn *et al.* 1994):

Percent un-ionised ammonia (NH_3) = $100\ (1 + \text{antilog}\ (pK_a s\ (T)\ (P)\ (I) - pH)^{-1}$

Where $pK_a s$ is the stoichiometric acidic hydrolysis constant of ammonium ions in saline water, T is the absolute temperature (°K), P is the pressure (1 atm), I is the ionic strength (M) = $19.9273\ S(1000\text{-}1.005109\ S)^{-1}$ and S is salinity.

Exposure of fish to acutely toxic concentrations of ammonia causes increased gill ventilation, hyperexcitability, convulsions and death (Russo 1985) and it is recognised that the toxicity of ammonia to aquatic organisms is due largely to the NH_3 fraction (Chipman 1934, Thurston *et al.* 1981, van Sprang and Janssen 1997), at least at pH values above 7 (Thurston *et al.* 1981, Erickson, 1985). Consequently most ammonia toxicity data are reported as just the concentration of the NH_3 fraction (Erickson 1985, Miller *et al.* 1990). Studies have been carried out to assess the relative toxicity of NH_3 to NH_4^+ on benthic and epibenthic macroinvertebrates (*Lumbriculus variegatus* and larval midge of *Chironomus tentans*) in order to establish quality criterion for ammonia in (freshwater) sediments (Schubauer-Berigan *et al.* 1995). As total ammonia was more toxic at high pH values (7.8-8.6 where NH_3 predominates) it was concluded that NH_3 was critical in determining the toxicity of ammonia to the 2 test species (Schubauer-Berigan *et al.* 1995).

However, some ammonia toxicity trials refer to total ammonia nitrogen or TAN (Ferretti *et al.* 2000) and some have reported that toxicity due to NH_4^+ can be significant in chronic toxicity trials (Erickson 1985, Borgmann 1994). Regardless of which scheme is adopted, or favoured, common techniques for measuring ammonia in water bodies and sediment porewaters e.g. indophenol blue (Aminot *et al.* 1997) will, by the high alkalinity of the assay, measure TAN as NH_3, with the proportionate amount of each fraction reported (NH_4^+ : NH_3) being calculated afterwards using *in situ* pH and temperature values. However, Miller *et al.* (1990) highlighted some important considerations when measuring ammonia toxicity in transitional waters; namely that such proportioning is highly reliant on accurate pH measurements, as a variation of just 0.1 pH units during a toxicity trial will result in a variation of 25 % in the quoted NH_3 concentration. Hence, ammonia toxicity studies that quote NH_3 concentrations to 3 decimal places should be viewed with some caution. In this review ammonia refers to total ammonia nitrogen and where data are available the proportionate value for each form i.e. NH_4^+ or NH_3 will be given. It should also be noted, with regard to sediment porewaters, that the sediment ammonia pool comprises both that in the porewater and that bound to sediment particles by ion exchange; these are not always distinguished.

Ammonia toxicity in relation to sediments

Pelagic / benthic perspective

Ammonia is present in transitional and coastal waters in the water column and in the sediment. When considering the interactions between benthic macroinvertebrates (and fish) and ammonia it is important to try and discriminate between these two sources, as water column ammonia (at appreciable concentrations) will most likely be due to, and close to, a point source discharge, whilst that in the sediment will largely be a result of natural organic ammonification (Blackburn and Henriksen 1983). However, there maybe indirect interactions whereby hypernutrification with ammonia feeds into eutrophication of, for example, macrophytes, which in turn, via their decay, leads to a substantial elevation of ammonia porewater concentrations (Trimmer *et al.* 2000a). In addition, the ammonia in the porewater will efflux to the overlying water and therefore, epibenthic organisms will be exposed directly to water column ammonia and that effluxing from the sediment. Whilst infauna will be exposed directly to porewater ammonia some will be exposed to that in the overlying water that they draw down to irrigate their respiratory surfaces or to feed from. For example, bivalve molluscs take water in through the inhalant siphon and pass it through their gills to trap particles of food and to facilitate gaseous exchange. In so doing a large surface area of gill tissue is exposed to any elevated concentrations of nutrients in the water.

Pollutants commonly accumulate in sediments to concentrations that are much higher than those found in the overlying water (Traunspurger and Drews 1996), primarily because many aquatic pollutants have a strong affinity for particulate material. This is also true for ammonia and it is likely that concentrations will be greater in sediment porewaters than in the overlying water. This is not due to an accumulation of water column ammonia in the sediment but from a production of ammonia within the sediment (organic ammonification). In addition a fraction of the ammonia produced within sediments is bound via cation exchange; the degree of which varies in transitional waters due to cation exchange sites being saturated by the cations dominant in marine waters e.g. Na^+ and K^+ (Gardner *et al.* 1991). There is a wealth of information from research into sediment toxicity tests (both acute and chronic) on meio and macrobenthic organisms focusing on either the toxic effect of contaminated whole sediment or whole sediment spiked with either heavy metals or sulphide or an extract of whole sediment i.e. an elutriate (see review Traunspurger and Drews 1996). Similar approaches have been adopted for assessing the toxicity of sediment ammonia to macro-epibenthic and infaunal invertebrates (see below).

Sources of ammonia within sediments

Estuaries and coastal margins are generally areas of intensive sedimentation, with the degree of sedimentation depending upon the specific physical characteristics of each area (Dyer, 1973), but the degree of sedimentation tends to decline with distance off-shore from the head of the estuary (Trimmer *et al.* 2000b). Deposition is largely due to the flocculation and settlement of colloidal organic and inorganic particles that are carried into the estuary by the freshwater or fluvial input, and is in turn aided by decreasing flow velocities at the freshwater-seawater interface (Wollast 1983; Forsgren *et al.* 1996). In estuarine and near-shore sediments this deposition of organically rich suspended material stimulates benthic respiration (Jensen *et al.* 1990; Sampou and Oviatt, 1991; Kerner and Gramm, 1995) and in turn its own mineralisation via aerobic and anaerobic microbial metabolisms (Fenchel and Blackburn 1979). Primarily, mineralisation results in the release of organically bound C, N and P, and to a lesser extent sulphur and silicate. The ultimate ratio of the end products depend upon the ratio of the constituent elements of the organic material and the ratio of organic material to electron acceptor. Hence, as a consequence, it is not uncommon to find concentrations of ammonia within interstitial porewaters exceeding 1 mM (14 mg l^{-1} N) and estuarine sediments are commonly sources of ammonia to the overlying water but the concentration and magnitude of flux varies widely depending on sediment type (Blackburn and Henriksen 1983, Nedwell 1982, Hopkinson 1987, Nedwell *et al.* 1993, Watson *et al.* 1993, Nedwell and Trimmer 1996, Trimmer *et al.* 1998). In addition, concentrations will be lowest at the surface (< 100 μM) and tend to increase linearly with depth (Nedwell and Trimmer 1996). Few seasonal or spatial data are available for proportionate porewater concentrations of NH_4^+ and NH_3, although Frazier *et al.* (1996) demonstrated significant temporal and vertical variation in sediment porewater distributions of total ammonia and unionised ammonia (NH_3) in sediments of the upper Mississippi River.

Despite the fact that many estuaries receive discharges of treated sewage, concentrations of ammonia will *naturally* be higher in sediment due to organic ammonification. For example, the Thames estuary receives some of the largest volumes of treated sewage in the world (1.3×10^6 m^3 d^{-1}) but concentrations of ammonia are usually < 100 μM immediately downstream of the main STWs and are diluted by the mixing seawater to < 1 μM by the mouth of the estuary (Middelburg and Nieuwenhuize 2000). However, the impact of STW discharges on estuarine ammonia concentrations varies according to the ratio of discharge to receiving water volume and can, in smaller estuaries, be more significant; with ammonia concentrations sometimes exceeding 1 mM, especially in summer (Ogilvie *et al.* 1997).

Ammonia toxicity

Assaying ammonia toxicity

The toxicity of ammonia to aquatic organisms has been recognised for many years (Chipman 1934) and has been fairly extensively researched for various freshwater fish (Thurston *et al.* 1981, Thurston 1983, Thurston *et al.* 1983, Erickson 1985). By 1985 the US Environment Protection Agency had published acute toxicity data for 49 freshwater species but only 9 marine species, while chronic data were available for 11 freshwater species but no marine organisms (US EPA 1985). The guidelines published by the US EPA (1985, 1989) for acceptable ammonia concentrations have been adopted by other nations as a means for assessing the potential quality of waters subject to some form of ammonia pollution. However, Hickey and Vickers (1994) carried out research on nine native New Zealand species of freshwater invertebrates (mainly epibenthic) and concluded, importantly, that the US EPA data did not provide adequate protection for their native species and that specific chronic toxicity tests were required for these species. Hence the application of US EPA guidelines to other non-US waters, be they freshwater, transitional or coastal, for setting 'safe' ammonia concentrations could be inappropriate.

A number of biotic indices have been adopted for assessing water quality and they are based on the well understood and basic principles that some benthic macroinvertebrates are more sensitive to (organic) pollutants than others, and that as concentrations of pollutants rise species will be eliminated progressively (Mackay *et al.* 1989). A lot of research into the toxicity of ammonia to benthic invertebrates (epibenthic and infaunal) arose out of the realisation that sedimentary ammonia could interfere with sediment bioassays for anthropogenic contaminants (Ankley *et al.* 1990). Indeed, in trials to identify the toxic component of sediment porewaters from the Upper Scheldt in Belgium, ammonia was confirmed as being responsible for all (or most) of the observed acute toxicity to the freshwater crustacean *Thamnocephalus platyurus* (van Sprang and Janssen 1997). Stemming from this has come research into methods for removing ammonia interference in marine sediment toxicity trials, for which the US EPA has published protocols (US EPA 1994, Davies *et al.* 1993) and an evaluation of the methods used has recently been published (Ferretti *et al.* 2000).

Traditionally the toxicity of ammonia to benthic organisms has been assessed in 96 h LC 50 water only exposures (Kohn *et al.* 1994, Schauer-Berigan *et al.* 1995). However, whilst such data are useful, as in the broader field of the application of bioassays for assessing water pollution, the use of acute toxicity trials has come in for increasing criticism (Mackay *et al.* 1989, Moore *et al.* 1997) and a move towards an understanding of sub-lethal effects (chronic) e.g. on reproductive capacity, embryogenesis and early development, and multi-species testing is called for (Mount 1984, Cairns 1985).

Pelagic and epibenthic ammonia toxicity

In 1989 the US EPA developed and summarised data for the acute toxicity of ammonia to 21 marine species in 18 genera. The test organisms exhibited a wide range of sensitivities to NH_3 (unionised ammonia) with the most sensitive being the winter flounder *Pseudopleuronectus americanus,* with a mean acute toxicity value of 0.492 mg l^{-1} (NH_3) and the most tolerant being adult molluscs e.g. the eastern oyster *Crassostrea virginica* (19.1 mg l^{-1} NH_3). Mean acute toxicity values for 7 crustaceans ranged from 0.773 mg l^{-1} (NH_3) for the sargassum shrimp *Latruetes fucorum* to 2.21 mg l^{-1} (NH_3) for the larvae of the lobster *Homarus americanus*. However, none of the organisms selected were infaunal benthic organisms (Kohn *et al.* 1994).

Miller *et al.* (1990) recognised the short comings of acute 'one-dimensional studies' and suggested a need to examine the interactions between ammonia toxicity, temperature, salinity and pH. Whilst it had been shown that acute sensitivity to ammonia decreased for two salmonids in saltwater relative to freshwater (Alabaster *et al.* 1979, Harader and Allen 1983), the influence of salinity on ammonia toxicity was not well known. The prawn *Macrobrachium rosenbergi* (Armstrong *et al.* 1978) and mysid *Mysidopsis bahia* (EA Engineering 1986) have been used to research the effect of pH on ammonia toxicity in seawater, with the effect being the same as for freshwater fishes (Erickson 1985) i.e. the toxicity of NH_3 increased as pH decreased below 8.0. Although this is not always the case and no discernible difference could be demonstrated for acute ammonia toxicity with the Atlantic silverside (*Menidia menidia*) over the pH range 7.0-9.0 (EA Engineering 1986, cited in Miller *et al.* 1990). Although the pH of coastal waters is stable at 8.2 and salinity varies only a little around 33‰ the potential for salinity, pH and temperature dependent ammonia toxicity could be of particular significance in transitional waters (Miller *et al.* 1990); where, for example, diel elevations of pH > 8.5

may occur due to phytoplankton blooms, and the influence of freshwater at the estuary head can lower the pH to < 7.6.

Miller *et al.* (1990) highlighted some of the complexities of multidimensional ammonia toxicity tests. Acute toxicity (96 h LC 50) for NH_3 to mysids (*Mysidopsis bahia*) and larval inland silversides (*Menidia beryllina*) was affected by both pH and salinity in a species specific manner (Miller *et al.* 1990). NH_3 was more toxic to mysids at pH 7 than at pH 8 or 9, but, in contrast, NH_3 was more toxic to the silversides at pH 7 and 9, than at pH 8. In addition salinity uniformly increased toxicity to mysids with a drop from 31 to 11‰, but in silversides, toxicity was less at pH 7, greater at pH 8 and slightly less at pH 9 at a salinity of 11‰ relative to 31‰. Such non-uniform and non-predictable differences between ammonia toxicity among species at different salinities and pH could make predicting the impacts of ammonia on organisms in transitional waters particularly difficult (Miller *et al.* 1990).

Chen and Lin (1992) clearly showed a similar effect to that of Miller *et al.* (1990) of increasing ammonia toxicity with decreasing salinity, though with a simpler set of experiments. *Penaeus chinensis* juveniles were exposed to ammonia for 24, 48, 96 and 120 h LC 50 trials and as salinity decreased from 30 to 10‰, susceptibility to ammonia increased 154 %. The threshold ammonia concentrations for rearing *P. chinensis* juveniles were estimated at 3.8, 3.6 and 2.3 mg l^{-1} ammonia at salinities of 30, 20 and 10‰ respectively.

Infauna

Wilson *et al.* (1995) documented the decline of various species of aquatic macroinvertebrates in the upper Mississippi River. In particular the finger clam *Musculium transversum* suffered very large declines in population densities from 30,000 m^{-2} in 1985 to undetectable abundances in 1990 (Frazier *et al.* 1996). Sediment porewater (interstitial) toxicity was suspected as a contributory factor to the decline of this benthic filter feeding invertebrate. In addition Sparks and Sandusky (1981) hypothesised that elevated porewater concentrations of ammonia were partly responsible for the marked decline in the fingernail clam population in the Illinois river in the 1950s. Fingernail clams have been demonstrated to be particularly sensitive to NH_3 with exposure to 0.03 to 0.14 mg l^{-1} for 4-8 weeks inhibiting growth and reproduction and higher concentrations of 0.6 mg l^{-1} inhibiting clams from reburrowing (Hickey and Vickers 1994, Sparks and Sandusky 1981).

Although the US EPA has developed, summarised and published data for the acute toxicity of ammonia to 21 marine species in 18 genera (US EPA 1989) none of the organisms selected for this summary were infaunal benthic organisms (see above). As organisms that are closely associated with marine or estuarine sediments are likely to be exposed to higher ammonia concentrations than those found in the overlying water, especially when sediments are disturbed, this was clearly an area that lacked research data. Ankley *et al.* (1990) showed that ammonia contributed to the toxicity of sediment porewater with trials on four freshwater species but although the species used were not common to sediment toxicity tests the link between sediment porewater, ammonia and toxicity was significant. Kohn *et al.* (1994) sought to discern the contribution of ammonia to sediment toxicity for marine benthic organisms, the rationale being that some of the observed toxicity in tests with dredged sediment was in fact due to ammonia and not the contaminated sediment *per se* (Jones and Lee 1978, Lee *et al.*

1978). Kohn *et al.* (1994) conducted research into the acute toxicity of ammonia to the benthic amphipods *Rhepoxynius abronius*, *Ampelisca abdita*, *Grandidierella japonica* and *Eohaustorius estuarius* as these organisms are commonly used in standard sediment toxicity tests (ASTM 1990). They are also key prey species for higher trophic organisms and represent two different life-strategies for benthic infauna: *R. abronius* and *E. estuarius* are mobile interstitial sediment dwellers being exposed to sediment, porewater and overlying water; whilst *A. abdita* and *G. japonica* are tube building amphipods (Kohn *et al.* 1994). The four amphipods were exposed to ammonia in seawater in the absence of sediment and the median lethal concentration (LC_{50}) for *A. abdita* was 49.8 mg l^{-1} TAN (0.83 mg l^{-1} NH_3), with similar values for *R. abronius* (78.7 mg l^{-1} TAN and 1.59 mg l^{-1} NH_3) whilst *E. estuarius* and *G. japonica* were less sensitive 125.5 mg l^{-1} and 148.3 mg l^{-1} TAN and 2.49 mg l^{-1} and 3.35 mg l^{-1} NH_3 respectively. Although the authors concluded that the absence of sediment did not influence the response of the amphipods over the 96 h exposure period, they acknowledged that further research was required to assess the significance of sediment to ammonia toxicity and that the actual concentrations of ammonia that organisms are exposed to in sediment bioassays needed to be researched. In addition, the effects of ammonia on benthic organisms under varied environmental conditions and the significance of intraspecific variability i.e. seasonal, age, size, sex and locality needed researching (Kohn *et al.* 1994).

Whiteman *et al.* (1996) acknowledged that porewater ammonia concentrations can be as high as 40-80 mg N l^{-1} and that such concentrations, based on water-only tests, exceeded those that might be expected to be toxic to a variety of aquatic organisms (US EPA 1989). However, there had been no direct test to extrapolate water only data to porewater ammonia concentrations in solid-phase (sediment) tests. Whiteman *et al.* (1996) assessed the route of porewater for exposure to ammonia for benthic macroinvertebrates and although they broadly concluded that ammonia bioavailability (i.e. what fraction of a contaminant is actually available) could be accurately predicted from porewater concentrations, and in turn water only trials, many of their porewater LC_{50} total ammonia concentrations were greater than their water only values. Ho *et al.* (2000) published data for the toxicity of ammonia in whole sediment samples (sediment spiked with NH_4Cl rather than amended seawater alone) to both amphipods and mysids (multi-species tests) and concluded that amphipods were more resistant than mysids, which although ecologically logical for sediment dwelling amphipods, contradicted the results of Miller *et al.* (1990) and Kohn *et al.* (1994) which indicated that mysids were more resistant to ammonia than amphipods. Ho *et al.* (2000) concluded that the sediment itself afforded the amphipods some protection from the ammonia via lower and more stable porewater pH. Ho *et al.* (2000) also demonstrated that their short (48-96 h) small scale sediment toxicity trials were predictive of results gained from longer (10 d) larger scale trials.

From the results of Ho *et al.* (2000) it could be argued that tests to discern the real toxic effect of ammonia on benthic infauna should be carried out within a sediment matrix; although differentiating the effect of ammonia from possible sediment synergistic factors such as hypoxia and sulphide concentrations would be difficult (Diaz and Rosenberg 1995). The conventional toxicity identification evaluation (TIE) approach combines numerous chemical and physical fractionation techniques to identify and characterise toxicants and has been extensively applied (van Sprang and Janssen 1997). However, van Sprang and Janssen (1997) developed a simplified and discriminative method for the detection, identification and confirmation of specific compounds responsible for sediment porewater toxicity, whilst removing the need to perform the time consuming identification and confirmatory procedures. Using this modified technique they were able to characterise ammonia as the primary toxicant to the freshwater crustacean *Thamnocephalus platyurus* in sediments containing multiple toxicants.

Moore *et al.* (1997) investigated the chronic effects (survival, growth, reproduction) of ammonia on the life-stages of the estuarine amphipod *Leptocheirus plumulosus* which has similar acute LC 50 concentrations to the crustaceans studied by Kohn *et al.* 1994. With 4 d water only exposures for 2 different age/size classes of *Leptocheirus plumulosus* the juveniles were affected more at lower concentrations than adults, similar age related effects of ammonia toxicity have been reported for other crustaceans. Lin *et al.* (1993) and Young-Lai *et al.* (1991) both reported decreasing sensitivity to ammonia with increasing size/age, respectively, for the shrimp *Panaeus japonicus* and lobster *Homerus americanus*. Such effects may be related to underdeveloped osmosregulatory systems and more frequent moulting in juvenile crustaceans (Prosser 1973, Wajsbrot *et al.* 1990).

Hyne and Everett (1998) discussed the impact of anthropogenic 'disturbances' on estuarine ecology and sought to assess the application of a new euryhaline *Corophium* sp. amphipod as a sediment toxicity testing organism for CuCl and NH_4Cl in both freshwater and estuarine sediments. However, the authors only report data for the toxicity of total ammonia in freshwater sediments and give an LC50 for acute toxicity trials for porewater NH_4Cl of 6 mg l^{-1} i.e. concentrations that would normally be found below 5 cm in estuarine sediments. Although the authors state that sediments act as sinks for toxic contaminants they gave no recognition to the fact that sediments can contain substantially higher concentrations of ammonia to those reported in their study, because of organic ammonification,. Given that *Corophium votulator* is usually found within the top 2 cm of sediment, but can burrow to 5-6 cm (Meadows and Reid 1966), and coupling this to the temporal and spatial changes that occur in sediment porewater ammonia concentrations (Frazier *et al.* 1996) it is difficult to assess how useful such single-point acute toxicity data actually are.

Nitrate and nitrite ecotoxicology

Aquatic sources and chemistry

In the absence of an anthropogenic source NO_3^- and NO_2^- are naturally generated in water via the chemoautotrophic oxidation of ammonia. In turn NO_3^- and NO_2^- are returned to the atmospheric pool of dinitrogen gas (N_2) via the anaerobic process of denitrification (see below), completing the nitrogen cycle. However, NO_3^- is now regarded as the most abundant form of ground water pollution (Pauwels *et al.* 2001) and, as discussed above, vast quantities of NO_3^- flow into estuaries from both fluvial and sewage sources (Nedwell *et al.* 1999). As a result of this it is not uncommon for winter NO_3^- concentrations to exceed 1 mM in the east coast estuaries of the UK (Trimmer *et al.* 2000b). The total annual load of NO_3^- flowing down UK east coast estuaries has been estimated at 5 Gmol N or 70000 t N (Trimmer *et al.* 2000b). Obviously NO_3^- concentrations are much higher within estuaries than the receiving coastal waters (Vannekom and Salomons 1980, Allen *et al.* 1998), as NO_3^- is diluted by the mixing seawater and invariably exhibits conservative mixing.

Ecotoxicology

Despite the scale of NO_3^- (and to a much smaller extent NO_2^-, on a molar, not toxicological basis) pollution in fresh, transitional and coastal waters very little is known about the toxicological impacts of NO_3^- or NO_2^- on aquatic life, particularly different life history stages (Camargo and Ward 1995, Scott and Crunkilton 2000). Nitrite toxicity is caused by it converting haemoglobin to methaemoglobin, a form of haemoglobin that is incapable of carrying oxygen, which results in anoxia (Huey and Beitinger 1980); though this may not always be the case (Wood *et al.* 1975, Smit and Hattingh 1978) and it is probably more complex than this from a biochemical point of view (Woo and Chiu 1997). In addition, though not surprisingly, the toxicity of nitrite is exacerbated by low oxygen concentrations (Lewis and Morris 1986). The mode of toxicity of nitrate has been studied in homeothermic animals and maybe due to an in vivo reduction to nitrite and subsequent nitrite toxicity (Kross *et al.* 1992, Shuval and Gruener 1972). By 2000 no data had been published on the acute or chronic toxicity of NO_3^- to *Pimephales promelas* and *Ceriodaphnia dubia*, species that are commonly used in aquatic toxicity trials (Scott and Crunkilton 2000, Camargo and Ward 1992). What is known, for NO_2^-, is largely restricted to either commercially important exotic crustaceans (Armstrong *et al.* 1976, Chen and Lin 1991, Chen and Chen 1992, Chen and Nan 1991), freshwater fish (Knepp and Arkin 1973, Alabaster and Lloyd 1982, Lewis and Morris 1986, Hilmy *et al.* 1987) or freshwater invertebrates (Anderson 1944, Anderson 1946, Camargo and Ward 1992, Carmargo and Ward 1995, Scott and Crunkilton 2000). As NO_3^- is considered less toxic than NO_2^- or ammonia to aquatic organisms it is probably this premise that has precluded research into the toxicity of NO_3^- and to a lesser extent NO_2^- (Russo 1985, Scott and Crunkilton 2000).

Woo and Chiu (1997) reported metabolic and osmoregulatory responses to exposure to NO_2^- for 4 days in marine water in the sea bass *Lates calcarifer*. Whilst technically beyond the scope of this review it is interesting to note that the fish in the Woo and Chiu (1997) trials were exposed to between 30-80 mg l^{-1} NO_2^- -N (2-6 mM) i.e. concentrations that are 1000 times greater than those measured immediately downstream of the largest sewage treatment works (Middelburg 2000). In a similar way Scott and Crunkilton (2000) measured the acute and chronic toxicity of NO_3^- to fathead minnows (*Pimephales promelas*), *Ceriodaphnia dubia* and *Daphnia magna* and reported a 96 h LC_{50} NO_3^- -N concentration for the larval fathead minnows of 1341 mg l^{-1} or 96 mM i.e. 100 times greater than the highest values measured in the Gt Ouse (Nedwell and Trimmer 1996); which is responsible for 16 % of the total UK east coast NO_3^- load. In addition 1341 mg l^{-1} of NO_3^- -N is equivalent to 8.14 g l^{-1} $NaNO_3^-$ or in other words a salinity of 8.14. (i.e. 23 % of seawater 35). Hence it is difficult to judge whether in fact the minnows were suffering from saline toxicity rather than NO_3^- toxicity or indeed a synergy of both. The use of high 'salt' concentrations is common throughout freshwater toxicity trials (mainly aquaculture). Indeed 96 h LC 50 concentrations for NO_3^- -N have been found to be similar for a variety of fish species. For example the Chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout (*O. mykiss*), channel catfish (*Ictalurus punctatus*) and Guadalupe bass (*Micropterus treculi*) all fall within the range 1250 – 1400 mg NO_3^- -N l^{-1} (Tomasso and Carmichael 1986, Colt and Tchobanoglous 1976, Westin 1974). In trials to determine the toxicity of nitrogenous compounds to the commercially important Australian crayfish *Cherax quadricarinatus* organisms were exposed to NH_4Cl, $NaNO_2^-$ and $NaNO_3^-$ at concentrations of 3.82, 4.92 and 6.07 g l^{-1} respectively (final N concentration of 1000 mg l^{-1} for each) and acute toxicity or respiration compared to that determined for ‘freshwater’ controls. It has also been concluded that many fish and crustaceans exhibit similar tolerances to ammonia (Meade and Watts 1995) and it follows therefore that such trials have also been conducted at artificially high salinities and, although remarked upon by Meade and Watts (1995), the toxicity results were held as valid. Hence, similar doubts could be levelled at all these trials that conduct ‘freshwater’ trials at such high salinities. Colt and Tchobanoglous (1976) commented on the fact that Trama (1954) had reported a 96 h LC50 for $NaNO_3^-$ with bluegills (*Lepomis macrochirus*) of 12 g l^{-1} and that they had also reported 96 h LC 50 for NaCl and Na_2SO_4 of 12.9 and 13.5 g l^{-1}, respectively. Hence the problem of inadvertently assaying the toxicity of inorganic nutrients on freshwater fish at artificially high salinities is known, but may not always be considered.

It is also interesting to note that Westin (1974) assayed the toxicity of NO_3^- to the Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*) in both freshwater and artificial estuarine water at a salinity of 15 (deionised water amended with Instant Ocean). They reported that the toxicity of NO_3^- was ~ 1.3 times more toxic in the saline water than in the freshwater for both fish, yet the median tolerance concentration was 4.5 g l^{-1} NO_3^- i.e. 6.2 g l^{-1} $NaNO_3^-$ or a total salinity of 21.2. Although Camargo and Ward (1992) did not use as high $NaNO_3^-$ concentrations as some of those reported here, they reported 72, 96 and 120 h LC 50 concentrations of up to 183.5 mg l^{-1} and 210 mg l^{-1} for the last instars of *Hydropsyche occidentalis* and *Cheumatopsyche pettiti*, or 13 and 15 mM NO_3^- respectively, which are still high with regard to NO_3^- for any receiving water.

Few data exist for either NO_2^- or NO_3^- (especially) toxicity for indigenous organisms at varied salinities i.e., data that could be applied (at least as a point of reference) to UK estuarine organisms. What data do exist come mainly from the estuarine and marine aquaculture literature (see below), because both NO_2^- and ammonia can rise to ‘stressful concentrations’ in intensive aquaculture systems and hence there is a need for toxicological data so that safe concentrations for nitrogenous waste compounds can be set. Again most of these trials examined the acute toxicity of nitrogenous compounds at very high concentrations, and it is doubtful whether such data are useful for assessing water quality in UK estuarine and coastal waters. Clearly there is a need for long-term chronic toxicity trials for benthic macroinvertebrates and fish in UK transitional and coastalwaters, with both NO_2^- and NO_3^- in realistic salinities (critical), with UK species and *in situ* (spatial and temporal) concentrations of these inorganic nutrients.

Chloride and nitrite interactions in transitional waters

It is known that the toxicity of NO_2^- to aquatic organisms can be attenuated by the presence of Cl^- ions (Lewis and Morris 1986); a factor that will be critical when designing long-term chronic toxicity trials for both NO_2^- and NO_3^- in estuarine waters. Lewis and Morris (1986) argued that the effect of Cl^- on the toxicity of NO_2^- is so great that experiments in which Cl^- concentrations are not determined are relatively meaningless and would at best be difficult to compare with other studies. Crawford and Allen (1977) first showed that the toxicity of NO_2^- to Chinook salmon was dependant on the salinity of the test water, and concluded that mortality in seawater occurred at 50 – 100 times the concentration of that in freshwater. Perrone and Meade (1977) suggested that the Cl^- ions compete with the NO_2^- ions for transport across the gills, hence uptake of NO_2^- is inhibited at higher Cl^- salinities. Lewis and Morris (1986) regressed the NO_2^- toxicity of Russo and Thurston (1977) for rainbow trout and used this to model the effect of Cl^- on NO_2^- toxicity, concluding that an increase of 1 mM in Cl^- raises the LC 50 for NO_2^- by 0.73 mM. Further research demonstrated that Cl^- concentrations were critical for methaemoglobin formation (Tomasso *et al.* 1979) and hence the resultant degree of hypoxia in fish exposed to acute concentrations of NO_2^- (For a more detailed discussion of the biochemistry and data see Woo and Chiu 1997 and papers therein.). Rainbow trout and channel catfish are more sensitive to NO_2^- toxicity than fathead minnows, although interestingly, the attenuating effect of Cl^- on the LC 50 for NO_2^- is greatest for the least sensitive species (Lewis and Morris 1986).

A similar consistent trend of attenuating NO_2^- toxicity by Cl^- ions has been demonstrated for commercial prawn species. Chen and Lee (1997) reported 96 h LC 50 NO_2^- concentrations for the giant prawn *Macrobrachium rosenbergii* of 8.48, 11.21 and 12.87 mg l^{-1} NO_2^--N at 15, 24 and 34 mg l^{-1} Cl^-, respectively. Similar results were reported for juvenile stages of *Penaeus penicillatus*, where NO_2^- toxicity increased by 20 % with a decrease in salinity from 34 to 25‰ (Chen and Lin 1991). However, only the experiments at salinities of 25 and 34‰ could be taken to represent transitional or coastal waters with corresponding Cl^- concentrations of 9 and 19 g l^{-1}, respectively.

Fate of nitrate and nitrite within sediments

Nitrate behaves markedly different to ammonia in estuarine and coastal waters, with concentrations of NO_3^- being predominantly higher in estuarine waters relative to sediment porewaters. For example, the Great Ouse estuary in Norfolk has high seasonal NO_3^- concentrations > 400 µM and the concentration in the surface 1 cm of sediment is consistently half that of the overlying water (Nedwell and Trimmer 1996). In fact, estuarine sediments act as 'sinks' for NO_3^- via the anaerobic respiration process of denitrification. Stimulation of organic mineralisation leads to a rapid consumption of dissolved O_2 from the water column by the surface sediments and estuarine (and coastal) sediments tend to be anaerobic below a few mm into the surface (Sørensen *et al.* 1979, Revsbech *et al.* 1980, Revsbech and Jørgensen 1986). In turn, any other oxidised molecules, such as NO_3^-, may be utilised as alternative electron acceptors to O_2 and reduced by anaerobic bacteria. This in turn sets up a vertical concentration gradient and maintains the flux of NO_3^- from the overlying water into the anaerobic sediment layers. It is also important to realise that within the surface 0.5 cm of sediment both NO_3^- and NO_2^- will be generated through nitrification of ammonia arising from organic ammonification. Indeed concentrations of NO_2^- (1-5 µM) in this surface layer may exceed those in the overlying water despite anthropogenic sources of NO_2^- to the overlying water. As with ammonia, when assessing or assaying for the toxicity of either NO_3^- or NO_2^- to benthic macroinvertebrates, it is important to appreciate the different exposures encountered by either epibenthic or infaunal organisms. Although concentrations of both oxidised species will decrease exponentially with depth below the sediment surface, penetration will be increased via bioturbation, benthic invertebrates not only irrigate sediments with their respiratory streams but also their physical mixing activity.

Phosphate ecotoxicology

The concerns and nature of phosphate pollution in aquatic environments have been dealt with in the introduction. With the exception of the physiological effects of organophosphorus pesticides (see references in Reynolds and Guillaume 1998), little is known about the ecotoxicology of phosphate to any aquatic organism, and as a result this section is presented without subdivisions. It has been demonstrated for fish that phosphate can be taken up directly from water by their developing embryos (Jana *et al.* 1986) and plays a significant role in the development of bone structure (Asano and Ito 1956). Lewis and Hanna (1995) demonstrated a significant decrease in electric organ discharge rate for two freshwater electric fish (*Gnanthomenus petersi* and *G. tamandua*, Mormyridea) at a phosphate concentration of 0.05 mg l^{-1} PO_4^{3-} -P. Reynolds and Guillaune (1998) reported a significant increase in the rate of premature expulsion, emergence of bitterling embryos and a concomitant increase in mortality at phosphate concentrations of 0.5 mg P l^{-1}. 0.5 mg P l^{-1} (16 μM) is a very high P concentration. For example, pristine estuaries are regarded as those with P concentrations of < 1 μM and industrialised estuaries are considered P enriched at concentrations > 5 μM (Nedwell *et al.* 1999).

4.4.3 Indirect relationships

Nutrient enrichment and enhanced primary production

Clearly, as a result of anthropogenic activities, concentrations of inorganic nutrients have increased throughout many estuarine and coastal systems around the UK (Nedwell *et al.* 1999) and whilst many factors may increase the supply of organic matter to estuarine and coastal systems the most common is inorganic nutrient enrichment (N and P) or hypernutrification (Nixon 1995). However, evidence to support a causative link between nutrient enrichment and elevated production can be contentious and inconclusive (Raffaelli *et al.* 1999).An increase in the amount of organic matter in an ecosystem can 'enrich' the system and support greater secondary biomass and higher trophic levels. Anecdotal studies have linked overall system production to nutrient inputs.

Experimental data linking increased nutrient availability to enhanced system production has come largely from mesocosm-based studies. Fulton (1984) measured a 30-50 % increase in copepod densities at a N enrichment of 2.3 mmol N m^{-3} d^{-1}, whilst Sullivan and Banzon (1990) recorded a 9 % increase in female copepods in 8 × enrichment MERL mesocosms. Growth of the Atlantic menhaden (*Brevoortia tyrannus*) in larval through to juvenile stages was enhanced by 50 % with an 8 fold increase in N loading (Keller *et al.* 1990). Linn and Hines (1994) and Breitburg *et al.* (1999) measured increases in phytoplankton abundance and production and growth of the clam *Macoma balthica* in nutrient enriched mesocosms. Indeed, Breitburg *et al.* (1999) measured increased densities of most phytoplankton taxa and significantly increased production, growth and or densities of most other organisms in their experimental estuarine food web mesocosms.

Detrimental effects of increased production

Estuarine and coastal waters are naturally productive and through a normal interplay of stratification, poor mixing, seasonal temperature and the mineralisation of organic matter can at times suffer from oxygen depletion. Although oxygen depletion (hypoxia) is a natural phenomena in estuarine and coastal waters throughout the world, what has come to be of major concern in recent years has been the increased spread, intensity and duration of hypoxic conditions as a result of increase inputs of anthropogenically derived nutrients (Turner and Rabalais 1994, Diaz and Rosenberg 1995). The situation is now regarded as critical and Diaz and Rosenberg (1995) argue that many systems are now severely stressed by hypoxic conditions, with only further small decreases in dissolved oxygen concentrations potentially having catastrophic impacts on system production and trophic state.

Estuarine and coastal sediments tend to be anaerobic (anoxic) below a few mm into the surface (Sørensen *et al.* 1979, Revsbech *et al.* 1980, Revsbech and Jørgensen 1986). However, if bottom waters become deplete in oxygen the entire sediment will become anoxic and the same situation can occur in sediments either impacted by large quantities of organic matter (Blackburn *et al.* 1988) or in those which support large biomasses of macroalgal mats (Viaroli *et al.* 1996, Trimmer *et al.* 2000a). Reports of either declining or significant changes in the spatial distribution of benthic macroinvertebrates (*Corophium volutator*) have been linked to changes in land-usage, estuarine nutrient concentrations and the consequent increase in the abundance of macroalgal mats which may cover intertidal sediments and smother benthic invertebrates and render the underlying sediment anoxic (see below). (Raffaelli 1999, Raffaelli *et al.* 1999). Below the surface few mm in estuarine or coastal sediments the mineralisation of organic matter will proceed via alternative electron acceptors (Fenchel and Blackburn 1979), with the predominant alternative mode of respiration being sulphate reduction (Jørgensen 1982) and, hence, sulphide will be present in anoxic sediments throughout the world. Benthic macroinvertebrates can tolerate low oxygen saturations, with their respiration remaining unaffected until extremely low concentrations are reached e.g. for many species this is about 2 ml O_2 l^{-1} (~ 45 µM) (Rosenberg *et al.* 1991). Due to its neutral charge H_2S diffuses easily through biological membranes and inhibits the last enzymatic respiratory pathway in mitochondria, resulting in total breakdown of oxidative metabolism. As they generally occur together the combined effects of H_2S poisoning and hypoxia are difficult to separate but their effects are at least additive (Vismann 1991, Diaz and Rosenberg 1995).

Within a hypoxic zone organisms have been seen to exhibit altered behavioural patterns such as decreased feeding and reproduction and changes in physiological function (Vernberg 1972, Davis 1975). As with the direct interactions between inorganic nutrients and both benthic macroinvertebrates and fish both the response and degree of tolerance elicited amongst benthic organisms to hypoxia and sulphide varies with species (Diaz and Rosenberg 1995).

Indirect detrimental effects of eutrophication

The greatest effects of increased concentrations of nutrients, particularly N and P, on the invertebrate and fish fauna in estuarine and coastal waters will be indirect, predominantly by changing the distribution and abundance of phytoplankton (potential food) and intertidal and shallow subtidal aquatic vegetation (macroalgae and seagrasses - see Section 4.3). Relating actual concentrations of nutrients to any effect they have in these ways will be at the least extremely difficult, not least because relationships may not be linear and quite small changes in concentrations may breach a threshold and cause quite large state changes.

The biological processes that affect the abundance and distribution of invertebrates in estuaries are complex. For example, the figure at the end of this section shows a simple interaction web for some common estuarine plants and invertebrates, in which only some of the positive and negative interactions are shown (Hughes, 2001). This can be compared to a food web but also includes other interactions, particularly disturbance through bioturbation. In general the invertebrates (here *Hydrobia*, *Corophium* and

Nereis, but which also could include bivalves such as *Macoma* and *Scrobicularia,* and other polychaetes particularly *Arenicola*) rely on the primarily producers for food but the relationships are often more complex.

For example, seagrasses (e.g. *Zostera* spp.) are important to invertebrates as a source of food, directly or indirectly via the decomposition cycle, but also in other ways. They are a habitat for obligate and facultative epiphytic species, gastropods may graze from the surfaces of the leaves, and the seagrasses may stabilise the sediment making it possible for some infaunal species to exist where otherwise they would not. Seagrass beds are used by fishes and mobile invertebrates, such as decapod crustaceans, some of which are of commercial importance, either throughout their lives or as nursery areas. Within the seagrass beds the juvenile fishes and crustaceans are protected from predation and utilise the higher abundance of invertebrates as a food source (see Hemminga and Duarte (2000), and references therein, for a review of the importance of seagrass beds for invertebrates and fishes). The stability of this system is fragile as it depends on the continued existence of seagrasses, which in this context may be regarded as keystone species.

However, the relationships between seagrasses and individual animal species are not always so straightforward. For example, an increase in the nutrient state of a water body can cause an increase in the abundance of epiphytic algae growing on the leaves of seagrasses. This would be to the benefit of the small gastropod *Hydrobia* which grazes them. However, if the nutrient enrichment is sufficient to cause a bloom in the phytoplankton and in the epiphytic algae this could result in a reduction in the abundance of seagrasses (see section 4.3) to the detriment of *Hydrobia*. The loss of seagrasses may affect the abundance of burrowing infauna negatively, if they depend on decomposing leaves for food, or positively if there is competition for space; burrowing fauna may be deterred by the roots of the seagrasses (Hughes *et al.* 2000). These cases exemplify the difficulties in relating changes in the abundance of fauna to nutrient concentrations.

A common effect of increased nutrient concentrations in coastal waters and estuaries is an increase in the growth of opportunistic filamentous green algae (particularly *Enteromorpha* and *Ulva*) (see Lopes *et al.* (2000) and references therein). These may form algal mats on the sediment surface or become detached and mobile. These may affect the invertebrates in several ways (Raffaelli *et al.* 1998), causing an increase or decrease in the abundance of infaunal deposit feeders, depending on the amounts of algae, and an increase in the abundance of epibenthic species, which eat the algae or use it as a habitat (or both). For example, in the Ythan estuary, widespread growths of *Enteromorpha* spp., associated with eutrophication, are having adverse effects on the numbers of mud-dwelling *Corophium volutator*, an important food source for fish and wading birds found in the area (Raffaelli *et al.*, 1991).

It has long been appreciated that interspecific interactions are major factors affecting the abundance of infaunal invertebrates in estuarine and coastal waters (e.g. Wilson 1991) especially since the trophic group ammensalism hypothesis was proposed by Rhoads and Young (1970). They suggests that the abundance of suspension feeders, which require a stable substrate, is reduced by the action of bioturbating deposit feeders, while the abundance of deposit feeders may be reduced by the presence of suspension feeders, which prevent burrowing and intercept potential food before it can be deposited.

Changes to the nutrient status of an estuary or coastal water body may change the balance of these interspecific interactions leading to changes in the relative abundance of the competing species. Although specific data are lacking any changes in these relationships would be difficult to relate to nutrient concentrations. A more significant impact would more likely be caused not by chemical nutrients but by increases in particulate organic material from sewage outfalls.

Extensive blooms of phytoplankton, caused by increased concentrations of nitrogen and phosphorus in the water column, can cause hypoxic or anoxic conditions severe enough to lead to the death of fish and invertebrates, especially shellfish (see Diaz and Rosenburg (1995) and Paerl *et al.* 1998 for reviews). This would be a particular problem in stratified waters, either deep thermally stratified coastal waters, or in some estuaries where stratification occurs as a result of strong density gradients in the water column (with freshwater overlying saline water). In stratified waters the decomposition of large amounts of organic material that sinks toward, and onto, the sea bed uses oxygen in the bottom water layers which is not readily replaced by mixing of the surface and bottom waters. The dynamics of dissolved oxygen in coastal and estuarine waters have been used to characterise the anthropogenic impacts on water quality (Paerl *et al.* 1998). In the Baltic Sea changes in the distributions of the benthic fauna have been related to hypoxia and anoxia (Bianchi *et al.* 2000) caused by nutrient enrichment and increased phytoplankton production (Rosenburg 1990).

Low oxygen concentrations in the bottom water may lead to increases in the concentrations of H_2S in the sediment, and to a lesser extent in the overlying water. H_2S is toxic to most invertebrates, fish and plants but the toxicity varies between species. In general the resistance to anoxia and to H_2S toxicity in invertebrate species are related (Diaz and Rosenberg 1995) but the effects of the two may act synergistically further increasing the effects of the hypoxic/anoxic event. Diaz and Rosenberg (1995) concluded that many ecosystems that are now severely stressed by hypoxia appear to be at or near a threshold. Any further decrease in oxygen concentrations, even small decreases, could have catastrophic consequences on the systems including those that are the foundation of commercial fisheries.

There are three main problems in relating water quality standards or nutrient concentrations to these effects. Firstly, relating the spatial and temporal variations of nutrients to those of other contributory factors (including other stressors) and then to low oxygen concentrations. Secondly, disassociating the anthropogenic from the natural effects, as estuaries and coastal waters are often eutrophic naturally. Thirdly, the effects of hypoxia/anoxia may vary depending on the species affected by it, its duration and severity (Diaz and Rosenberg 1995). Moreover the effects may be sub-lethal, for example in depressing reproductive activity in which case the effects on population densities may not be apparent for several months. Thus any given condition of nutrient enrichment may or may not lead to hypoxia/anoxia depending on the coincident occurrence of other physico-chemical conditions (e.g. temperature and salinity gradients) and may or may not have an effect on the invertebrates and fish and any effect may not be detected easily.

Breitburg *et al.* (1999) examined the variability of the effects on an estuarine food web of elevated concentrations of trace elements (As, Cu, Cd, Ni) and nutrients. They concluded that the response to trace element additions varied more than that of nutrient additions, both spatially and between species. Most taxa increased in abundance with nutrient additions, but there were also nutrient/trace element interaction effects. In particular nutrient additions sometimes increased the toxicity effects of the added trace elements and this led the authors to conclude that high trace element concentrations may mask the effects of high nutrient loading in some estuaries. A general consequence of conclusions such as these are that the effects of one series of experiments or observations may not easily be extrapolated to other natural situations where other compounding factors may be present.

The Infaunal Trophic Index (ITI UK) uses benthic invertebrates in sub-tidal soft sediments to assess whether sites are 'polluted', or not (Perkins M, personal communication). This index does not specifically relate changes to nutrient concentrations, but could be used to indicate indirect effects of eutrophication, such as increased organic matter from decaying macroalgal blooms.

Migratory Fish

Several fish species of rivers and lakes are migratory. Their populations in fresh waters being dependent on water quality in transitional waters. Many estuaries in the UK have been severely affected by nutrient and organic pollution to the extent that their resident fish populations have completely disappeared and migratory fish movements completely blocked (Elliot Griffiths and Taylor 1988). Some of the changes and mortalities concerned have been due to toxins (e.g. Mersey estuary), but others have been due largely to the organic load, with the resultant severe or complete oxygen depletion. For example, loss of fish species in the Forth Estuary were due to the discharge of organic matter, the historic loading from enriched sediments and consequent low dissolved oxygen concentrations. Over the last decade, there has been an overall trend for increasing dissolved oxygen concentrations in the estuary and a good indication of the continuing long-term improvement has been the return of the Sparling *Osmerus eperlanus*, which now has a flourishing population there (Forth River Purification Board. 1993).

4.4.4 Summary

Despite the large-scale spread of inorganic nutrient pollution in estuarine and coastal waters there is a marked lack of data with which any realistic assessment of the relationships between either benthic macroinvertebrates or fish and (inorganic) nutrients in UK transitional and coastal waters could be based. What data do exist are based largely on 'water only' acute toxicity tests, mainly in the US, (although there are some U.K. sediment data for ammonia) and hence do not reflect benthic conditions for either epifaunal or infaunal species. There is some evidence that the ecotoxicology of indigenous species for one country cannot be applied to those of another e.g. between the US and New Zealand. In addition, the majority of tests have been confined to freshwater aquaculture, either fish or commercially important crustaceans. However, the validity of some of the freshwater (and estuarine) experiments maybe bought into doubt when the real salinity of the water used in the toxicity trials is considered. For example, the addition of 8 g l^{-1} $NaNO_3^-$ to freshwater or estuarine water will increase the salinity of the test water by 8‰, whilst this may seem obvious, it is certainly not appreciated by many of the researchers or agencies who have conducted toxicity tests. Some data indicate that juvenile organisms are more susceptible than adults. There is a clear need for long-term chronic toxicity trials for both benthic macroinvertebrates and fish of transitional and coastal waters with ammonia, NO_2^-, NO_3^- and phosphate, under conditions that reflect realistic salinities (critical), using UK species and *in situ* (spatial and temporal) concentrations of these inorganic nutrients (critical). In any long-term toxicity trials attention will have to be paid to the maintenance of pH and appropriate concentrations of Cl- ions, as both are critical to determining the toxicity of both ammonia and nitrite. The data are now available for UK estuaries that would enable the selection of both high and low nutrient toxicity studies on comparative species.

The indirect interactions that may arise from the hypernutrification of estuarine and coastal waters, namely hypoxia and sulphide poisoning via the degradation of organic matter, have received a great deal of attention and the subject has previously been well reviewed. However, there is little information available concerning the direct association between nutrient concentrations and the distribution and abundance of invertebrates and fish in estuaries and coastal waters, and even less data from experiments that might have ecological realism. The greatest effects of increased concentrations of nutrients, particularly N and P, on the invertebrate and fish fauna in estuarine and coastal waters will be indirect, predominantly by changing the distribution and abundance of phytoplankton (potential food) and the intertidal and shallow subtidal aquatic vegetation. Relating concentrations of nutrients to any effect they have on the invertebrates in these ways will be difficult, if not impossible because of the unpredictability of the relationships and the presence of many other confounding factors that also affect invertebrate distribution and abundance.

4.5 Summary

Salinity is the primary determinant of species composition in transitional waters; very few species can tolerate such fluctuating salinity regimes. Despite this, changing nutrient conditions can have major effects on species compositions, but more significantly on species abundances. In fact, transitional waters can exhibit the greatest range of nutrient concentrations of any surface water type.

4.5.1 How sensitive are different groups of organisms to increased nutrient concentrations?

It is generally accepted that the primary producers are likely to be the most sensitive group to increased nitrogen and phosphorus concentrations. There are a number of examples where increased nutrients have resulted in increases in phytoplankton and macroalgal biomass. The clearest relationship between nutrient conditions and biological quality exists for phytoplankton abundance, with a number of studies demonstrating significant relationships between nutrient load to an estuary and the mean chlorophyll *a* concentration. That a number of estuaries do not show such a clear response suggests that not all estuaries can be treated the same. It appears that those estuaries with a macrotidal range are less sensitive to increased nutrient inputs and do not exhibit increased chlorophyll concentrations. In terms of macrophytes, seagrasses, brackish charophytes and other slow-growing macroalgae are the most sensitive to nutrient enrichment with numerous recorded losses around the UK and European coasts in response to eutrophication.

Invertebrate and fish responses to increased algal biomass can be separated into two types, direct and indirect. Direct, when an increase in algal biomass stimulates a proportional increase in an algal grazing organism, a response seen in mussels. Indirect responses largely result from the decomposition of algal biomass, when the organic C is concentrated at the bottom of the water column, resulting in anoxia. There is, however, little data from UK sources on this subject area.

4.5.2 Is ecological status influenced more by factors other than nutrients?

Salinity is the primary determinant of species composition in transitional waters; very few species can tolerate such fluctuating salinity regimes. The salinity at a fixed point in a transitional water body (estuary or lagoon) will vary according to the tide and also seasonally in response to variations in freshwater inputs. This means that the species composition at a fixed point is likely to change in response to variables other than nutrients.

Climate variability (rainfall, light and temperature) will also affect responses to nutrient conditions, particularly the spatial and temporal dynamics of phytoplankton.

4.5.3 To what extent can current EA monitoring and assessment practices fulfil the requirements of the WFD with respect to nutrients ?

Current monitoring is not sufficient for the WFD and there is an urgent need to develop standards methods and investigate the influence of sampling frequency. A full review of the extent of monitoring in marine waters and recommendations for future is provided in Annex 3 of the proceedings of the National Marine Monitoring Programme (NMMP) indicators workshop (National Marine Monitoring Programme 2001). Recommendations include monitoring of inputs of nutrients to the marine environment discharged by rivers, planktonic algal biomass (annual maximum chlorophyll *a*), duration of algal blooms, phytoplankton composition, opportunistic green macroalgal blooms and fish kills.

4.5.4 How and with whom (pan-European?) should the EA progress the future developments of monitoring and assessment practises?

To achieve a consistent approach to monitoring and assessment for the Great Britain Ecoregion, the EA needs to progress future research developments with SEPA and SNIFFER. Awareness of, and involvement in, EC coastal research programmes is also required to develop standardised and comparable approaches. Developments are being co-ordinated by the NMMP (http://www.marlab.ac.uk/NMPR/NMP.htm)

4.6 Recommendations for Phase II

Given that our transitional waters are such dynamic systems, monitoring and classification schemes must be structured in such a way as to take account of the great spatial and temporal variability that exists. We have very limited data that combines well-documented biological data with chemical data for transitional waters. It is imperative that long-term databases are built up so that we can properly quantify the natural range of variability that transitional waters exhibit. The rarity of baseline data or undisturbed sites is also likely to hamper the identification of reference biology or nutrient conditions.

Research requirements for WFD monitoring have been reviewed in Annex 3 of the proceedings of the National Marine Monitoring Programme (NMMP) indicators workshop (National Marine Monitoring Programme 2001). Recommendations included:

- Developing standardised methods for chlorophyll *a*, particularly identifying sampling frequency required for measuring the duration and magnitude of phytoplankton blooms
- Agreement of threshold algal densities for measuring the duration of algal blooms
- Inter-calibration between automated fluorometer measurements of chlorophyll *a* and Continuous Plankton Recorders (CPRs)
- The development of a UK phytoplankton trophic index (UK PTI)
- Developing remote sensing for monitoring the cover of macroalgae in inter-tidal areas
- Monitoring fish kills in association with harmful algal blooms

This study supports the development of chlorophyll *a* concentration as the most readily usable indicator for development in the short-term as it has already been shown to be significantly related to nutrient conditions in certain transitional waters and it is a relatively straightforward, rapid and cheap measure, requiring little expert knowledge. It must, however, also be stressed that high phytoplankton growth rates associated with nutrient enrichment do not always correspond to large final biomass. Factors such as predation and nutrient recycling greatly control the balance between growth rates and final biomass.

Abundance of bloom-forming macroalgae is similarly recommended as being sensitive to nutrient conditions. Macroalgal abundance is a little more difficult to quantify, but is less dynamic. More predictable patterns in their development may make them more suitable indicator organisms in the long-term in a monitoring scheme. Monthly monitoring of standing crop during the summer growing season has been shown to be effective in the monitoring of macro-algal blooms in the UK (Ythan Estuary and Langstone Harbour). The implications of reducing the frequency of this monitoring should be investigated and as highlighted in the NMMP report (2001), the effectiveness of remote sensing in mapping macroalgal blooms needs to be further explored.

There are a number of new approaches and other issues that are also worth considering in terms of the needs of the WFD:

Although benthic algae are not explicitly mentioned in the WFD, the use of diatoms as indicators of nutrient conditions is well documented for lakes and rivers. A diatom-phosphorus transfer function has recently been developed for coastal lagoons (Martin, 2002). It quantitatively relates diatom compositional data to nutrient concentrations and separates out the effects of nutrients from salinity. Its advantage in transitional waters is that it uses surface sediment assemblages which integrate the ecological response over space and time. It is possible that this approach could be adapted for assessing ecological responses to nutrient conditions in estuaries. Another alternative is the trophic diatom index, developed to monitor rivers (see Section 2.3). A study of its suitability for benthic diatom species in estuaries has recently commenced (Underwood G, personal communication).

Delivery of nutrients from underwater sediments may extend the eutrophication process for many years. The use of nutrient concentrations within sediments as an indicator of *potential* response could be further investigated and could be developed to identify sites that may have problems achieving good ecological status.

The issue of how water quality barriers in transitional waters affects fish species diversity in fresh waters upstream needs to be addressed in the WFD. A review of methods currently being employed in measuring fish populations in transitional waters in the UK would be of great value. Currently, much of the sampling is done by trawling and we need to understand what the results from this mean. Data from the Forth estuary, comparing demersal trawling with entrainment at power staions, indicated clearly that the former was inadequate in terms of understanding fish communiuties in the estuary (Maitland P, personal communication). The more recent use of pelagic trawls can overcome some of these problems. A better link with catches from commercial fishermen may also help fill in the picture.

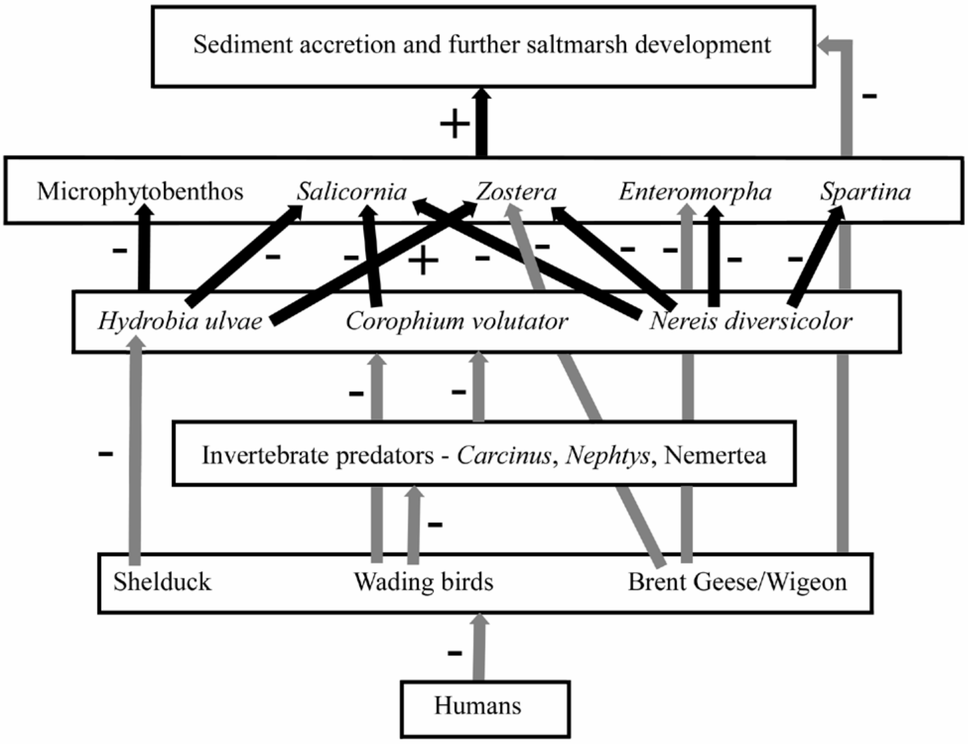


Figure 4.2 A simple interaction web for some common estuarine plants and invertebrates (Hughes, 2001)

5 COASTAL WATERS

5.1 Introduction

While our understanding of the effects on increased nutrient loadings have on algal growth and species composition in freshwater systems is based on a number of decades of research, it is only relatively recently that the potential problems associated with increased coastal nutrient loadings have been considered (Nixon 1995). A widely accepted position is that the algae from temperate coastal zones are limited by the amount and availability of nitrogen. The consequences of increased nutrient loading can range from the minor to the extreme, depending on the local conditions. Increased nitrogen loading can lead to a stimulation of algal growth, with possible biomass accumulation. Under some conditions, if production exceeds consumption, then increased growth will be followed by increased sedimentation of algal derived organic matter. The increased sedimentation of carbon can stimulate microbial decomposition and consume oxygen. With thermal stratification in the spring or summer months, bottom water oxygen concentrations can be greatly depleted leading to anoxia. This also occurs following the crash of macroalgal mats, such as *Ulva rigida* (Ceccherelli, Ferrari and Viaroli 1994).

Two important points need to be considered when discussing the relationships between chemical and biological measurements of water quality. Firstly, the range of nutrient concentrations in most coastal zones is smaller than those found in transitional waters, the low nutrient marine waters dilute the riverine inputs. Concentrations of nitrate are rarely greater than 20 mmol m^{-3}. Algae have high affinities for nitrate, ammonium and phosphate and ability to increase the efficiency of acquisition when the nutrient availability declines (Berman-Frank, Erez and Kaplan 1998; Collos *et al.* 1997; Harrison, Parslow and Conway 1989). The increased nutrient load is likely to affect the total biomass production, rather than the rate at which this biomass is achieved calculated on a biomass basis. It is therefore important to understand the differences between growth rates and final biomass. In general increases in nutrient concentrations in coastal waters have not been of the same magnitude as lake and transitional waters because the algae are acclimated to relatively low concentrations, small increases can potentially have a disproportionate response. Secondly, while the WFD restricts itself to nitrogen and phosphorus as the key nutrients in influencing biological change, it must be remembered it is difficult to separate the direct effects of nutrients on the growth of algae from light and temperature, which also play a vital role in the photosynthetic process.

There is potentially some confusion as to the definition of coastal waters. As described by the WFD “coastal waters” means surface water on the landward side of a line every point of which is at a distance of one nautical mile on the seaward side from the nearest point of the baseline from which the breadth of territorial waters is measured, extending where appropriate up to the outer limit of transitional waters. In “Marine and Coastal Environment Annual topic update, 1999” (Topic Report No11/2000) the EEA reported on an indicator questionnaire sent to each EEA member country. They considered the width of the coastal zone as being 20 km from the outer coastline. For the purposes of this review phytoplankton will be restricted to continental shelf waters mainly in the temperature latitudes.

5.2 Phytoplankton

5.2.1 Composition

Initial studies of the effects of nutrient enrichment on phytoplankton productivity concentrated on the straightforward link between nutrient load and phytoplankton response. While this revealed considerable information, it soon became clear that there are a number of interacting parameters that influence the phytoplankton response. One such is the ratios of nutrients involved in growth. The cellular composition of organic material under normal growth conditions is 16 N atoms for every atom of P. This ratio is termed Redfield ratio after the original observation. For diatoms, which have an absolute requirement for Si, the normal ratio of Si:N is 1. Si is unlike N and P because the Si concentration of river and estuarine water entering the coastal zone has not increased. The Si biogeochemical cycles have not been altered to the same extent as those of N and P by human activities. So time series that show trends of increasing N concentration usually show trends of decreasing Si:N.

- Irish Sea in 1960 had a Si:N ratio of 1.3 but by 1982 the Si:N ratio had declined to 0.9 (Allen *et al.* 1998b).
- German Bight, (Radach, Berg and Hagmeier 1990) reported that by 1984 the Si:N ratio had fallen to less than 0.1.
- Bay of Brest the Si:N ratio was 2.0 in 1976 and had declined to 0.4 in 1992 (Le Pape *et al.* 1996).
- Mississippi River the Si:N ratio steadily declined from 4.5 in 1960 to less than 1 by 1980, after which there was no further change (Rabalais, 1996).

This alteration in nutrient resource ratios can have a profound effect on the selective advantage of diatoms over other algal groups. Diatoms are frequently the primary blooming group. By reducing the relative availability of Si, increasing N and P concentrations allow the progressively increasing importance of other groups, such as dinoflagellates and phytoflagellates. For example the proportion of diatoms in the Black Sea has declined from 90% of the assemblage in the 1960's to less than 40% in the 1980's. Since European settlement of the Chesapeake Bay area began in the 1600's the proportion of centric to pennate diatoms in the Bay sediments has increased from less than 1 to the current ratio of 8 (Cooper 1995). In the Wadden Sea the change in nutrient ratios has seen the appearance of the prymensiophyte *Phaeocystis* sp. In this case there was a significant change in the N:P ratio in the late 1970's, from 40 to less than 16. *Phaeocystis* sp. is efficient at growing under N-limited conditions where nitrate, rather than ammonium, is the source of N. There was a steady increase in the duration of the bloom season, from 40 days in 1975 to over 300 days in 1984 (Cadee 1992; Philippart and Cadee 2000; Philippart *et al.* 2000).

While we have some understanding as to what the prevailing conditions are when a phytoplankton bloom occurs it is still a mystery as to why one species will become the dominant representative of an assemblage at a given time and place. Recent studies have begun to reveal that individual species have different requirements for nutrient resources and respond differently to environmental changes. As already mentioned phytoplankton groups appear to have different light growth responses, Dinophyceae (dinoflagellates) being able to grow at lower light intensities than the Bacillariophyta (diatoms), which are able to grow at lower light intensities than the Chlorophyta (green algae) (Raven and Richardson 1986). From a slightly different perspective,Tang (1996) concluded from a literature review that Dinoflagellates have substantially lower growth rates than other taxa of similar size and related this reduction in algal growth to two parameters, the carbon content and the ratio of Chlorophyll *a* : carbon. The basis of most of these differences is cell size, the ability of a cell to assimilate nutrients by diffusion is proportional to the inverse of the radius^2 (Raven 1984). A clear example of nutrient requirement is that of silica by diatoms. For micronutrients, such as vitamins, clear differences in requirements have been reported at the species level ((Guillard *et al.* 1991), (Honjo 1993)), and trace metals ((Sunda 1989), (Price and Morel 1991)). What is still to be understood is how these resource requirements are influenced by the environmental cues, such as light intensity and temperature.

Phytoplankton have different strategies in responding to varied nutrient supply. Nutrients can be quite patchy, for example, clouds altering light intensity, waves acting as lens for light, the mixing of waters with different N and P concentrations. The spring bloom in temperate regions is often dominated by large diatoms that are able to accumulate nutrients and use them as required, termed the storage response by Collos (1986) where there is an uncoupling between uptake and growth. Sommer (1989) used the terms “growth strategists" for those algae with a rapid increase in growth after enrichment and "affinity strategists" or "storage strategists" for those with a weak or no growth response to enrichment. It should be noted that there is some confusion in the terminology in this field of study. Smayda (1997) when considering harmful algal blooms, made the distinction between “growth strategy species” with high Vmax, “storage strategy species” with high internal stores and “affinity strategy species” with low Ks. Smayda possibly confused uptake (Vmax) with growth (μ), because a high Vmax does not necessarily mean a high growth rate. This is probably a result of the paper by McCarthy and Goldman (McCarthy and Goldman 1979) who assumed an instantaneous response of growth rate to elevated uptake rate. Later, Goldman (1984) acknowledged the error and wrote (p. 147) that the relationship between Vmax and growth rate in McCarthy and Goldman (1979) "obviously was inconsistent", but this review article probably had less exposure than the original paper, and so the misconception still survives today.

That different species of phytoplankton have different affinities for the same substrate has been repeatedly demonstrated for a wide range of nutrient resources. One such example is that of Collos *et al.* (1997) who reported that the diatoms *Chaetoceros* sp. and *Skeletonema costatum* had high affinities for nitrate during a spring bloom where as *Thalassiosira* blooms later in the year had lower affinities for nitrate. They were able to show that the uptake characteristics of samples taken from natural populations were similar to those observed in laboratory culture. The work of Harrison *et al.* (1989) should always be kept in mind during discussion on the subject of resource uptake characteristics because the conditions under which the algae are grown in the laboratory will play a large part in deciding the observed parameters.

Having discussed the range of resource acquisition strategies exhibited by phytoplankton the complex matrix of nutrient demand, availability, light and temperature makes profiling each species a formidable task (Richardson 1997). The understanding of the seasonal phytoplankton succession of lakes is more advanced than the marine system probably because lakes are more accessible and have been studied to a greater degree for a longer time. In general the spring bloom is associated with larger cells while smaller cells dominate the summer stratified period when nutrients are not so plentiful. The general pattern of phytoplankton succession, at least for seasonally stratified waters, has emerged but not yet at the species level.

An important contribution to this field is the work of Smayda and Villareal (1986) who studied the relatively long species database on species distributions of the Narragansett Bay where in 1985 the "brown tide" bloom of *Aureococcus anophagefferens* occurred. They suggested that a characteristic feature of this area was the existence of an "open" niche during the summer months in which flagellates or other non-diatomaceous phytoplankton often formed blooms. They point out that this "open niche" makes for a poor predictive capability with respect to which species will dominate the bloom. Further they concluded that the predictability is compromised by interannual variations in key determinants of niche availability, occupancy and interactions and hystersis effects from the spring diatom bloom. In the 1985 "brown tide" they reported that 15 species bloomed between late July and the end of October. Two species, *Minutocellus polymorphus* and *Fibrocapsa japonica* were just as novel blooming species as the case of *Aureococcus anophagefferens* was for this region.

Harmful phytoplankton blooms

The relationship between increased nutrient concentrations in estuaries and coastal systems and the perceived increase in harmful phytoplankton blooms is still strongly debated by the various groups involved in this area of study. This discussion will restrict itself to a few points that highlight some of counter positions that have been put forward.

Harmful algal blooms describe a condition when some phytoplankton reach critical concentrations and have a detrimental effect on the ecosystem. This effect can range from a slight inhibitory effect on other algae (Windust, Wright and McLachlan 1996) or zooplankton, to the extreme toxic effects of amnesic shellfish poisoning (ASP) and paralytic shellfish poisoning (PSP). There are a number of phytoplankton species, which can cause considerable economic damage to the aquaculture industry. One example was the *Chrysochromulina polylepis* bloom in the Skagerrak / Kattegat during May 1988. Massive losses were incurred by the salmon farms along the Swedish and Norwegian coasts (Moestrup 1994). There are numerous examples of such events, (see Richardson 1997).

The question as to whether anthropogenic increases in nutrient concentrations have stimulated the frequency of harmful phytoplankton blooms has been repeatedly answered in the affirmative by a number of workers in the field (Anderson, 1989; Smayda 1990; Hallegraeff 1993; Burk, Usleber and Martlbauer 1998) and commentators (MacGavin, 2000). It is very difficult to decide if the perceived increase is due to increased nutrients or increased monitoring. It is clear we are much more aware of such events, as each year brings notification of closure of shellfish areas in response to ASP and PSP. Some have argued, typified by MacGavin (2000) that on the balance of probabilities it seems that increased nutrient concentrations benefit many toxic species and even though there will not be an increase in toxins per cell the increase in cell density will have the same effect with an increase in overall toxicity. It is not clear whether this means that toxic algae have some sort of competitive advantage over non-toxic species or whether some toxic species will be stimulated by increased nutrient inputs. Richardson (1997), in a careful study of the literature suggested there was little evidence to suggest that toxic species had any competitive advantage over non-toxic species. Indeed, it seems that of 15 orders listed by Sournia (1995), 9 orders possessed harmful bloom species and 5 orders possessed toxic blooms species. The number of species that have the potential to form harmful or toxic blooms represent great taxonomic diversity. Sournia (1995) argued that there was “no hope of defining a single algal type or target organism for use in understanding, modelling or protection against” harmful or toxic algal blooms.

Influence of factors other than nutrients

While much attention has been directed towards the response of biology to changes in nutrient concentrations there are other factors that can influence phytoplankton biomass and species composition. Climate is probably the most variable parameter. Reid, Holliday and Smyth (2001) analysed the Continuous Plankton Recorder data for the North Atlantic and North Sea and has suggested that in 1988/9 and 1998 the North Sea experienced two episodic shifts in plankton abundance and composition that had little to do with nutrient change. These shifts were caused by inputs of oceanic waters into the North Sea. The CPR is primarily used for zooplankton as the mesh size, 260 µm, is much greater than the majority of phytoplankton but it is still useful as the zooplankton are dependent on phytoplankton as a food source.

Tett (1992) and Smayda and Reynolds (2001) have both suggested that there is a high degree of unpredictability about which species will be the blooming species, selection being stochastic, e.g. bloom species are often selected as a result of being in the right place at the right time at a suitable inoculum level. Tett (1992) goes so far as to suggest there is little evidence of site-specific flora, assemblages being more diverse than would be expected, hence the composition being largely decided by chance. The dispersive nature of the pelagic environment may reduce the sensitivity of plankton to nutrient enrichment but the combination of eutrophication and climate change can alter pelagic assemblages, especially in enclosed waters.

Studying the distribution of fossil dinoflagellate cysts Dale and Nordberg (1993) suggest that *Gymnodinium catenatum* bloomed in the Kattegat-Skagerrak region periodically over the last 2000 years ago, long before anthropogenic activities could have influenced these waters. For the last 300 years these workers found no evidence of this dinoflagellate. They consider the most important regulating factor of these ancient blooms was climate change with the blooms occurring during periods with relatively warm water temperatures.

Classification and monitoring schemes

As discussed in the transitional waters section, to be able to monitor changes in species composition requires a high degree of technical expertise and is labour intensive. Certain species will only bloom for a short period of time, days to a couple of weeks. This needs to be considered when species compositional changes over a growing season and longer term studies are being instigated. In general increased nutrient concentrations or changes in nutrient ratios will alter species composition, with smaller faster growing species out competing larger slower growing species in the spring bloom.

5.2.2 Abundance

Changes in phytoplankton assemblages with increased nutrient loading
There are numerous examples of increases of nitrate concentrations in rivers of Europe and North America. In some rivers this increase has been relatively recent, for example it was only after 1975 that an increase in nitrate concentrations was observed in the Vilaine River (Moreau, Bertru and Buson 1998). The increases in concentration have shown a very strong seasonal spread. In 1972 the nitrate concentrations were less than 200 mmol m^{-3} with little seasonal variation. By 1992 the maximum concentration was greater than 1800 mmol m^{-3} and yet the minimum concentration was still less than 200 mmol m^{-3}. For the Ythan river in the North East of Scotland, Balls *et al.* (1995) reported that the nitrate concentration increased 4 fold between the late 1960's and the early 1990's. Most of the acceleration in the increases in nitrate concentration has been linked to changes in land use.

There are few continuous observational programs but some have clearly reported significant increases in N and or P concentrations in coastal ecosystems:

- The Black Sea: from 1970 to 1990 the concentration of N increased from 60 to 180 mmol m^{-3} nitrate and from 1 to 9 mmol m^{-3} phosphate. It is interesting to note that the concentration of phosphate had been low and constant throughout the 1960's but while there was a steady increase in nitrate, the increase in phosphate was abrupt and concentrations then varied throughout the 70's. This is an indication that the processes of biogeochemical cycling N and P often show differing patterns of activity (Mee, 1992).

- The Baltic Sea: in the bottom waters of the central Baltic Sea between 1970 and 1990 the concentration of nitrate increased from 4 to 11 mmol m^{-3}, while between in 1960 and 1990 the concentration of phosphate increased from 1 to 3 mmol m^{-3} (Nehring, 1992).

- The Outer Archipelago Sea, northern Baltic: between 1970 and 1992 the concentration of Total N increased from 13 to 28 μM and Total P increased from 0.4 to 0.9 mmol m^{-3} (Bonsdorff, 1997).

- Irish Sea: between 1960 and 1984 the total organic N increased steadily from 4 to 8 mmol m^{-3} and soluble reactive P from 0.5 to 0.9 mmol m^{-3} (Allen *et al.* 1998b).

There are other observations that indicate that not all coastal zones have experienced increases in nutrient concentrations. For example (Bock, Miller and Bowman 1999) reported that between 1982 and 1996 there was no evidence to suggest that nutrient concentrations had changed significantly with time in the Firth of Clyde.

For many locations where the problems associated with increased coastal nutrient loading are studied it is important to understand that because continuous monitoring has only started recently there is no baseline data available. Most of the conclusions derived from these studies are based on a situation where the nutrient concentrations are already relatively high.

To investigate the effect of increased nutrient loading it is necessary to have a measurable response to the signal. These typically include biomass determined as the proxy of chlorophyll *a*, primary production or oxygen concentration. Each parameter has its strength and weaknesses. The chlorophyll a content of a phytoplankton cell shows a remarkable range depending on the algal group, the size of the cell and the prevailing light and nutrient status of the water. Primary production can be defined in a number of different ways and measured using a variety of techniques, which can make comparisons between research groups remarkably difficult.

Chlorophyll concentrations

There are some reports that show coastal systems respond to increased nutrient loading with increased biomass (Boynton *et al.* 1996 and Meeuwig 1999), but Cloern (2001) suggests that there might be a significant difference between freshwater and marine systems as the models which come from these studies show the chlorophyll yield, per unit N delivery, is 10 times smaller than the mean chlorophyll yield derived from comparable lake models. Borum (1996) in a literature study of coastal water and mesocoms studies reported that there was only a weak correlation between nutrient loading and the phytoplankton responses of biomass and primary productivity.

Again it has to be stated there are very few examples of time series that show clear trends of change in phytoplankton biomass with increased nutrient loading.

- Irish Sea: Allen *et al.* (1998b) reported chlorophyll concentrations of 1 mg m^{-3} in 1966 and as high as 5 mg m^{-3} in 1986, but with a very high degree of interannual variability, for example, 1 mg m^{-3} in 1985.
- Wadden Sea: Cadee (1992) reported that chlorophyll concentrations increased from 4 mg m^{-3} in the early 1970's to greater than 10 mg m^{-3} in the 1980's. Again the interannual variability was considerable with values as low as 5 mg m^{-3} being recorded in the 1980's.

It is noteworthy that the Bay of Brest, which showed decades of increasing nutrient concentrations did not show a response in the phytoplankton biomass (Le Pape *et al.* 1996).

Primary production

- Kattegat, by comparing mean annual monthly cycles for 1954 to 1960 with 1984 to 1993, Richardson and Heilmann (1995) were able to show there had been a considerable increase in the spring bloom, 8 to 47 g C m^{-2} mo^{-1} and the summer bloom, 10 to 45 g C m^{-2} mo^{-1}.

- Wadden Sea, de Jonge (1990) reported that the annual production increased from 35 g C m^{-2} y^{-1} in 1950 to 200 g C m^{-2} y^{-1} in 1974 and reached 540 g C m^{-2} y^{-1} in 1982.

- Adriatic Sea, Solic *et al.* (1997) reported a steady increase in annual production from 1962 (100 g C m^{-2} y^{-1}) to 1982 (250 g C m^{-2} y^{-1}).

Most primary productivity measurements are based on ^{14}C assimilation studies. Richardson and Heilmann (1995) were able to do this for the Kattegat because the methodology was developed by Steemann Nielsen (1952) in these waters. Richardson

(1991) makes the point that the methodology has been adapted by many labs to "local conditions" such that the inter laboratory comparison resulted in a very wide range of estimated primary production values.

As there is so little firm documentation of direct responses of phytoplankton biomass or productivity it is worthwhile considering potential proxy measures. One such measure is the sedimentary record. Cooper and Brush (1991, 1993) have reported on variations in pollen, diatoms, concentration of organic carbon, nitrogen, sulfur, acid-soluble iron, and an estimate of the degree of pyritisation of iron as indications that sedimentation rates, anoxic conditions and eutrophication have increased in the Chesapeake Bay since the time of European settlement. For example, there was a steady increase in organic carbon between the 1920's up until the 1980 after which there was a rapid increase.

Influence of factors other than nutrients
Another source of information concerning the status of phytoplankton biomass is the Continuous Plankton Recorder (CPR) survey, which started for zooplankton in the North Sea in 1950 and for phytoplankton in 1958. Jickells (1998) concluded that while there were changes in phytoplankton abundance the scale and timing were much more likely to be the result of climatic factors than a response to nutrient inputs. This opinion is slightly contradicted by Berry (2000) who citing the work of Reid *et al.* (1998) concluded that between 1948 and 1995 there has been a growing trend in the average amount of phytoplankton in the North Sea and central north-east Atlantic, and that this increase has been very rapid since the mid-1980s. At the same time a decrease in the average amount of phytoplankton has been witnessed in the northern reaches of the Atlantic between Iceland, Norway and northern Scotland. Berry (2000) agreed with Jickells (1998) that the changes recorded in the CPR were likely to be related to climatic changes, specifically those influenced by the North Atlantic Oscillation (NAO). These include such factors as wind strength, temperature, circulation and wave height, which influence the status of phytoplankton.

Classification and monitoring schemes
The two primary techniques for determining algal abundance are based on chlorophyll *a* and primary productivity measurements. While both have their limitations they have an advantage over species composition analysis in that they are straightforward and require little technical skill. To be able to fully integrate the course of a bloom using these techniques, intenive sampling would be required. This can become expensive.An alternative technique is to use continuous fluorometry measurement (Allen *et al.* 1998a; Althuis *et al.* 1994; Tett *et al.* 2001). These recordings generate considerable data; the relationship between fluorometric measurements and chlorophyll concentrations needs to be calibrated for the prevailing conditions.

5.2.3 Bloom frequency and intensity

Phytoplankton blooms generally show a latitudinal effect. This is primarily as a result of hydrographical conditions. A bloom will take place when the conditions of light and nutrients are such that production will exceed consumption. In arctic latitudes there is a single bloom event with development of the grazing population occurring as the phytoplankton bloom reaches its biomass maximum. In temperate regions there are frequently two phytoplankton blooms, one in the spring and then in the late summer to early autumn. In this region the surface waters are well mixed with nutrient rich bottom water in the winter, and with increased light intensity and duration in the spring, blooms will occur. The grazing population increases soon after the bloom is initiated. During the summer months the phytoplankton population is limited by nutrient availability. As microbial decomposition of the spring bloom releases nutrients back into the water column a secondary bloom will occur towards the end of the year. It should be noted that during the summer months, when nutrient availability is low, fresh pulses of nutrients can be introduced to the coastal surface waters over a short period of time. Such pulses are often caused by a change in the climate, which lead to mixing of stratified waters and can stimulate a phytoplankton bloom. In tropical areas the water column is often stratified so that the surface waters have a high light environment with low nutrients. The grazing population is tightly coupled to the phytoplankton so the intensity of blooms in this region is not nearly as great as the arctic and temperate regions.

The occurrence of the temperate spring bloom is predictable but the timing of it can vary from year to year. This has been long recognised (Bigelow, Lillick and Sears 1940) but it is only recently that the consequences of the time of bloom can have on the role of the bloom in the marine ecosystem. As mentioned previously, the lag between the initiation of the phytoplankton and the grazing population increases towards the arctic region. The growth rate of zooplankton grazers is temperature dependent. Townsend *et al.* (1994) developed a numerical model to investigate the effect of temperature at the time of bloom initiation in the Gulf of Maine. Comparing a cold year, 1974 with water temperatures during the bloom of 0-2°C with a warm year, 1978 with water temperatures 2-4°C revealed that the zooplankton bloom was delayed by two weeks in the cold year compared to the warm year. This had the effect of increasing the net phytoplankton production by 7% over the warm year.

Generally phytoplankton blooms have been studied in the surface waters of the coastal zone. This is probably a reflection of the ease of sampling and that high biomasses are usually found in this location. It has become increasing clear that once stratification of the water column occurs and nutrients in the surface layer become depleted, subsurface chlorophyll maxima can be found at the pycnocline during the late spring and summer months. The concentrations of chlorophyll can be similar or greater than those of the spring bloom. Richardson and Pedersen (1998) have estimated that 20% of new production in the North Sea takes place in subsurface chlorophyll peaks, which occur in the summer months. The ability to grow at subsurface locations is a reflection that many dinoflagellates have light requirements best met by the lower photon flux densities found at the pycnocline, (Raven and Richardson 1986). These pycnocline locations can be important sites where surface blooms are initiated. The cells are mixed into surface waters in frontal zones where stratified and mixed waters meet (Holligan 1979). Often coastal blooms are not initiated where they are observed.

An important feature of phytoplankton blooms is that it is necessary to make the distinction between an increase in an individual species representation in a bloom and whether there is an overall increase in primary production in a series of blooms. An example of this is the May 1988 Chrysochromulina polylepis bloom in Skagerrak – Kattegat. In the years between 1987 and 1993 the concentrations of surface chlorophyll and primary production showed little interannual variability (Heilmann, Richardson and Aertebjerg 1994), yet in only a single year C. polylepis was identified as a major component of the phytoplankton assemblage. Richardson (1997) has suggested that the reduced variability seen in primary production rates relative to abundances of individual species may make it possible to identify trends with shorter time series from data.

There are a number of frequently cited examples of areas exhibiting an increase bloom frequency. One such is the Seto Inland Sea in Japan (Prakash 1987) where the number of red tide outbreaks increased from 45 in 1965 to over 300 in 1976. Following reduced chemical oxygen demand values with increased water quality in the late 1970's the number of outbreaks declined. Most of the other examples of increased harmful blooms also come from semi-enclosed marine areas, such as Baltic, Black and North Sea (see above) but there is little quantitative data to suggest an absolute increase in harmful algal blooms. Richardson (1997) concluded that the time series upon which these increases have been demonstrated are not, biologically speaking, very long. Until longer data series become available, it is difficult to ascertain with any degree of certainty that the increases observed are true increases and not simply an expression of the naturally occurring changes in species abundance.

Influence of factors other than nutrients

The question for this section is what effect have increases in riverine nutrient concentration had on the coastal zone where the residence time is short. There are three properties of estuaries that can significantly attenuate the impact of increased riverine nutrient concentrations. The first, conservative mixing behaviour, occurs when the decrease in nutrient (N, P or Si) is the same as the increase in salinity the nearer the mouth of the estuary. A deviation from this relationship indicates that additional processes are influencing the cycling of nutrients. If there were more nutrients at a particular point in the estuary than would be expected this would indicate production of the nutrient, i.e. phosphate release from sediments. Similarly, if the concentration were less than expected this would indicate assimilation of the nutrient and its removal from the system. There are two sites where this can happen, the pelagic and the benthic zones. The pelagic is dominated by the phytoplankton and the benthic environment strongly influenced by the bacteria.

A number of researchers have shown that the various parts of estuaries can act as sinks for nutrients, i.e. they remove nutrients from the water column, or they can act as sources for nutrients, i.e. they introduce nutrients into the water column (Magnien, 1992; Morris, 1995; Nedwell, 1996; Ogilvie, 1997; Sanders, 1997; Trimmer, 1998; Cabecadas, Nogueira and Brogueira 1999). Phytoplankton and sediments can be sinks for nitrate while sediments can be sources of ammonium and phosphate. The ability of an estuary to attenuate the nutrient concentrations will influence the amount of nutrient entering into the coastal waters. For example Sanders, Klein and Jickells (1997) reported that the Great Ouse estuary, Norfolk UK was a minor sink for nitrate and nitrite (8%), a modest sink for silicate and phosphate (12 and 22%, respectively) and a large source of ammonium (95%). Trimmer *et al.* (1998) reported that substantial amounts (49%) of nitrate flux into the sediment are lost to the system when converted to ammonium by denitrification. Estuaries have the potential to greatly reduce the amount of N, P and Si entering the coastal zone. Jickells *et al.* (2000) estimates that 90% of the intertidal and sediment accumulation capacity of the Humber estuary has been lost to reclamation since the end of the Holocene, 3000 years ago. They speculate that with today's nutrient loading budget and the Holocene geomorphology the amounts of nutrients entering the coastal waters would be greatly less than today's fluxes.

The potential for changes in the biogeochemical cycling of nutrients in an estuary influencing the timing, species composition and size of coastal phytoplankton blooms is considerable. One example is from the Great Ouse, which Fichez, Jickells and Edmunds (1992) divided into two periods. The first, between September and January phytoplankton development inhibited by high suspended particulate matter loads and decreased light penetration. The estuary is characterised by low chlorophyll *a*, an increase in POC:PON ratio and conservative pattern of nitrate and silica. The second period, between March and August, is marked by a phytoplankton bloom associated with high chlorophyll with phytoplankton development confined to the estuary. The timing and composition of this river estuary bloom can influence the characteristics of the plume bloom. While the nutrients are sequestrated in the estuarine phytoplankton their movement into the coastal zone is delayed. The growth of large diatoms and their deposition into sediments will reduce the amount of silica entering the coastal zone thus altering the N:P:Si ratio to favour non-silica requiring species.

Responses such as changes in biomass, primary production and dissolved oxygen are the last visible responses to chronic nutrient enrichment. Cloern (2001) suggests that it is important to understand that there are early responses to enrichment that can be detected. These include changes in assemblages at species level, changes in the rates of biogeochemical cycling and shifts in seasonal patterns or magnitude of variability. One such example is the shift in the seasonal development of phytoplankton biomass in the Bay of Brest (Chauvaud *et al.* 2000) where the spring blooms have become smaller in amplitude and the summer biomass has become greater. The shift appears to be caused by changes in the elevated inputs of N from the watershed and increased biomass and grazing by benthic suspension feeders, resulting in removal of Si from the pelagic zone.

There are a number of reasons why some coastal zones are more sensitive to nutrient enrichment than others. These include: (i) the tidal amplitude - the San Francisco Bay and Chesapeake Bay systems have similar nutrient loads but the former has lower phytoplankton biomass because the tidal amplitude is greater. This explains why the primary responses to enrichment is most extreme in areas small tidal energies such as the Baltic, Adriatic, Wadden and Black Seas; (ii) the horizontal transport processes that determine the residence time with in coastal basins (Lucas *et al.* 1999). Ecosystems with slow transports and long residence times tend to retain exogenous nutrients (Nixon *et al.* 1996); (iii) the optical properties of the coastal zone will greatly influence the level of primary productivity.

5.2.4 Summary

Phytoplankton in coastal waters have exhibited a range of responses to increased nutrient concentrations. These range from no observable effect to the replacement of all species by one particular species. What is clear is that it is often the local physical environment which will determine whether there is an observable response to increased nutrient concentrations. Such conditions are the restricted exchange of water so that marine waters do not dilute the riverine nutrient inputs. There are very few long term records which have the combination of nutrient and biological data. This has been a serious handicap in developing methods to monitor the biological response to increased nutrient concentrations.

The relationship between nutrient concentrations and phytoplankton species composition, abundance and bloom frequency is complex as a number of other physical and chemical components are involved in algal productivity. Being able to separate the individual effects requires a greater understanding of the mechanisms of resource acquisition than we currently have. Blooms of individual species can last for periods as short as a week. It is unlikely that the monitoring procedures adopted will be able to fully cover the wide range of natural variability exhibited by phytoplankton. The long term objective of data analysis must be to extrapolate from relatively few sampling events the difference between natural variations with those caused by anthropogenic increases in nutrients. To be able to do this we require robust long term databases. It has been estimated that it will take up to 11 years to detect changes in the Kattegat arising from reductions in nutrient inputs over the natural variability.

For surveillance monitoring analysis of phytoplankton species composition is a useful guide to the physical and chemical environment, but this needs to be carried out over a long period of time before the true benefit can be gained. It requires a high degree of specialist knowledge and training and is labour intensive. Phytoplankton abundance is normally determined by chlorophyll *a* and or primary productivity measurements. Chlorophyll *a* determinations are by far the most common, the technique being simple and robust. Primary productivity measurements, while in principle are straightforward, can be beset with problems relating to definitions of what has been measured and artefact effects. For the monitoring of bloom frequency and intensity, the main techniques are again chlorophyll and primary productivity measurements. All of these techniques can deliver large amounts of information but inevitably they will reveal snap shots of the environment. New continuous monitoring technologies may overcome this but greatly increase the requirement for improved data handling procedures.

It should be understood that phytoplankton blooms are natural phenomena and are absolutely essential to the health of a wide range of ecosystems. There are reports from the paleorecord that show large blooms of dinoflagellates in areas where today they only form a small component of the species assemblage.

5.2.5 Recommendations for Phase II

A major undertaking would be the assessment of the available data relating algal species composition, abundance and bloom characteristics with the chemical environment. The objective of such a study would be to collate the data in a consistent database that would be freely available to researchers in the field. It is essential that the long-term databases be maintained so that methods can be developed to separate out changes in the natural variability of algal populations from that induced by changes in nutrients and climate.

The use of macroalgae as bioindicators using C:N ratio to show that the physiological status of the alga has been altered by changes in the chemical environment, needs development. As a refinement of this technique Rogers (1999) was able to demonstrate a clear link between the isotopic composition of *Ulva* sp. and their position relative to a sewage outlet. The use of stable isotope analysis has been limited due to the lack of suitable methods for measuring the isotopic composition of source inorganic nitrogen. These problems were recently overcome by Johnston (1999) who developed a robust method for measuring the $\delta^{15}N$ of nitrate.

The development of continuous fluorometry recordings to monitor coastal blooms in areas that are likely to be sensitive to changes in the chemical environment.

Major blooms are associated with river plumes entering into coastal waters. It has been suggested (Jickells *et al.* 2000; Jickells 1998) that changes in the sedimentary processes in estuaries could have a profound effect on the dynamics of blooms associated with river plumes. The suggestion that the reduction of sandbanks in estuaries has the effect of reducing the ability of estuaries to act as sinks for nutrients means that the export of riverine nutrients into the plume will increase. If this process is continuing it would be an opportunity to study over the next two decades how changes in the chemical environment influence biological quality in British coastal waters.

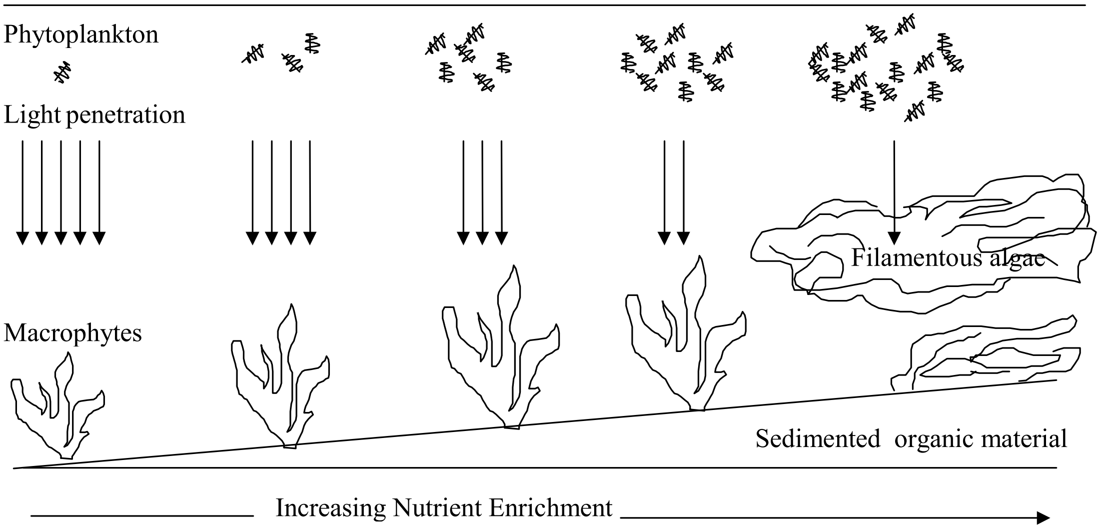
It would increase our understanding of the importance of interannual variability in algal population dynamics if there was better data concerning algal responses to moderate increases in nutrient concentrations. While it is more straight forward to observe responses to large chemical changes. We need to understand the processes relating to moderate enrichment and hopefully the responses to reductions in nutrient concentrations.

5.3 Macrophytes

5.3.1 Composition

When significant enrichment of a body of water occurs, increased productivity is often reflected in changes to local macrophyte communities. This is shown schematically in Figure 5.1.

Figure 5.1 Changes in productivity in coastal waters with increased nutrient loading (Adapted from Funen Island Council 1991, Raffaelli *et al.* 1998)



Schramm (1996) suggests that there is a series of successive phases with increased nutrient loading. Phase I represents marine or brackish shallow coastal waters characterised by a balanced nutrient regime i.e. the nutrient load is low and nutrient concentrations may temporarily become limiting to primary production. In shallow near-shore systems, the dominant primary producer communities usually consist of perennial benthic macrophytes such as seagrasses and other phanerogams on soft bottoms, or long-lived seaweeds on hard substrates, whereas seasonal macroalgae or phytoplankton play a lesser role in terms of biomass and production. Increased nutrient loading, however, favours these seasonal forms.

A second phase (II) from slight to medium increases in nutrient loading is therefore characterised by increasing blooms of 'nutrient opportunists', in particular fast-growing epiphytic macroalgae and bloom-forming phytoplankton taxa. In contrast, phanerogam and perennial macroalgal communities gradually decline, usually combined with a change in their depth distribution limits, and finally disappear. During phase II, parallel to the blooms or mass development of seasonal fast-growing seaweeds, an increase in unattached or free-floating macroalgae can often be observed.

With further increasing nutrient loads (phase III - eutrophication) free-floating macroalgae, alternating with heavy uncontrolled phytoplankton blooms, dominate and more or less completely replace the perennial and slow-growing benthic macrophytes. Finally, in phase IV, under hypertrophic conditions with continuously high nutrient concentrations, phytoplankton constitute the dominant primary producers.

As nutrient loading increases, there is a generally a corresponding increase in the productivity of benthic macroalgae, but, as previously discussed, some species flourish only at the expense of others (Schramm 1996). At nutrient-enriched sites, quantitative increases in benthic algal production are generally tempered by a number of qualitative changes, many authors reporting a general impoverishment of the flora and an overall reduction in species numbers and diversity (Fletcher 1996a). The loss of perennial macroalgae and phanerogams is particularly detrimental to the ecology of local waters as they provide a stable environment and invariably support a wide variety of associated marine benthic plant and animal communities. Ultimately, the altered ecological state of coastal waters could have a cascading effect on ecosystem functioning (Pihl *et al.* 1999). Annual green algae compete with other vegetation such as seagrasses, perennial macroalgae and benthic microaglae (Sündback *et al.* 1990, 1996, Vogt and Schramm 1991, Fong *et al.* 1993, Munda 1993, den Hartog 1994), and thereby alter the structure of associated benthic and epibentic faunal communities (Nicholls *et al.* 1981, Reise 1983, Hull 1987, Hall and Bell 1988, Raffaelli *et al.* 1989, 1998). A shift from late-successional species of brown algae or seagrasses to ephemeral algae often results in dramatic reductions in the distribution of littoral flora and drastic changes in the complexity of the vegetated habitat (Pihl *et al.* 1999).

Valiela *et al.* (1997) report on the extensive loss of seagrass habitat that has taken place as a result of the increased nutrient loading of many shallow, temperate coastal areas. This conversion from seagrass to macroalgal habitats is often considered a degradation of coastal environments because seagrass beds support a wide variety of consumers – some of commercial interest (Valiela *et al.* 1992) – and are nurseries for juveniles of many deeper water fish species (Valiela *et al.* 1997). In addition, both seagrasses and the larger perennial macroalgae provide sediment stabilisation and physical support for coastal macrophyte communities.

Influence of factors other than nutrients

The effect of increased nutrient loading on marine algae was established during the early part of this century when excess macrophyte growth in Belfast Lough, Northern Ireland, was attributed to the high concentrations of nutrient salts in the water originating from sewage (Letts and Richards 1911). The relationship between nutrients and excess algal growth was also established shortly after for several sites (Weymouth, Southampton Water) on the south coast of England (Letts and Richards 1911, Cotton 1910, 1911). Since that time, numerous authors have suggested a relationship between increased nutrient input and local changes in algal populations.

Although high concentrations of nutrients are generally recognised as the *prima facie* reason for many of the observed changes in benthic macrophyte populations, other environmental factors can also play a major role (Fletcher 1996a). As discussed in the section on transitional waters, probably one of the most important environmental factors that determine the response of algal communities to excess nutrient loading is the degree of 'exposure' at the locality. A characteristic of many of the affected areas, for example, is their relatively enclosed and sheltered aspect. In the British Isles these include the quiet Backwater area of Weymouth Harbour, the very enclosed, shallow harbour systems of Poole, Langstone and Portsmouth on the south coast of England and large, but enclosed areas such as Dublin Bay, Ireland. Under these conditions there may be restricted tidal flow and water exchange, whilst the absence of strong currents and breakers allow nutrients to accumulate in the water column. These conditions can cause changes in macrophyte community structure and, in particular, may promote the growth of bloom-forming macroalgae.

Classification and monitoring schemes

Several recent studies have documented comparisons of vegetation in areas of different trophic status. This provides a useful indication of how increased nutrient loading may affect our marine ecosystems. In the USA, Lapointe *et al.* (1994) describe differences in productivity, biomass and epiphyte densities of the seagrasses *Thalassia testudinum* and *Halodule wrightii* along onshore-offshore transects representing hypereutrophic, eutrophic, mesotrophic and oligotrophic communities. Seagrasses at the hypereutrophic and eutrophic strata had low shoot densities, low shoot production rates, low areal biomass values, low areal production rates, and high densities of attached epiphytes and mat-forming macroalgae. Seagrasses at the oligotrophic strata, on the other hand, had the highest shoot densities, highest areal biomass values, highest areal production rates, and typically the lowest or second lowest epiphyte level of all strata. A similar report has been made from the Aegean Sea, Greece, where Belegratis *et al.* (1999) examined epiphytic macroalgal assemblages growing on the brown alga *Cystoseira* at both eutrophic and oligotrophic sites. The authors suggest that the species composition of the epiphytes was exclusive for each locality, reflecting differences in the trophic staus of the two sites. In a subsequent study by Bachelet *et al.* (2000), the structure of macrophyte communities at three French lagoons with differing degrees of eutrophication was examined. The authors report that the intertidal area of Arcachon Bay (the least nutrient enriched site) was covered by dense, stable beds of *Zostera noltii*. In the Cerets Lagoon, described as marginally eutrophic, vegetation was dominated by *Ruppia cirrhosa*, whilst sporadic development of green macroalgae occurred in the spring. In the hypereutrophic Prevost Lagoon, macrophytes were represented only by opportunistic macroalgae that out-competed the other benthic macrophytes.

5.3.2 Abundance

Many of the reports highlighting the response of benthic algal communities to the impact of nutrients refer to changes in the abundance of certain algal taxa. Specifically, with increasing nutrient load the occurrence of excessive growths or blooms of certain macroalgae has been reported. Notably, these excessive growths are largely comprised of green algae (predominantly *Enteromorpha*, *Ulva*, *Chaetomorpha* and *Cladophora* spp.) and are referred to as 'green tides' (Fletcher 1974, 1996a, b). Table 5.1 lists coastal localities relevant to this review where an increased abundance of 'green tide' macroalgae has been reported.

Table 5.1 Examples of green tides reported from the coastal waters of the British Isles and Europe (Adapted from Fletcher 1996a)

Location	Algal taxa	Reference
British Isles		
Langstone Harbour, England	*Enteromorpha* *Ulva*	Nicholls *et al.* 1981, Dunn 1972, Soulsby *et al.* 1985, Montgomery and Soulsby 1980
Portsmouth Harbour, England	*Enteromorpha* *Ulva*	Soulsby *et al.* 1985
Thanet Bay, England	*Ulva*	Fletcher 1974
Holes Bay, England	*Ulva*	Southgate 1972
Dublin Bay, Ireland	*Enteromorpha* *Ulva*	Wilson *et al.* 1990, Jeffrey 1993
Europe		
Saint-Brieuc Bay, France	*Ulva*	Briand and Morand 1987, Le Bozec 1993
Alfaques Bay, Spain	*Ulva*	Martinez-Arroyo and Romero 1990
Corsica	*Chaetomorpha*	Grimes and Hubbard 1971
Naples Bay, Italy	*Ulva*	Golubic 1970
Laholm Bay, Sweden	*Cladophora*	Fleischer *et al.* 1985, Rosenberg 1985, Wennberg 1992
Island of Sylt, Germany	*Cladophora*	Grenager 1957, Bokn and Lein 1978,

The presence of macroalgal blooms or green tides along the coast may have profound effects on local macrophyte communities. As discussed by Schramm (1996), fast-growing, opportunistic ephemeral algae are often observed to out-compete the larger slow-growing benthic plants and seagrasses by utilizing available nutrient sources and reducing light availability by shading.

Influence of factors other than nutrients

With respect to macroalgal abundance, many parameters control and dictate the response of coastal and marine flora to increased nutrient loading. These include light and oxygen availability, substrate availability, grazing and wave action. The direct and indirect effects such forces may have on macrophyte communities is discussed inSection 4.3.

Sedimentation is a further factor to be considered, with increased sediment loading having both direct and indirect effects on macrophyte communities in the marine environment. Direct effects include the physical covering of available substrata by sediment which can interfere with the processes of spore attachment (Devinny and

Volse 1978). Sediments may also smother young germlings and inhibit their growth and development (Devinny and Volse 1978) and may act abrasively to scour surfaces of settled spores (Fletcher 1996a).

Also reported are the indirect effects of sedimentation, whereby increased silt deposition results in decreased light penetration. This can cause changes in algal zonation patterns and depth distributions. Fletcher (1996a), for example, describes a reduction in the kelp depth range in the vicinity of sewage outfalls where plants are restricted to shallow water (Clendenning and North 1959, Bellamy *et al*. 1972) and very few new kelps are present under the original canopy. Decreased water transparency has also been implicated in changes in the depth penetration of eelgrass in the south Kattegat region of the Baltic (Nielson and Knudsen 1990), in decreased depth penetration of *Fucus vesiculosus* in the Baltic (Vogt and Schramm 1991, Kautsky *et al*. 1986, 1992) and in vertical distribution changes of macroalgae on the Swedish west coast (Rosenberg *et al*. 1990).

Classification and monitoring schemes

Fletcher (1996a) suggests that one of the most noticeable floristic changes associated with increased nutrient loading is a decline in the number of perennial macrophytes contributing to the so-called climax communities of both the intertidal and sub-tidal regions. Table 5.2 documents such changes in algal communities as documented for the British Isles and Europe.

Table 5.2 Coastal waters around the British Isles and Europe where the marine benthic flora have been affected by nutrient loading

Location	Available N and P data	Changes in local flora	Reference
British Isles			
Shetland Isles, Scotland	Nutrient-enriched waters due to sewage input	Abundant occurrence of *Fucus disticus* subsp. *edentatus*	Powell 1963 Russell 1974
		Decline in *Zostera marina* beds due to prolific growth of green filamentous algae	Bunker *et al.* 1995
Montrose Basin, Scotland	Elevated concentrations of nitrogen	Large increases in green algae (notably *Enteromorpha*) between 1981 and 1991. Considerable decline in eelgrass beds	MacGarvin 1995
Belfast Bay, Ireland	-	Blooms of *Ulva lactuca*	Wilkinson 1963
Dublin Bay, Ireland	Nutrient enrichment	Dense blooms of *Ectocarpus siliculosus, Enteromorpha* and *Ulva* spp.	Wilson *et al.* 1990 Jeffrey *et al.* 1992

Location	Available N and P data	Changes in local flora	Reference
Portsmouth Harbour, England	Input of sewage effluent Nutrients in water column: 0.085-0.794 $mgl^{-1} NH_4 + NO_2$ (1988-93 data)	Appearance of green algal mats	Soulsby *et al.* 1978 Parr and Wheeler 1996
Chichester Harbour, England	Nutrients in water column: 0.004-0.1 mgl^{-1} NO_3 0.04-0.3 mgl^{-1} PO_4 (1985-90 data)	Changes in benthic flora growth and species composition	MacGarvin 1995
Langstone Harbour, England	Input of sewage effluent – 5-fold increase in amount of effluent entering the harbour between 1959-1981	Excessive growth of *Enteromorpha* and *Ulva*	Montgomery and Soulsby 1981 Tubbs and Tubbs 1983 Montgomery *et al*. 1985
	Nutrients in water column: 0.121-0.788 mgl^{-1} $NH_4 + NO_2$ (1988-93 data)	Increase in *Zostera marina* and *Zostera noltii* during 1960's, 70's and 80's Suffocation of *Zostera* by *Enteromorpha radiata* during 1990's	Lowthion *et al.* 1985 Fletcher 1996a, b Parr and Wheeler 1996 Tubbs 1977 Tubbs and Tubbs 1993 den Hartog 1994
Poole Harbour, England	-	Proliferations of green algae	Farnham 1986
Thanet, Kent, England	-	Abundance of *Ulva lactuca* and *Griffithsia floculosa*	Fletcher 1974

Location	**Available N and P data**	**Changes in local flora**	**Reference**
Europe			
German Wadden Sea	-	Explosive growth of *Enteromorpha* forming thick algal mats since late 1970's. Loss of red algae from deeper waters and disappearance of *Zostera marina* coinciding with dramatic increase in green algal mats	Reise 1983, 1994 Nienhuis 1996
Kiel Bay, Germany	'eutrophication'	Drastic decline in *Fucus* biomass since 1950's and 1960's due to enhanced growth of opportunistic algae	Breur and Schramm 1988 Schramm 1988 Vogt and Schramm 1991
Island of Sylt, Germany	-	Blooms of *Enteromorpha*, *Ulva*, *Chaetomorpha*, *Cladophora* and the red alga *Porphyra*	Reise 1983
Baltic Sea - general	-	Reduction in *Fucus, Zostera* and *Chara*. Increased growth of filamentous algae *Pilayella*, *Ceramium, Cladophora, Enteromorpha* and *Monostroma*	Schramm 1996
Baltic coasts – Germany, Sweden and Finland	Increased nutrient concentrations	Decline in *Fucus vesiculosus* since early 1970's Replacement of *Sphacelaria arctica* by *Ceramium tenuicorne* and *Rhodomela confervoides*	Kangas *et al.* 1982 Kangas and Niemi 1985 Haathela and Letho 1982 Rönnberg 1984 Kautsky *et al.* 1986
Coast of Denmark	Increased nutrient loading	50% reduction in areas covered by *Zostera marina* since 1960's	Funen Island Council 1991 Nienhuis 1996

Location	**Available N and P data**	**Changes in local flora**	**Reference**
Dutch coast, including Dutch Wadden Sea	Increasing nutrient concentrations Nutrients in water column: 0.5-4.6 mgl^{-1} N	Decline in *Zostera marina* and *Zostera noltii* and increasing floating algae, *Ulva, Enteromorpha* and *Chaetomorpha*	den Hartog and Polderman 1975 Nienhuis 1996
Brittany coast, France	Intensive agriculture contributing to high N and P loading	Blooms of *Ulva* and some blooms of *Pilayella littoralis* since late 1970's	Briand 1989 Dion and Le Bozec 1996
Bay of Saint-Brieuc, Brittany, France	-	Proliferation of free-floating *Ulva*	Menesguen and Piriou 1995
Bay of Arcachon, France	Increased N loading due to mariculture Nutrients in water column: 0.2 mgl^{-1} TON	Mass production of *Enteromorpha* since 1982. Increase in *Monostroma obscurum* since 1989	Auby *et al.* 1994 Dion and Le Bozec 1996
Spanish Atlantic coasts	Increase of nitrogen	Proliferation of *Ulva* blooms	Fuentes 1986 Niell *et al.* 1996
Alfacs Bay, Spain	Nutrients in water column: 0.2-0.9 mgl^{-1} NH_4 0.6-3.1 mgl^{-1} NO_3 0.1-0.2 mgl^{-1} PO_4	Domination of green algal communities, predominantly *Ulva* sp.	Romero *et al.* 1996
Gulf of Trieste, Northern Adriatic	Increased eutrophication due to sewage input Nutrients in water column: 0.21-4.39 mgl^{-1} NO_3 0.01-0.28 mgl^{-1} PO_4	Profound changes in benthic algal vegetation since late 1970's. Reduction or absence of Fucoids linked with increasing *Enteromorpha, Ulva, Blidingia* and *Cladophora*. Appearance of turf-like mats of *Gelidium*. Reduction or absence of *Cystoseira* stands, replaced by populations of *Halopteris scoparta, Dictyota dicotoma, Halopithys incurvus, Padina pavonica* and under extreme conditions by *Ulva rigida, Scytosiphon lomentaria* and *Enteromorpha* spp.	Munda 1993, 1996

Location	Available N and P data	Changes in local flora	Reference
Coast of Greece	Increased nutrient loading due to discharge of sewage	Reduction or absence of some genera or species (e.g. *Cystoseira*). Increase in biomass of *Ulva rigida*, *Enteromorpha compressa* and *Gracilaria verrucosa*	Haritonidis 1996
Gulf of Thessaloniki, Greece	Nutrients in water column: $0.02\text{-}0.1\ \mathrm{mgl}^{-1}$ N $0.03\text{-}0.27\ \mathrm{mgl}^{-1}\ PO_4$ (1988-89 data)	Macroalgal blooms of *Ulva, Enteromorpha, Cladophora* and *Chaetomorpha* since late 1980's	Haritonidis 1996
		Mass development of *Codium* spp. since 1980	Nikolaidis and Moustaka-Gouni 1990
		Reduction of number of macrophyte species from 127 to 30	Nikolaidis 1985
		Decline in *Posidonia oceanica* and *Cymodocea nodosa* over last 20 years	Haritonidis 1996
		Disappearance of perennial brown algae (e.g. *Cystoseira* spp.) and replacement by *Ulva, Enteromorpha, Cladophora, Chaetomorpha* and *Ectocarpus*	Lazaridou *et al*. 1997

As noted for the case studies in the transitional waters section of this review, descriptions of both changes in nutrient loading and macrophyte communities listed in Table 2 are largely qualitative. In relatively few of the scientific publications pertaining to nutrient enrichment and macrophyte growth, has emphasis been placed on quantifying changes in N and P in local marine systems. Further detail may be found in the 'grey' literature however, Crouzet *et al*. (1999) providing a comprehensive review of the nutrient status of a range of European seas including the Mediterranean Sea, Black Sea, Baltic Sea, North Sea, Barents Sea, Norwegian Sea and the north-east Atlantic Ocean.

As shown by the case studies listed in Table 5.2, changes in macrophyte communities with nutrient loading are both variable and dependent on a range of environmental and physical parameters. The frequency with which the monitoring of coastal flora subject to different and/or variable nutrient loadings is conducted must hence take this into account. This, and the recommended frequency of monitoring with respect to macroalgal communities is discussed in Section 4.3.

5.3.3 Bloom frequency and intensity

Annual blooms of green macroalgae (Chlorophyceae) have been documented at a range of localities both within Europe and world-wide. The development of macroalgal blooms and green tides in nutrient enriched waters is discussed in sections 5.3.1 and 5.3.2.

Influence of factors other than nutrients

The frequency and intensity of macroalgal blooms is known to be governed by a range of environmental parameters including nutrient availability, irradiance and temperature. At sites recognised as being nutrient enriched or eutrophic, however, the role of the sediment in nutrient recycling also appears to be a critical factor in determining the extent of the bloom from one year to the next. At such sites, local sediments may represent a significant recyclable source of N and P (e.g. Trimmer *et al.* 2000, Taylor and Raven 2001). Reducing conditions, under anoxia, accelerate nutrient release from sediments, which in turn raises the eutrophication level (Schramm 1996). In Dublin Bay, Jeffery *et al.* (1992) report that nutrients released from the sediments play a major role in determining spatial variations in algal biomass. Similarly, Pihl *et al.* (1999) suggest that in shallow coastal areas, storage of nutrients in the sediment could constitute the basic pool for future algal production.

Klein and van Buuren (1992) propose that sediments play an important role in the nutrient budgets of the North Sea because of the exchange of nutrients between the water column and the sediments. Sediment-water interactions may hence be important in both nitrate and phosphate budgets at nutrient-enriched sites. Studies on sediments may be particularly important with regard to nitrogen cycling and nutrient supply for macroalgal growth since increased sediment nitrogen mineralisation rates are associated with areas of increased intertidal growth (Parr and Wheeler 1996). These authors note that to detect the initial stages of nutrient enrichment and eutrophication on a small scale requires specialist knowledge and frequent biological monitoring. The use of mineralisation rates as a measure of the trophic status of sediments is hence suggested as a monitoring tool. This finds some support in the literature, Jeffrey *et al.* (1991) having suggested that the best predictor of *potential* algal production in Dublin Bay, for example, is the nitrogen mineralisation rate in the intertidal sediments in late spring. The use of sediments in predicting the ecological status of the marine environment is further discussed inSection 4.3.

Classification and monitoring schemes

Since the degree of eutrophication and the proliferation of certain macroalgae at nutrient-enriched sites appear to be correlated, seaweeds have the potential to be good indicators of nutrient change in the local environment.

Kiirikki and Blomster (1996), for example, suggest that the brown filamentous alga *Ectocarpus* can be used as an indicator of short nutrient pulses. Such nutrient pulses are not detected in standard water sampling as they are utilised by both pelagic and benthic organisms within a few hours or days, but promote a growth response in the alga that can readily be monitored. Constanzo *et al.* (2000) similarly describe investigations into the applicability of the red alga *Gracilaria edulis* as a marine bioindicator at two oligitrophic locations, and show that the alga responds to nutrient pulses that analytical water sampling techniques fail to detect. The authors suggest that the response of the alga as a bioindicator of variable nutrient pulses may provide a useful tool for investigating the source and geographical extent of nutrients entering transitional and coastal waters.

The use of ephemeral, bloom-forming algae as biological indicator species has also been suggested by several workers. Levine and Wilce (1980) and Ho (1987), for example report a correlation between sewage pollution of coastal water and the abundance of *Ulva*, and suggest the possibility of using this alga as a bio-indicator of coastal water quality. Fong *et al*. (1998) similarly suggest that *Enteromorpha* may be used as an indicator of N enrichment and has a potential use as a bioassay organism for detecting eutrophication. The use of *Ulva lactuca* in the bioassessment of changes in nutrient concentrations has also been reported on (Lyngby *et al*. 1999), the authors suggesting that measuring algal tissue N and P concentrations is a biomonitoring technique well suited for direct nutrient detection and for providing a time-integrated measurement of nutrient availability in coastal waters and thus for assessing ecosystem health with regard to eutrophication.

The distribution of green algae such as *Cladophora, Ulva* and *Enteromorpha* spp. has been used as a quality indicator in an extensive survey of the NE Mediterranean coast (Ballesteros *et al*. 1984), but the appearance of algal blooms may be the ‘final step’ in the eutrophication process, indicating the reduction of a particular site to poor or even bad ecological status (major or severe alterations in biology). There are serious implications for wildlife long before algal blooms become evident, especially for those species best suited to nutrient-poor conditions (Irving 1993). For biological indicators to be of any practical use to scientists and managers, therefore, they would need to be indicative of the trophic status of a body of water before this stage was reached.

There is a noticeable lack of sound historic data for evaluating the occurrence and extent of eutrophication in both our transitional and coastal waters (Irving 1993). Whilst there is a general agreement as to the effects of eutrophication, the concentrations of nutrients required for effects to become apparent are both disputed (Irving 1993) and dictated by a whole host of other interacting physical and environmental factors.

At marine and estuarine sites alike, the effects of eutrophication that have attracted most attention include the growth of algal weed mats and the smothering of larger macrophytes by ephemeral fast-growing algae. Less or scarcely understood effects of eutrophication are those on macrophyte community structure, including changes in species diversity and macroalgal dominance (MacGarvin 1995, Parr and Wheeler 1996). Even those issues to which most attention has been devoted remain poorly understood (MacGarvin 1995), with descriptions of changes in nutrient loading and the corresponding macrophyte changes at most sites in Europe largely being qualitative. Although this data may allow qualitative predictions of the changes of submerged vegetation and associated ecosystem properties with respect to nutrient loading, qualitative predictions are of little use to managers responsible for coastal ecosystems and legislators responsible for setting tolerable nutrient loadings (Duarte 1995). There is hence a need for quantitative models that predict how much nutrient loading may increase before changes in macrophyte communities become evident. The diversity of processes involved in the response of macrophytes to nutrient regime renders prediction of plant community changes very difficult, and to date the development of models to provide the required answers has been slow (Duarte 1995).

Numerous models have been proposed linking algal standing crop or primary production to nutrients. For the most part, however, these have focused on phytoplankton growth (e.g see Cullen 1990, Fasham *et al.* 1990) and little data are available with respect to macroalgae. Amongst the relatively few workers in this field, Solidoro *et al.* (1996) have produced a eutrophication model describing the shallow water environment of the Venice lagoon colonised by *Ulva*, and linking yearly patterns of temperature, light intensity, phytoplankton and nutrient concentration with the growth of the alga. Valiela *et al.* (2000) have developed a model in order to establish the relationship between N loading rates and mean annual concentrations of N in the estuaries of Wasquot Bay, Massachusetts. The authors suggest that this data can be related to mean annual production, with phytoplankton, macroalgal and eelgrass production representing suitable end-point measures.

Cloern (2001) points out that coastal research in recent decades has identified key differences in the responses of lakes and coastal-estuarine systems to nutrient enrichment, and proposes a Phase II conceptual model to reflect these differences. The model takes into account the distribution of vascular plants and biomass of macroalgae and recognises that their abundance will be affected by a complex suite of direct and indirect responses to a number of variable parameters. Evidence has suggested for some years that macrophyte response is not directly proportional to nutrient loading (Duarte 1995) but the development of non-linear models is still very much a challenge for future work.

5.4 Benthic Invertebrates

See Section 4.4

5.5 Summary

Phytoplankton and macroalgae are the major primary producers in coastal waters. Responses to changes in the chemical environment are most likely to be observed in these biological groups. Yet while the increases in nutrient concentrations in coastal waters are not as great as those observed in lakes, rivers and estuaries, the relative increases in some areas have been considerable. Where this has occurred, and there have been changes in species composition and abundance, it is normally associated with restricted exchange water bodies.

5.5.1 How sensitive are different groups of organisms to increased nutrient concentrations?

With respect to macroalgae, the consequence of increased nutrients in localities where water exchange is limited, or where the coast is particularly sheltered, has been a reduction in species numbers. This is often coupled with the replacement of slow-growing, long-lived macroalgae with faster growing annual species, particularly green macroalgae such as *Ulva* and *Enteromorpha* spp. For phytoplankton, the natural succession of species change following the spring diatom bloom shows considerable regional variation. Again, where there is restricted water exchange and an increase in nutrients, the water body can become dominated by a single individual species, for example the dominance of *Phaeocystis globosa* in the Wadden Sea.

Algal blooms are natural events; they have been observed in the sedimentary record in times before the recent anthropogenic increases in nutrient concentrations. There are, however, clear observations of increased nutrients in recent years, resulting in significant increases in algal biomass. The actual number of these observations for phytoplankton is remarkably few. In particular, we lack observations of a clear direct phytoplankton response to moderate increases in nutrient concentrations in coastal waters.

In addition, the short life cycle of phytoplankton make them poor candidates as indicators of nutrient change unless intense monitoring procedures are in place. Macroalgae are likely to prove a more reliable alternative. For example, indicator species, such as *Ectocarpus*, are reported to respond to short nutrient pulses whilst the macroalga *Gracilaria edulis* appears to be a good indicator of oligotrophic locations.

With respect to the invertebrate fauna of coastal waters, the greatest effects of increased nutrient loading will be indirect, predominantly through changing the distribution and abundance of phytoplankton and macrophytes. Relating concentrations of nutrients to any effect they have on the invertebrates in these ways will be difficult, if not impossible, because of the unpredictability of the relationships and the presence of many other confounding factors that also affect invertebrate distribution and abundance.

5.5.2 Is ecological status influenced more by factors other than nutrients?

It is impossible to consider the growth of algae purely in terms of the effects of changing nutrient availability. Nitrogen and phosphorus are just two of a number of important resources needed to satisfy the requirements of growth and reproduction. While diatoms have an absolute requirement for silica, all algae need appropriate levels of light to drive photosynthesis. Light availability can be influenced by processes which are quite independent of nitrogen and phosphorus supply, for example cloud cover and silt loading. One climatic variable that is vital but whose impact is very poorly understood is temperature. Increased temperatures will stimulate growth but more importantly increased winter minimums may have the effect of reducing the losses in biomass between growing seasons making for a higher initial population in the spring. There is evidence that suggests that changes in the ratios of nutrients, particularly N, P and Si, can influence the species composition of a bloom rather than the concentrations of nutrients.

5.5.3 To what extent can current EA monitoring and assessment practices fulfil the requirements of the WFD with respect to nutrients ?

As discussed for transitional waters, current monitoring is not sufficient for the WFD and there is a clear need to develop routine sampling methods and programmes that can be applied to a range of localities nationwide. A full review of the extent of monitoring in coastal waters and recommendations for future monitoring of eutrophication impacts is provided in Annex 3 of the proceedings of the National Marine Monitoring Programme (NMMP) indicators workshop (National Marine Monitoring Programme 2001). Recommendations include monitoring of planktonic algal biomass (annual maximum chlorophyll *a*), duration of algal blooms, phytoplankton composition, opportunistic green macroalgal blooms and fish kills.

5.5.4 How and with whom (pan-European?) should the EA progress the future developments of monitoring and assessment practises?

A more consistent approach to monitoring is necessary (see above), not only within Great Britain but within all EC coastal zones. As is the case for transitional waters, increased awareness of relevant EC research and monitoring programmes is necessary, as well as a more holistic approach to monitoring more local coasts. This would involve considerable liason between organisations such as the EA, SEPA, EN, SNH, CCW etc. and their European counterparts in order to set up guidelines and specific monitoring programmes appropriate to a wide range of localities. Developments are being co-ordinated by the NMMP (http://www.marlab.ac.uk/NMPR/NMP.htm)

5.6 Recommendations for Phase II

As previously discussed, very limited data exists relating nutrient conditions to biological quality in coastal waters. This is even more true for baseline data required for the setting of reference conditions. A key issue that should be considered in Phase II is the development of standardised monitoring protocols, particularly with respect to phytoplankton and macroalgae. A more consistent and unified approach is needed to monitoring a range of 'reference' and impacted sites around the UK coast. To date, even at sites that have been well studied, there are inconsistencies in monitoring objectives and methods. The Ythan, for example, is one of the most documented sites in the UK with over 30 years of data pertaining to nutrient loading, algal blooms and invertebrate-algae interactions. Even at this site it is difficult to characterise ecological changes due to the inconsistencies in monitoring methods and frequency.

Research requirements for WFD monitoring have been reviewed in Annex 3 of the proceedings of the National Marine Monitoring Programme (NMMP) indicators workshop (National Marine Monitoring Programme 2001). Recommendations included:

- Developing standardised methods for chlorophyll *a*, particularly identifying sampling frequency required for measuring the duration and magnitude of phytoplankton blooms
- Agreement of threshold algal densities for measuring the duration of algal blooms
- Inter-calibration between automated fluorometer measurements of chlorophyll *a* and Continuous Plankton Recorders (CPRs)
- The development of a UK phytoplankton trophic index (UK PTI)
- Developing remote sensing for monitoring the cover of macroalgae in inter-tidal areas
- Monitoring fish kills in association with harmful algal blooms

This study supports the development of chlorophyll *a* concentration as the most readily usable indicator for development in the short-term. It is a relatively straightforward, rapid and cheap measure, requiring little expert knowledge.

Abundance of bloom-forming macroalgae is similarly recommended as being sensitive to nutrient conditions. Macroalgal abundance is a little more difficult to quantify, but is less dynamic. More predictable patterns in their development may make them more suitable indicator organisms in the long-term in a monitoring scheme. As highlighted in the NMMP report (2001), the effectiveness of remote sensing in mapping macroalgal blooms needs to be further explored.

In general, however, a much greater understanding is required of the factors affecting sensitivity of sites to nutrient pollution. Why do some sites have micro/macro algal blooms whilst others do not, despite a similar nutrient loading? The use of sediments as indicators of potential nutrient availability may be a way of assessing areas receiving a high nutrient loading before they actually show ecological responses.

6 PROCEDURES AND METHODS FOR ESTABLISHING NUTRIENT REFERENCE CONDITIONS

6.1 Introduction

The WFD aims to achieve good ecological water quality in all relevant surface waters by 2016. It stipulates that ecological water quality will be assessed by the degree to which present day conditions deviate from those that would be expected in the absence of significant anthropogenic influences. These are termed 'reference conditions'. This review focuses on the determination of *nutrient* reference conditions for each type of waterbody covered by the WFD (i.e. rivers, lakes, transitional waters and coastal waters).

The first question that needs to be asked is whether it is actually necessary for Member States to determine a set of nutrient reference conditions for each type of surface water. Strictly speaking, the WFD refers to the determination of *ecological* rather than *nutrient* reference conditions for each surface water type. However, as *nutrient* reference conditions are more tangible than *ecological* reference conditions, the authors consider that the EA should continue to address the issue of establishing *nutrient* reference conditions for surface waters as a useful stepping-stone between management and ecology. The remainder of this chapter evaluates the possible methods for these determinations.

Another key question that needs to be addressed is which historical baseline should be used to define these conditions. This issue is discussed in detail by Johnes (1994) who suggest that, for the UK, there are many nodal points in history that could be used. These include:

- the pristine state immediately after glacial retreat and settlement of the initial clay turbidity (*ca*. 9000 BC),
- before the earliest land clearances for farming by Neolithic invaders (*ca*. 3000 BC),
- before the main effects of the industrial revolution (*ca*. 1800 AD),
- before the onset of marked changes in pH in acidified waters
- before the major land use changes brought about by post-war agricultural policy (*ca*. 1930 AD).

In practice, the choice of historical baseline for nutrient reference conditions probably depends on the planned use of this baseline and practical considerations such as data supply and the availability of suitable methodologies. For example, palaeoecological evidence indicates that, although serious acidification problems probably began in the mid-1800s, most rapid changes in ecological water quality due to nutrient enrichment probably began only 20 to 30 years ago. If nutrient reference conditions are to be set as a realistic target for the restoration of nutrient enriched surface waters, then it might be better to aim at these more recent 'pre-disturbance' conditions, rather the more pristine state that existed ca. 1850, when land use and management practices within the catchment were very different from those in place today. This agrees with Moss *et al*. (1997) who suggest that "the philosophy of the baseline should be one of determining the state which reflects the highest possible quality, consonant with maintenance of current populations and of agricultural use of the catchment".

Battarbee (1999) argues that, although it is not always possible (or even desirable) to restore a lake to its previous undisturbed condition, knowledge of that condition is essential in defining one of the options for management and in dictating the limits of what can be achieved. In this context, a historical baseline of 1850 would provide a scientifically derivable reference conditions against which changes could be assessed, while a more recent historical baseline could be used to set ‘achievable’ restoration targets that reflect current socio-economic pressures within the catchment and take account of the need for sustainable development. It is important that reference conditions and class boundaries are not set at levels that prevent good status from being realistically achieved, otherwise the resource implications of attempting to restore these waters would become economically unsustainable.

In general, there appears to be no single, ideal definition of a ‘reference condition’ in terms of its historical baseline. This suggests that a range of nutrient reference conditions might be a more useful and practical starting point, as follows:

- Natural - the lowest level expected based on catchment geology, soils, climate and topography.
- Background - the lowest level expected under present day land use.
- Threshold - the level at which high or good ecological status is sustainable and above which ecological changes are likely to occur.
- Least disturbed - the level at the least affected sites among the current population of surface waters.

On this basis, it would not be necessary to fix a single date for defining a reference condition. The ‘natural’ condition would act as a scientifically derivable reference state against which change could be assessed, while the ‘background’ condition could be used as a restoration target that would be achievable under current land use and management practices. This could be important in terms of the practical implementation of the WFD.

Whatever the choice of historical baseline, there is still a need to determine appropriate methodologies that can be used to set baseline or reference conditions, especially where there are no historical records. The WFD identifies three principle approaches that can be used:

i) Palaeoecological methods
ii) Spatial-state schemes
iii) Mathematical modelling approaches

These approaches are reviewed below (Sections 6.2 - 6.4) in the context of establishing nutrient reference conditions for rivers, lakes, transitional and coastal waters. Section 6.5 compares available methods where possible, Section 6.6 summarises the key findings of this review and Section 6.7 makes recommendations for further work in Phase II.

It should be noted that data and information from a closely related, European Union (EU) funded, project on the ’Development of a Protocol for identification of reference conditions, and boundaries between high, good and moderate status in lakes and watercourses' (REFCOND) were not available to the authors during the course of this review. Also, it is not known, at present, how closely the protocols for identifying reference conditions developed within the REFCOND project will have to be followed by each Member State. Further details of this work are available at http://www-nrciws.slu.se/REFCOND.

6.2 Palaeoecological Methods

6.2.1 Introduction

This section reviews the palaeoecological methods available for establishing nutrient reference conditions for each of the four water body types covered by the WFD. The sediment accumulated in a water body can provide a good historical record of its past floral, faunal and chemical changes (e.g. Smol 1992). Interpretation of these sediments (palaeoecology) allows scientists to quantify and evaluate environmental change, assess its causes, and determine baseline or reference values for ecosystem status that can guide the setting of restoration targets (Battarbee 1999). Lakes are considered first because, in a palaeoecological context, they are the best studied of all of the surface water categories.

6.2.2 Lakes

Hydro-chemical transfer functions

Over the last few decades, a quantitative approach to the reconstruction of past environments has been developed which is based on a predictive equation, or transfer function, that models the relationship between species composition and water chemistry (see Birks 1998). This transfer function is generated from a calibration, or training, dataset that is generated from the biota in present day surface-sediment samples and contemporary water chemistry measurements from a large number of water bodies spanning the environmental gradient of interest. Once calibrated, it is then applied to the fossil assemblages in a dated sediment core to provide quantitative inferences of past water chemistry. The technique of weighted averaging (WA) regression and calibration (e.g. ter Braak and van Dam 1989), and its extension to a method called WA partial least squares (WAPLS) (ter Braak and Juggins 1993), have now become standard techniques in palaeoecology for the reconstruction of past environmental conditions (Anderson 1995, Battarbee, 1997, 1999). Modern environmental optima are calculated for each taxon based on their distribution in the training set, and then past chemical conditions are derived from the weighted average of the optima of all taxa present in a given fossil sample. The methodology and the advantages of WA over other methods of regression and calibration are well documented (e.g. ter Braak and van Dam 1989, Line, ter Braak and Birks 1994).

Diatom-phosphorus transfer functions

Diatoms (Bacillariophyceae) are the most commonly used biological indicators in palaeolimnological studies. Their unicellular, siliceous algae and their glass (silica) cell walls are generally well preserved in most lake sediments and they are very sensitive to changes in water quality. This makes them good indicators of past conditions such as pH, nutrient concentrations and salinity (Battarbee 1986). A particular success has been the development of transfer functions for quantitatively inferring lake total phosphorus (TP) concentrations in lakes in northwest Europe (e.g. Bennion *et al.* 1996a), central Europe (e.g. Wunsam and Schmidt 1995, Lotter *et al.* 1998), Canada (e.g. Reavie *et al.* 1995) and the United States (e.g. Dixit *et al.* 1999). This approach can provide not only estimates of baseline (reference) TP concentrations in lakes but also the timing, rates and possible causes of enrichment at a particular site if the sediment cores are dated (e.g. Bennion *et al.* 1996b, Anderson 1997a).

A northwest European calibration set developed by Bennion *et al.* (1996a) is currently the best available diatom model for establishing nutrient reference conditions in relatively small, shallow (< 10 m maximum depth), productive lakes in the UK. It is based on 152 lakes from six regions of Western Europe (south-east England, the Cheshire and Shropshire Meres, Wales, Northern Ireland, Denmark and Sweden) with annual mean TP concentrations ranging from 5-1200 µg TP l^{-1}, and a median value for the dataset of 104 µg TP l^{-1}. The relationship between diatom-inferred TP (DI-TP) and measured TP, based on $\log_{10}$-transformed annual mean TP data, is strong (apparent r^2= 0.85 and r^2= 0.91 for WAPLS component 1 and WAPLS component 2, respectively) with low errors of prediction (apparent root mean square error (RMSE) of 0.19 and 0.15, and a cross-validated RMSE (RMSE-P) of 0.22 and 0.21 for WAPLS component 1 and WAPLS component 2, respectively), indicating that the models perform well. Recently, a new training set has been developed as part of a palaeolimnological study of Scottish lochs (Bennion *et al.* 2001b). This is based on 56 relatively large, deep (> 10 m maximum depth), unproductive lakes from the United Kingdom (UK), southern Norway and central Europe with annual mean TP concentrations ranging from 1 to 73 µg TP l^{-1}, and a median value for the dataset of 22 µg TP l^{-1}. For this dataset, the best model was generated with simple WA and inverse deshrinking (apparent r^2= 0.75, apparent RMSE=0.20, RMSE-P=0.25). The errors are slightly higher in this model than in the Bennion *et al.* (1996a) model largely due to the relatively low number of lakes in the training set. Further expansion of the model to include more lakes may result in improved performance.

The surface area, depth and basin shape of a lake influence its mixing regime, degree of stratification, ratio of littoral to profundal habitats, and extent of wind disturbance. These factors, in turn, affect the potential for growth and survival of any particular plant or animal species, including diatoms (Hutchinson 1957). The geology and topography of the catchment influences the type of use to which land can be put; this determines the chemistry of the lake water, which, in turn, influences the biota. Consequently, both the chemistry and biology of large, deep lakes in largely upland catchments tend to be different from those of small, shallow lakes in lowland regions. For this reason, two different models are required for reconstructing TP: one for deep, large, relatively unproductive lakes and another for small, shallow, productive waters.

Quantitative reconstructions of TP have been carried out, using these models, on 97 lakes in the UK (see Appendix 1). In the majority of cases, the transfer functions appear to give reliable results in that the diatom predicted TP for the surface samples corresponds closely with current measured TP, and the inferred trends are supported by other historical data. The models can, therefore, in most cases be used with confidence for establishing nutrient reference conditions.

As with all modelling techniques, there are certain limitations associated with the development and application of the diatom-TP transfer function. Firstly, it can only provide single estimates of lake water chemistry characteristics so the TP data collected for model calibration must be summarised as a single estimate for each lake. Any difficulties associated with estimating the current mean TP in a seasonally varying system will introduce errors into the models. In contrast to the winter maxima commonly observed in deep, stratifying lakes, shallow waters usually experience highly fluctuating nutrient concentrations throughout the year. Some of these, especially the summer peaks, are associated with internal recycling (e.g. Bailey-Watts *et al.* 1987, Bennion and Smith 2000). Such marked intra-annual variability in TP concentrations tends to be greatest at the more productive sites (e.g. Gibson *et al.* 1996, Bennion and Smith 2000).

A second source of error is in the estimation of the species optima. Although the DI-TP values are well correlated with observed TP in the training sets, there is some scatter in the relationship. Inverse de-shrinking, rather than classical de-shrinking, is often used in the development of transfer functions as it reduces the overall predictive error of the model. Unfortunately, however, there is an inherent bias in inverse de-shrinking techniques, which results in over-estimation at the low end of the environmental gradient and under-estimation at the high end (e.g. Martens and Naes 1989, ter Braak and van Dam 1989). This is the most likely cause of over-estimation by the model for a number of nutrient-poor sites. The species optima errors are primarily due to the complexity of lake ecosystems and the fact that factors controlling algal abundance in lakes are multivariate. Light, temperature, flushing rate, substrate and grazing pressure have all been shown to influence diatom distributions in lakes (e.g. Anderson *et al.* 1993, Bennion 1994).

The models estimate TP with reasonable accuracy when the errors in predicted DI-TP values are compared with the inter-annual TP range in the lakes concerned. Furthermore, when the predictive ability of the diatom transfer functions are compared to OECD-type regression relationships, the errors associated with DI-TP are as good or better (Anderson *et al.* 1993). Given all the possible sources of error, the predictive errors of the diatom models can, therefore, be considered to be satisfactory and more than acceptable for management requirements. Indeed DI-TP records have been validated against long-term monitoring data with encouraging results (e.g. Bennion *et al.* 1995, Rippey *et al.* 1997, Bennion *et al.* 2000b).

A larger European diatom database for palaeolimnological reconstruction is currently being developed as part of an EU funded multi-partner project known as EDDI which is being coordinated by the Environmental Change Research Centre (ECRC) at University College London (Bennion *et al.* 1999). This will generate a diatom-TP training set of over 400 lakes across Europe and will enable the generation of tailor-made transfer functions for the reconstruction of environmental conditions at individual sites. The project aims to further refine the numerical methods for quantitative reconstructions and to provide a guide on best practice for those wishing to apply the models.

The diatom-based hydrochemical transfer function approach is one of the most powerful techniques for establishing nutrient reference conditions in lakes and, for this reason, has become a standard tool in lake management for setting chemical targets for restoration. There are, however, a number of other biological groups that preserve in the lake sediments and represent a range of biological elements relevant to the WFD. The use of these organisms for assessing nutrient reference conditions is discussed below.

Chironomid-phosphorus transfer functions

Chironomids (Diptera) are non-biting midges whose larval head capsules are preserved in lake sediments. Although they have traditionally been used to classify lakes according to their hypolimnetic oxygen concentrations (e.g. Sæther 1979, Hofmann 1988), chironomid transfer functions have recently been developed for inferring past oxygen conditions in lakes (Quinlan *et al*. 1998, Clerk *et al*. 2000, Little *et al*. 2000, Ruiz *et al*. 2001). As profundal oxygen concentrations are a function of trophic status and basin morphometry, it would be expected that a relationship between chironomid assemblages and nutrient concentrations should also exist. However, it should be noted that chironomid midge larvae do not take up phosphorus directly from the surrounding water; instead, they respond to changes in availability of food and oxygen as a consequence of nutrient input.

A number of chironomid-TP transfer functions have recently been developed. Lotter *et al*. (1998) found a statistically significant relationship between chironomid assemblages and $\log_{10}$TP in a training set of 68 small lakes in the Alps and went on to develop an inference model. The two-component WAPLS model performed well with an apparent r^2 of 0.84 and an RMSE-TP of 0.14. Brooks *et al*. (2001) showed that $\log_{10}$TP was the most important factor in explaining the distribution of chironomids in surface sediments from 44 lakes in England and Wales. They produced a model based on simple WA with inverse de-shrinking, which had good predictive power (apparent r^2=0.71, RMSE-TP=0.34). This was applied to a core from Betton Pool, Shropshire, to reconstruct the trophic history of the lake. Inferred TP values from the chironomid model agreed well with diatom-inferred TP values.

These studies illustrate that fossil chironomid assemblages can be used to establish nutrient reference conditions in a quantitative way. In the studies cited above, both chironomids and diatoms are used in palaeolimnological assessments of eutrophication. This multi-proxy approach, using a number of fossil indicators to independently infer nutrient concentrations, clearly has advantages over the single indicator approach as the data can be used in a complementary and corroborative way. At present, only the chironomid inference models of Brooks *et al*. (2001) and Ruiz *et al*. (2001) exist for UK lakes, and even these would need to be expanded to include a greater number of sites from a broader range of lake types in order to be properly applicable at the UK level.

Cladocera-phosphorus transfer functions

Cladocera are microscopic crustaceans and are represented in lake sediments by a variety of body parts. There has been very little work on the use of cladocera for quantitative reconstruction of nutrient concentrations. Lotter *et al*. (1998) demonstrated a moderately strong and statistically significant relationship between $\log_{10}$TP concentrations and benthic cladocera in a training set from 68 small Alpine lakes. However, the relationship was not as strong as that between diatoms and TP in the same training set and large secondary gradients were observed indicating that variables other than TP strongly influence cladoceran distributions (e.g. temperature, conductivity, alkalinity). Nevertheless, the one component WAPLS model performed reasonably well with an apparent r^2 of 0.63 and an RMSE-TP of 0.28. In contrast, the planktonic cladocera showed very weak relationships with TP in the Alpine training set and thus no inference model was developed. Jeppesen *et al*. (1996), in a study of planktivorous fish and trophic structure in Danish lakes, also concluded that there was no significant influence of TP on planktonic cladocera. It is likely that other factors such as fish and copepod predation exert influence on the composition and abundance of the planktonic forms. Currently there is no cladocera-nutrient training set for lakes in the UK and further work is clearly required to explore the potential of this group in the development of a technique for determining nutrient reference conditions more fully. However, given that robust diatom and chironomid models already exist for reconstructing TP concentrations in UK lakes, the development of similar quantitative models using cladocera remains is unlikely to become a research priority.

Chrysophyte-phosphorus transfer functions

Chrysophytes are planktonic algae whose cysts and scales are preserved in lake sediments. Their use in palaeoecological models is limited because their taxonomy is based on descriptions of their morphotype, which is difficult to link to the source taxa. Furthermore, as chrysophytes are generally associated with cool, nutrient-poor waters (Rosen 1981), low abundance can be a problem in nutrient-rich waters. Chrysophytes have been used mostly in palaeoecology to study lake acidification (e.g. Facher and Schmidt 1996) and salinity changes (e.g. Cumming *et al.* 1993), whereas nutrient studies have been relatively few (Smol 1985, Zeeb *et al.* 1994). In a training set of 68 small lakes in the Alps, Lotter *et al.* (1998) found large gradients in the chrysophyte cyst data unrelated to TP. Consequently the resulting TP inference model had poor predictive ability and was considered unsatisfactory to be used for reliable nutrient reconstructions. The available evidence, therefore, indicates that chrysophytes are unlikely to be as useful a tool as diatoms or chironomids for establishing nutrient reference conditions in lakes.

Ecological reference conditions: multi-proxy palaeoecology and trophic-web interactions

The hydrochemical transfer function approach, described above, is not appropriate for all lakes, especially small, shallow, waterbodies where, in addition to nutrient effects, top down factors such as predation influence the ecological structure and functioning of the system. The concept of alternative stable states (Scheffer *et al.* 1993) appears to hold true in shallow lakes where either a submerged plant or phytoplankton community can dominate the system over a wide range of nutrient concentrations. Each of these states is buffered by mechanisms involving food-web and lake water-sediment interactions (e.g. Irvine *et al.* 1989, Moss *et al.* 1996b). Such complexities reduce the ability of diatom-TP transfer functions to reconstruct the nutrient history of these systems (Bennion *et al.* 2001a, Sayer 2001).

An alternative approach is to examine the relationship between surface-sediment fossils of a range of food-web components (such as plankton, aquatic pollen, plant macrofossils, chironomids, fish-scales etc.) and contemporary chemical and biological data (e.g. Jeppesen *et al.* 1996, Sayer *et al.* 1999, Sayer 2001) to develop ecological targets for shallow lakes. In sites such as the Norfolk Broads, where either a phytoplankton or macrophyte dominated community can exist over a range of TP concentrations, a combination of ecological and chemical reference conditions may be more appropriate than chemical conditions alone. Carl Sayer, a NERC fellow at the ECRC, in association with colleagues at University of East Anglia and Queen Mary College London, is currently working on 30 shallow lakes in Norfolk to establish contemporary ecological relationships amongst the lakes, determine how well these are reflected in the sediment records of aquatic organisms, and develop a methodology for lake restoration using combined ecological and palaeoecological approaches (*cf.* Sayer and Roberts 2001).

Analogue-matching techniques: space-for-time substitution

Analogue matching is a form of space-for-time substitution modelling. It is a statistical technique that compares degree of floristic or faunistic similarity between a fossil sample (in a sediment core) taken from an impacted lake and a range of modern (surface sediment) samples. The best modern analogues for the pre-disturbance assemblages are identified. These analogue lakes are then assumed to have similar community composition to those present in the pre-disturbance period of the impacted lake in biological groups other than those analysed in the palaeo-record (e.g. fish, benthic macroinvertebrates). The technique has, to date, only been developed for identifying restoration targets for acidified lakes based on diatom analogues (Flower *et al*. 1997). This method is currently being further developed and refined at the ECRC based on diatoms and cladocera from 83 upland surface waters in the UK (Simpson 2001). This is proving to be a powerful technique for identifying site-specific ecological reference conditions in these waters, but requires further development before it will be applicable to a wider range of UK lake types. In order to establish pre-disturbance communities in enriched lakes, the analogue matching approach must be extended to lowland waters spanning the full trophic gradient from oligotrophic to hypertrophic conditions.

The role of palaeoecology in establishing nutrient reference conditions for lakes in the United States EMAP-SW

In 1989, the USEPA began the Environmental Monitoring and Assessment Program for Surface Waters (EMAP-SW). This aimed to:

(i) Catalogue the present geographical distribution and current ecological conditions of the nation's lakes and rivers,
(ii) Determine the proportion of sites that are degrading or recovering,
(iii) Determine where, and at what rates, these changes are occurring
(iv) Identify the likely causes of these changes (Larsen *et al*. 1991, Dixit *et al*. 1999).

The program set out to develop methods of achieving these goals in a statistically and ecologically rigorous way (Hughes *et al.* 2000).

The objectives of EMAP-SW have many parallels with those of the WFD and the approaches used to establish nutrient reference conditions in this programme are reviewed, here. Palaeoecological techniques were combined with a 'top and bottom' sediment sampling scheme that had been successfully developed for, and applied to, the Palaeoecological Investigation of Recent Lake Acidification (PIRLA-II) Project in the USA (Cumming *et al*. 1992, USEPA 1998). The 'top and bottom' sampling approach has the advantage that it can be applied easily to a large number of lakes because it involves the analysis of only two samples per site from a single sediment core. Biological assemblages in the top (0-1 cm) sediment sample are taken to represent present-day conditions, while those of the bottom sample (from > 30 cm deep in the core) are generally taken to represent pre-disturbance conditions. The latter can be analysed for ^{210}Pb activity to confirm the chronology. If the ^{210}Pb activity in the bottom sample is close to background activity for lakes in that region, then it can be reasonably assumed that the sample was deposited prior to 1850 AD. Hydrochemical transfer functions are then applied to infer the lake water parameters of interest, with the bottom samples identifying the reference conditions.

EMAP employed a probability-based sampling design that would allow lake-specific data to be scaled up to the entire population of lakes in a country or geographical region. 'Top and bottom' diatom data were generated from 257 lakes distributed across three broad eco-regions of northeastern USA and representative of the target population of over 10,000 lakes. Diatom inference models were successfully applied to reconstruct lake pH, TP and chloride concentrations and the study demonstrated that a sample of a few hundred sites is sufficient to infer current and past conditions of thousands of lakes (Dixit *et al.* 1999). With the development of a standing waters database for the UK in progress, this probability-based sampling design approach could very easily be adapted for lakes in the UK if a similar palaeolimnological study of a few hundred UK lakes were undertaken to provide the necessary data.

Sedimentary-phosphorus reconstructions: geochemical techniques

Geochemical records are valuable for monitoring eutrophication histories. However, interpreting sedimentary TP profiles in terms of changes in the historical TP load is difficult due to variable retention and post-depositional diagenesis (Anderson *et al.* 1993). A number of studies have shown that sedimentary TP concentrations are highly variable, probably due to redox-derived effects, and exhibit only a vague relationship to the timing of increased TP inputs and even less to changes in the whole-lake TP load (e.g. Engstrom and Wright 1985, Anderson *et al.* 1993, Anderson and Rippey 1994, Bennion *et al.* 1995). Nevertheless, there are some sites in which an accurate, relatively undisturbed record of epilimnetic TP concentrations is preserved in the geochemical sediment history (e.g. Shapiro *et al.* 1971, Foster *et al.* 1996). In summary, sedimentary TP profiles must be interpreted with caution and the difficulties described here probably preclude this from being a viable technique for establishing nutrient reference conditions in the context of the WFD.

Palaeolimnological TP load reconstructions

In the first study of its kind, Jordan *et al.* (2001) have recently employed diatom-TP transfer functions and geochemical TP measurements in combination with mass balance equations to provide a novel approach to modelling historical TP loads to lakes from diffuse sources. The model was applied to one site in Northern Ireland to quantify diffuse TP loads over approximately the last century. Diatom-TP transfer functions have previously been widely applied to sediment cores to reconstruct *epilimnetic* TP concentrations as described above, and this study makes the logical, yet original, step of using them to estimate historical TP outflow losses and TP storage changes for inclusion in TP *loading* calculations. The palaeolimnological model is verified by comparison with contemporary data and model performance is sufficiently described using regression and significance statistics. Importantly, the study demonstrates concordance between the palaeolimnological model results and those of soil-TP models, both supportive of soil-TP threshold exceedance at the study site.

This appears to be a powerful technique for establishing nutrient reference loads rather than concentrations. One disadvantage of the approach is that it requires analysis of a number of sediment cores from a single site, to represent all water depths and the major sediment depositional zones, in order to obtain reliable results. Indeed, Jordan *et al.* (2001) analysed eight cores from their study site. It is unlikely that such detailed studies could be undertaken at a large numbers of lakes owing to resource implications.

6.2.3 Rivers

Although palaeoecological techniques provide a powerful tool for establishing nutrient reference conditions in lakes, this approach has limited use in lotic ecosystems owing to the lack of continually accumulating sediments. However, suitable undisturbed sediments can sometimes be found where rivers drain into reservoirs or deltas (e.g. Amoros and van Urk 1989), and in palaeo-channels and floodplain lakes in lowland river systems.

Reference conditions for lowland floodplain rivers cannot easily be defined using the spatial approach because almost all lowland rivers have been affected to some degree by flow regulation, agricultural development or point source pollution and few exist in a minimally impacted state (Thoms *et al.* 1999). Reference conditions for these systems can, however, be established using palaeo or historical records.

In terms of palaeoecological methods, the floodplains of relatively large, lowland rivers often have old channel systems (palaeo-channels) and associated oxbow lakes (or billabongs as they are known in Australia). Such systems are low energy and thus provide suitable sedimentary environments for palaeoecological study. For example, Ogden (1996) examined historical variation in billabong macrophyte cover in response to European settlement on the southeast River Murray floodplains, Australia, using the fossil cladoceran record. Likewise, Reid (1997) determined the impact of agriculture and river regulation on water quality in billabongs of southeastern Australia using the fossil diatom record. These two studies provide only a qualitative interpretation of the changes observed in the cores and do not attempt to perform quantitative reconstructions of nutrient concentrations in the waterbodies. Nevertheless, they illustrate the potential of the palaeoecological approach for establishing reference conditions in lowland river environments (Thoms *et al.* 1999, Reid *et al.* in press). Reavie and Smol (1997) developed reliable transfer functions for reconstructing past habitat conditions based on diatom habitat preferences (rock, *Cladophora*, macrophytes) in the St. Lawrence River, Canada. The models were applied to a sediment sequence from a fluvial section of the river and showed promising results with a marked increase in macrophyte-associated diatom taxa that the authors attributed to accelerated nutrient inputs.

However, there are a number of potential problems associated with sediment records of floodplain lakes. These include variable sediment sources and transport of allochthonous material, water level fluctuation, and sediment scouring caused by floods. So, some floodplain lakes may not provide reliable records of environmental change and data must be interpreted with caution. Hughes *et al.* (2000) state that the use of palaeoecology in rivers is largely confined to oxbow lakes, sloughs or other habitat types where sediments may accumulate for hundreds of years. It is not likely, therefore, to offer a widely applicable method for determining nutrient reference conditions in UK rivers.

Where undisturbed reference sites no longer exist, an alternative approach is to use epiphytic diatoms on macrophyte herbarium records. A pilot study applying the Trophic Diatom Index (TDI) to herbarium records from chalk streams successfully highlighted the potential of this approach (Hourigan, 1999; see Section 2.3 for details of TDI).

6.2.4 Transitional waters

Coastal lagoons

Long-term water quality data for coastal lagoons are scarce in the UK and thus a palaeoecological approach is a valuable way of defining reference conditions. Lagoons provide a suitable sedimentary environment for palaeoecological study because they are relatively sheltered from wave and tide exposure (Carvalho *et al.* 2000). They act as natural traps for organic material derived from both their own system and from the surrounding environment (Martin, 2002). In a study of diatoms and charophyte oospores from a sediment core taken in the Fleet lagoon, Dorset, Carvalho *et al.* (2000) illustrated the potential of the sediment record for providing a long-term perspective on the salinity variability in the system.

Despite the potential of lagoonal environments for palaeoecological research and the susceptibility of their specialist plant communities, particularly charophytes (e.g. Martin and Carvalho submitted) to enrichment, quantitative reconstructions of nutrient concentrations in brackish systems have only recently been developed. Martin (2002) generated the first diatom transfer functions for reconstructing both salinity and nutrient conditions in coastal lagoons. The training set comprised 36 lagoon sites from Hampshire, Isle of Wight, Dorset, Norfolk, Kent and West Sussex in England, and North and South Uist in Scotland, spanning a gradient of 12-740 µg TP l^{-1} and a salinity gradient of 2-32 g l^{-1}. Ordination analyses indicated that both TP and salinity were independently statistically significant in accounting for floristic patterns in the diatom data, with few inter-related effects. Subsequently, a diatom WA-PLS model was generated for reconstructing TP with a good correlation between observed and predicted values for TP concentrations of less than around 160 µg TP l^{-1}. At higher TP concentrations, the model consistently underestimated TP concentrations in the lagoons.

The CASSARINA (ChAnge, Stress and Sustainability: Aquatic ecosystem Resilience In North Africa) project (Flower 2001) highlighted a number of benefits and problems associated with palaeolimnological studies in brackish wetland lake systems. The project uses a suite of multi-proxy palaeoecological indicators (diatoms, zooplankton, higher plants, benthic animals, pollen) in dated sediment cores to infer past water quality and biodiversity changes at nine North African wetland lakes during the 20th century. Dissolution of diatoms is often an important issue in brackish lake sediments and, indeed, only two of the sediment cores collected contained full and generally well preserved diatom sequences. At two sites, diatom preservation problems severely degraded the sedimentary diatom record. Planktonic diatoms, which are generally good indicators of changes in trophic status, were rare. At two sites, planktonic diatoms indicated some recent eutrophication but, generally, the assemblages were more indicative of salinity changes. Zooplankton remains, however, were generally well preserved in all the CASSARINA sediment cores and the Cladocera were particularly useful for assessing changes in trophic status. Despite some problems with sediment dating and microfossil preservation, recent biological changes at the CASSARINA sites were generally well documented by palaeoecology. The project combined the results with those of modern survey to provide an integrated assessment of recent environmental change.

Coastal wetlands and estuaries

Palaeoecological studies in estuarine environments have lagged behind those in freshwater lake systems largely because of the more dynamic nature of coastal ecosystems (Cooper 1999). In coastal sediments of tidal channels, sand and mud flats, and saltmarshes, diatoms are typically used to record palaeoenvironmental changes in salinity and tidal exposure (e.g. Juggins 1992, Vos and de Wolf 1993) but have not been widely used for establishing information on changes in trophic state. One difficulty of using diatoms as water quality indicators in tidal environments is that their distribution is determined by a complex of ecological factors and thus the ecology of marine and brackish species is perhaps less well understood than that of freshwater taxa (Sullivan 1999). Additionally, sediment re-suspension and transport poses problems and differential silicification and preservation of diatom valves can occur (Cooper 1999). Furthermore, the salinity gradient is likely to be stronger than the nutrient signal making it difficult to establish the relationship between nutrient concentrations and particular taxa. A few studies have shown that nutrients, such as phosphate and ammonia, do influence the composition of diatom assemblages in tidal ecosystems although they are few in number and, therefore, the relationships remain poorly understood (e.g. Colijn and Dijkema 1981, Admiraal 1984). In the UK, Underwood *et al.* (1998) studied the distribution of epipelic (mud-associated) diatoms along salinity and nutrient gradients in the Colne Estuary, Essex. They found a significant correlation between the diatom species and both gradients. However, an earlier study by Underwood (1994) indicated that the epipelic and epiphytic (plant-associated) diatom distributions were largely explained only by salinity. More studies on the autecology of estuarine diatoms are needed (Cooper 1999).

A second problem is the distinction between allochthonous species (transported from elsewhere) and autochthonous species (living at the place of deposition). Only the latter will provide information on local conditions whereas the former will have been introduced by tidal currents or rivers and will only supply information about the wider environment. In high energy, tidal environments the allochthonous influx of diatoms, comprised largely of marine planktonic taxa, can outnumber the autochthonous population (Simonsen 1969), thus causing a major problem for establishing site-specific reference conditions. For this reason, Simonsen (1969) proposed that only benthic taxa should be used in palaeoecological reconstruction in estuarine systems. The problem is most acute in the sediments of tidal channels and inlets because high current velocities provide unfavourable conditions for the development of an *in situ* benthic or epiphytic population. The allochthonous component is also commonly significant in the sediments of tidal mud flats, salt marshes and tide-influenced lagoons and ponds (Vos and de Wolf 1993). On intertidal sand flats, however, the representation of the autochthonous benthic community is less problematic.

Despite these difficulties, a number of studies have employed palaeoecological techniques to assess nutrient enrichment in estuarine environments (e.g. Brush and Davis 1984, Wendker 1990, Cooper 1995). These are reviewed by Cooper (1999). Currently, however, there are no training sets for inferring nutrient concentrations in coastal wetlands and estuaries. Some basic research would need to be undertaken to explore the potential of quantitative palaeoecology in these systems. Given the range of problems described above it is unlikely that high performance models for determining nutrient reference conditions for these waters could be produced within the time frame required by the WFD.

6.2.5 Coastal waters

Coastal waters have experienced massive changes in nutrient loading from anthropogenic activities during the history of human occupation. Most monitoring programs, however, began only in the last 30 years, thus severely limiting our perspective on when changes began, and on nutrient reference conditions prior to anthropogenic impact. Given the lack of long-term chemical or biological monitoring, palaeoecological methods provide a means of obtaining such information.

Anderson and Vos (1992) discuss the potential role of diatoms as palaeoecological indicators of changes in marine environments, with particular emphasis on their role in defining nutrient reference concentrations in the North Sea. They identify a number of problems with sediment records in such environments including high-energy inputs, dynamic and heterogeneous sedimentation, and diatom dissolution. Nevertheless, they argue that the scale of coastal marine systems and the nature of the problems are similar to those encountered in palaeo-studies of the North American Great Lakes where sediment records have shown clear enrichment in response to anthropogenic activity (e.g. Schelske 1991). A number of qualitative sediment studies in the Baltic (e.g. Risberg 1990, Grönland 1993, Korhola and Blom 1996) and the Adriatic (Puskaric *et al.* 1990) demonstrate the value of a palaeolimnological approach for assessing eutrophication in coastal environments. Furthermore, nutrient transfer functions have been developed for oceanic surface sediment diatom assemblages (e.g. Pichon *et al.* 1987) and have been successfully applied to address climate change issues in particular. Anderson and Vos (1992) conclude their review by suggesting that there are possibilities for the application of palaeoecological methods to eutrophication problems in coastal systems, and they call for the development of suitable training sets and transfer functions to allow quantitative estimates of past concentrations of key nutrients.

In contrast to the wide application of quantitative palaeolimnological techniques in fresh water systems, the potential to reconstruct past nutrient concentrations in coastal environments has only recently been explored. Two diatom-nutrient training sets have been developed for inferring long-term trends in nutrient concentrations in the coastal zone of the Baltic Sea (Weckström *et al.* 2000). The first is a Finnish dataset of 49 sheltered, small and shallow embayments with a mean depth for the dataset of 4.3 m. The second is from Denmark and comprises 70 sites from a range of coastal environments from shallow, sheltered fjords to deeper, open waters of the Belt Seas. Between them, the datasets cover a long salinity gradient (0.6-31.1‰). Ordination analysis showed that water depth explained the most variance in the diatom data in both datasets, and salinity was also an important and independent variable. Both total dissolved nitrogen (TDN) and TP significantly and uniquely explained an equal amount of the variation in the Finnish dataset but only total nitrogen (TN) accounted for a significant and unique part of the variation in the Danish data. Thus transfer functions were developed for both TDN and TP for Finland and for TN for Denmark. All three models showed a good correlation between observed and inferred values indicating that they were robust and that they were appropriate for predicting nutrient concentrations in these waters. The models were subsequently applied to a short core from a Finnish coastal site and another from Denmark to infer changes in nutrient concentrations over the last 100-200 years. The results suggested that both sites have experienced eutrophication over the last 50-100 years associated with an increase in planktonic taxa. These studies demonstrate that palaeoecological techniques provide a powerful means of defining reference conditions for nutrients in coastal waters.

An EU funded programme called MOLTEN (Monitoring Long-term Trends in Eutrophication and Nutrients in the coastal zone: Evaluation of background conditions, anthropogenic influence and recovery) aims to develop a novel palaeoecological methodology for assessing reference conditions, the degree of past and present departure from these conditions, and likely recovery with reduced nutrient loads of coastal systems. The project will use the high resolution record of environmental changes recorded in coastal sediments to reconstruct the long-term changes in nutrient loading in four representative European coastal basins: Mariager Fjord, Denmark; Himmerfjarden, Sweden; Laajalahti, Finland; and the Elms-Dollard Estuary, The Netherlands. Transfer functions for nutrient concentrations (N and TP) will be developed from the four European basins representing approximately one third of the total European coastal zone. Diatom-nutrient transfer functions have already been developed for two of the basins (Weckström *et al*. 2000) but two additional basins will be added to this training set by sampling a broad range of coastal ecosystems and utilising water quality data from contemporary monitoring programs. The project will also assess the associated biological responses to changes in nutrient loading in each area using a multi-proxy approach (phytoplankton, littoral macrophytes, zoobenthos). Ultimately, the project aims to use the palaeoecological data for the period 1850-1900 to establish ecological and chemical reference conditions for each basin under minimal human impact. In summary, MOLTEN will provide an evaluation of the effectiveness of the different palaeoecological records for assessing the ecological status of coastal waters and, importantly, will derive a minimum, parsimonious sampling strategy appropriate to provide a cost-effective tool for addressing the key points of the WFD in a standardised way across a large part of the European coastal zone. Specific guidelines will be prepared for management authorities to use in implementing the WFD.

6.3 Spatial-state Approaches

6.3.1 Introduction

This section reviews existing spatial-state (or reference state) schemes. The major literature sources used to compile this review are the technical guidance manuals developed by the USEPA for lakes and reservoirs (USEPA 1998, USEPA 2000a), estuarine and coastal marine waters (USEPA 2000b) and streams and wadeable rivers (Barbour *et al*. 1999), and the proceedings of a workshop on RIVPACS and other techniques for assessing the biological quality of freshwaters (Wright *et al.* 2000). The most accessible information on the use of spatial-state reference schemes for coastal marine wetlands is the web page of the Massachusetts Coastal Zone Management (www.state.ma.us/czm/wastart.htm) but is considered here to be outside the terms of reference of the current review.

For all water body types (lakes, rivers, transitional and coastal) work has tended to concentrate on the establishment of the reference condition itself rather than on unified schemes or programmes in which different biological components (e.g. macrophytes, diatoms, benthic invertebrates or fish) from test sites are directly compared to the reference condition to evaluate ecological quality status of the site. In running watercourses, the exception to this has been the development of the RIVPACS, AUSRIVAS and the BEAST programmes, which are spatial-state reference schemes whereby macroinvertebrate communities are compared with reference sites. A similar scheme, PSYM, for assessing the ecological status of ponds in England and Wales is under operational testing. In contrast, many North American test sites are evaluated using customised multimetrics that best meet the needs in individual areas. An area of analysis receiving increased attention is artificial intelligence, with promising developments in both the United Kingdom and more widely in the EU.

There are two primary approaches to establishing spatial-state reference conditions using biological survey. The first approach uses selected best-quality sites as the basis for determining reference conditions. The second approach does not use reference sites, but draws its reference conditions directly from those found in a sample of many water bodies of varying quality. Candidate metrics are developed from the key biological attributes, and the effects of stressors on specific metrics must be understood (USEPA 1996a). Those measurements that have a monotonic response to a gradient of conditions (from unimpaired to heavily impaired) will be the best candidates for assessing biological impairment. Therefore, ambient sites other than reference sites should be surveyed as part of the database. Selection and confirmation of the measurements must address the ability to differentiate between impaired and unimpaired sites.

6.3.2 Lakes

For lakes and reservoirs it has been suggested that biological surveys yield the best obtainable data on the current status of the water body (Cardoso *et al.* 2000; USEPA 1998, 2000a). Any biological assemblages deemed to be important can be used.

Selected reference sites approach

Selection of reference sites based on a prior definition of reference site criteria is used when a sufficient number of lakes exist that are minimally impacted. Since nearly all lakes are affected by human activities to some degree, the lakes need not be pristine or unimpacted, but the level of impact must be minimal relative to lakes in the region. Reference sites are selected using local expert knowledge on candidate sites, mapped information such as land use and roads, and other existing databases.

In the United Kingdom, the Pond Conservation Trust: Policy and Research Section (formerly Pond Action) has been developing a predictive model (PSYM: Predictive SYstem for Multimetrics) for assessing the ecological status of lowland ponds based on reference samples (Williams *et al.* 1998). PSYM integrates the multivariate prediction procedures used in RIVPACS (Wright *et al.* 2000, Section 6.3.3) and the multimetric approach adopted by the USEPA (Barbour *et al.* 1995, Section 6.3.3). This system, which links environmental characteristics of ponds to both their macrophytic and macroinvertebrate assemblages, is being developed in collaboration with the Environment Agency and an early operational version is currently being tested. The operational version integrates the information content of a range of metrics to compare the existing characteristics of the flora and fauna with the reference metric values to be expected in ponds of similar environmental character but subject to no significant environmental stress.

Percentile data selection approach

The determination of reference conditions can also be based on the best conditions found in a representative sample of lakes within a class. The approach does not involve the identification of reference lakes, but sets reference conditions by using an entire population of lakes from within a region. This approach is used when few appropriate reference sites exist, when they cannot be suitably defined, when a fixed definition of a reference condition is restrictive or is an impractical ideal. A number of lakes within the class are surveyed, and the best conditions for each measurement are determined from the entire sample of lakes. These best conditions are then used as the reference for biological assessment within that lake class. The approach is especially relevant for reservoirs, where no unimpaired systems exist, as well as for lakes that are subject to strong and relatively uniform human impacts, such as lakes in large urbanised areas or in heavily agriculturally dominated regions.

A representative sample of lakes is taken from the entire lake population excluding lakes that are known to be severely impaired. The population distribution of each selected variable is determined, and the best quartile of each variable is taken as its reference value. The reference value is a reasonable upper limit excluding outliers of the population distribution. The chosen reference value is critical. Lakes exceeding the value are in unacceptable (eutrophic) condition and should be dealt with through restoration, while those achieving less than the reference value are supposed to be in acceptable condition and their nutrient concentration should not be allowed to increase. Therefore, the point where the line is drawn for the best quartile determines the effort necessary for lake restoration. The establishment of reference conditions through other methodologies would help to set the position of the line as objectively as possible.

There is an underlying assumption that at least some of the lakes are high quality, minimally impacted waterbodies. The method also relies on each geographical region having sufficient standing waters for those with naturally different levels of eutrophication (e.g. upland vs. lowland) to be analysed separately, otherwise a naturally eutrophic lowland lake could be set a nutrient reference condition that would be impossible to achieve. It would be difficult to find sufficient high quality waterbodies for this approach to work on British lakes, especially in intensively farmed, lowland areas.

6.3.3 Rivers

Selected reference sites approach

There are, to date, three spatial-state reference schemes comparing macroinvertebrate community data from test sites with data from similar but unimpacted or minimally impacted reference sites. These are the River InVertebrate Prediction And Classification Scheme (RIVPACS) (Wright *et al.* 2000), the AUStralian RIVer Assessment Scheme (AUSRIVAS) (Davies 2000), and the BEnthic Assessment of SedimenT (BEAST) program (Reynoldson *et al.* 2000). These are described in turn in the following sections.

RIVPACS

RIVPACS was the first integrated spatial-state reference scheme for predicting water quality using macro-invertebrates. It is a software package developed by the Centre for Ecology and Hydrology (CEH). Wright *et al.* (2000) describe the background to the RIVPACS programme (Wright 2000), discuss the evolution of the statistical methods used (Moss 2000) and the uncertainty in estimates of biological quality (Clarke 2000), describe the methodology for classification of rivers in England and Wales (Hemsley-Flint 2000), explore quality assurance issues (Dines and Murray-Bligh 2000), discuss the application of RIVPACS to headwater streams (Furse 2000), and examine the potential of RIVPACS for predicting the effects of environmental change (Armitage 2000). Only the key findings of these papers are summarised here.

The primary application of RIVPACS is to assess the biological quality of rivers within the UK (Wright 2000). The programme offers site-specific predictions of the macroinvertebrate fauna to be expected in the absence of major environmental stress. The expected fauna is derived using a small suite of environmental characteristics and the biological evaluation for a given site is then obtained by comparing this with the observed fauna. RIVPACS also includes a site classification based on the macroinvertebrate fauna of the component reference sites. New sites, judged by their fauna to be of high biological quality, may be allocated to classification groups within the fixed RIVPACS classification. This has potential for evaluating sites for conservation.

Major scientific and operational developments were made over the life of the project. For RIVPACS I and II, TWINSPAN was used for classification of the reference sites. This was followed by a prediction procedure based on the use of multiple discriminant analysis (MDA), and a new method for predicting the probability of occurrence of individual taxa. Prior to the development of RIVPACS III some alternative methods for site classification, and a novel approach to prediction using logistic regression, were examined. These methods were tested internally on the RIVPACS II dataset and on an independent dataset. Although some methods showed promise, the method eventually chosen for RIVPACS III was a variation of the original TWINSPAN/MDA approach.

Uncertainty in estimates of biological quality based on RIVPACS is discussed by Clarke (2000). RIVPACS derives estimates of the macroinvertebrate fauna to be expected at a river site in the absence of environmental stress. Biological quality indices can be derived from statistics, which measure some aspect of lack of agreement between the observed and the expected fauna. To assess the significance of spatial or temporal differences in the values of an index requires knowledge and quantitative estimates of the sources of variation and errors in both the observed and expected fauna, jointly referred to as the uncertainty. Detailed procedures are proposed to assess the uncertainty in quality indices based on the ratio of observed (O) to expected (E) values of the BMWP indices. Studies involving a replicated sampling programme across a range of types and qualities of site were used to derive estimates of sampling variation, sample processing bias and the effect of errors in measuring the environmental predictor variables. The latest version of the RIVPACS software package, RIVPACS III+, uses statistical simulations which integrate these sources of variation to provide an assessment of the uncertainty in O/E values, the assignment of sites to quality bands, spatial and/or temporal differences in O/E values, and potential changes in quality band.

The Environment Agency, with the aid of RIVPACS has defined a scheme for categorising river sites into six quality grades, according to their macroinvertebrate communities. The Environment Agency has adopted quality assurance procedures for freshwater macroinvertebrate sampling and analysis in order to obtain data of known and appropriate quality on which to base river management decisions. Audits by the IFE (now CEH) provide an analytical yardstick against which the performance of Agency biologists is measured (Dines and Murray-Bligh 2000). The adoption of standard methods was the starting point for producing reliable information. Error terms for sampling variability were estimated in a one-off investigation, and these, together with data from audits, are now used in RIVPACS III and the General Quality Assessment Biological Classification scheme. An analytical quality control scheme has been developed to enable laboratories to ensure that they meet the quality standards.

Trials using RIVPACS II to evaluate the quality of headwaters suggested that ASPT values could be predicted with reasonable accuracy, but the numbers of taxa to be expected were almost always over-predicted because RIVPACS lacked adequate reference sites (Furse 2000). A headwaters version of RIVPACS was subsequently developed and tested. This was based on the application of MDA to a TWINSPAN classification of the macroinvertebrate assemblages of 214 essentially unpolluted headwater sites.

RIVPACS has been used successfully for biological assessment of river quality but its potential for forecasting the effects of environmental change has not been investigated. Armitage (2000) showed that it is possible to simulate faunal changes in response to environmental disturbance, provided that the disturbance directly involves the environmental variables used in RIVPACS predictions. These variables relate to channel shape, discharge and substratum. Many impacts, particularly those associated with pollution, will not affect these variables and therefore RIVPACS cannot simulate the effects of pollution. RIVPACS is essentially only sensitive to major changes in substratum. It was concluded that, because of the static nature of RIVPACS, it cannot respond to the dynamic effects and processes associated with environmental disturbance (Armitage 2000). Thus RIVPACS, while showing direction of change and indicating sensitive taxa, cannot be used to predict or forecast the effects of environmental impacts.

AUSRIVAS and BEAST

The British RIVPACS programme has largely formed the basis for two further spatial-state reference schemes: AUSRIVAS and the BEAST. The AUStralian RIVer Assessment Scheme (AUSRIVAS) (Davies 2000) has been developed in a cooperative effort between federal and state government agencies, and a variety of researchers. The RIVPACS framework was selected for adaptation to Australian conditions under the nationally managed Monitoring River Health Initiative (MRHI), and over 1500 reference sites were sampled over a two-year period for invertebrates and environmental variables, under a common protocol.

The development of AUSRIVAS models and outputs (Simpson and Norris 2000) illustrates several important differences between AUSRIVAS and RIVPACS. Australia is much larger and has a more varied landscape than Britain. Australia's size required, initially, development of 48 models for individual states or distinct geographic areas, in order to achieve better resolution for assessing sites within a particular region. In Australia, habitats are sampled separately, allowing both single and multiple habitat assessments. Rules have been developed for combining the assessments obtained from different habitats as well as assessments from different seasons. Extensive testing is still required to ensure confidence in these 'combined' assessments.

Separate classifications and sets of predictor variables are used for each of the 48 AUSRIVAS models currently being developed, but latitude, longitude and alkalinity were common to two-thirds of them. Only those taxa having >50% probabilities of occurrence are used in the AUSRIVAS models for calculating the expected number of taxa and SIGNAL index at a site. Large numbers of models, and the proposed extensive number of users, led to the development of AUSRIVAS as an internet-based program. The First National Assessment of River Health in Australia, for which sampling was to be undertaken in 1997-1999, would be the first large-scale test of AUSRIVAS in providing useful information on the ecological condition of Australia's rivers and streams.

Operator sample processing errors and temporal variability had implications for model sensitivity in AUSRIVAS (Humphrey *et al.* 2000). At the onset of AUSRIVAS, it was recognised that any serious compromise to the sensitivity of derived models was most likely to arise from errors in field live-sorting procedures and from temporal variability of macroinvertebrate communities. Sorting error and the degree of temporal variability have been quantified using family-level presence/absence data, to assess the implications for the development of models. External Quality Assurance/Quality Control (QA/QC) audits of state/territory sample processing procedures (laboratory subsampling and sorting of preserved samples, and field live-sorting procedures) have confirmed the potential of the live-sort procedure to result in high error rates, particularly as the result of poor recovery of small and cryptic taxa. Many of these missed taxa are common in samples.

Temporal variability of macroinvertebrate communities has been shown to be high for much of Australia, at least on a seasonal basis. This is attributable largely to the vicissitudes of the Australian climate. For the most part, current operator sorting errors and temporal variability are unlikely to prevent successful model development, but either in isolation or combination could lead to increased frequency of misclassification and inaccurate model predictions. Simulations based on actual errors recorded by QA/QC audits support this conclusion. Refinements are currently being made to live-sorting protocols, to reduce sample processing errors. Combined seasons models, together with concurrent repeated sampling of selected reference sites to adjust models, may offer the only suitable solutions to temporal variability. The results are consistent with the promotion of AUSRIVAS as a coarse, broad-scale screening tool for biological monitoring in Australia.

A new development of the AUSRIVAS approach, recently presented to the 2001 Annual Meeting of the North American Benthological Society by Richard Norris (www.benthos.org/database/allnabstracts.cfm/db/lacrosse200/abstracts/id/345) is to use whole-reach reference conditions and assessments of ecological status. In this approach the biota remain the key measure of river condition but the predictive variables include catchment variables such as sediment and nutrient exports from land uses, catchment land cover and catchment infrastructure together with habitat variables such as hydrology, riparian vegetation, water quality and channel condition. The researchers are currently developing the model for benthic invertebrates but foresee the potential for models predicting the reach reference conditions for birds, fish, aquatic macrophytes and diatoms.

Another new direction of the Australian programme is the development of a so-called "dirty-water" model for predicting the benthic diatom and macroinvertebrate communities for streams in urban situations. Although the models proposed may be considered as spatial-state reference schemes, in this context all sites, not just those considered to be minimally impacted, are used as reference sites of a state of ecological quality (Section 6.3.3.5). In these models the predictor variables used are not exclusively those un-influenced by human activities, as in the normal AUSRIVAS models, but also incorporate those that can be potentially altered by catchment managers. By incorporating sites over a range of ecological quality conditions, it is hoped that the model will be capable of use for answering questions of the “what if?” variety if particular new catchment management strategies, such as reduction in nutrient inputs, are planned. In a similar fashion, in the UK the Centre for Ecology and Hydrology, Dorset are undertaking a research project for the Environment Agency (E1-007), commonly known as “dirty water RIVPACS” in which a model, comparable with the proposed Australian system is being built using the biological and chemical results of the National River Authority’s 1990 River Quality Survey and 1995 General Quality Assessment.

The BEnthic Assessment of SedimenT (BEAST) program (Reynoldson *et al.* 2000) was originally developed for lentic rather than lotic environments as a predictive approach for assessing sediment quality in North American Great Lakes. Subsequently it was used to establish reference conditions in the Fraser River catchment, British Columbia, Canada by Reynoldson *et al.* (2000). The BEAST model is a three-stage multivariate approach to establishing reference conditions. Reference sites (representative of the ‘best available condition’) were chosen using ecoregions and stream orders within ecoregions. A small number of test sites (suspected of being impacted) were used for comparison with the reference sites when developing the model. Environmental variables representing map, site, channel and water-column scales were measured at each site. Single macroinvertebrate samples were collected from riffle habitats at each site using a kicknet sampler (400-um mesh) for three minutes.

Sites in the Fraser River catchment were sampled in the autumn of 1994, 1995 and 1996. Results from 1994 and 1995 field seasons used 127 reference sites and produced five reference groups based on macroinvertebrate community structure. Ten optimum predictor variables were identified for matching new sites to the appropriate reference group, and included map, site, channel and water-column measurements. The error rate for predicting test sites to particular reference groups in cross-validation studies was 37%. The model was capable of discriminating the most impacted of the test sites used.

Multimetrics and rapid bioassessment protocols

Rapid bioassessment protocols are widely used in the United States, based on the multimetric approach in which scores for individual metrics (or measures) are evaluated against thresholds developed from reference sites, and a composite score is then calculated (Barbour *et al.* 1999; Resh *et al.* 2000). The multivariate approaches (such as RIVPACS, AUSRIVAS and the BEAST) that use benthic macroinvertebrates in assessments of water quality are similar to the multimteric approach in their data collection methods, but differ in the way that reference sites are selected, and test sites are classified and assessed. The conclusion to a study of forty-four metrics calculated from collections of benthic macroinvertebrates at seventeen sites in the Fraser River catchment in British Columbia (Resh *et al.* 2000) was that multimetric approaches should consider incorporating multivariate analyses for defining reference conditions and assessing impairment of test sites. Collaboration among users of multimetric and multivariate approaches can enhance both types of water quality monitoring and assessment programs.

The potential for integrating multivariate and multimetric approaches, advocated by Resh *et al.* (2000) and already adopted for English and Welsh ponds (Williams *et al.* 1999), is now a key area of research in another important EU Framework V project entitled AQEM. The aim of the project is to develop and test an assessment procedure for streams and rivers, which meets the demands of the WFD using benthic macroinvertebrates. The 16 project partners represent a transect from northern to southern Europe (Scandinavia to Central-Mediterranean area) and another transect from the Iberian Peninsula to the Eastern Mediterranean. The assessment system will be based on the outlines of a European stream typology and on the fauna of near-natural reference streams. In a manner similar to RIVPACS, multivariate analyses will be applied to the reference site data and the contemporary environmental data collected for each site in order to provide predictive models that provide site-specific standards of ecological quality and a mechanism for classifying sites into grades of ecological status. However, unlike RIVPACS, the project intends to use a broad range of multimetrics in order to diagnose the probable cause, including nutrient enrichment, of environmental degradation at sites that fail to meet their ecological targets.

This dual combination of multivariate and multimetric techniques in a diagnostic fashion may lead to the development of a suite of spatial-state reference schemes specific to individual eco-regions and potentially to individual stressors. However, it is the aim of AQEM and its possible successor project STAR to ensure that all such models are fully inter-calibrated to provide common European standards for the categorisation of rivers to the five grades of ecological status required by the Water Framework Directive. Whilst the current AQEM project, which will be completed in spring 2002 is concerned specifically with lotic benthic invertebrates, the STAR project, if funded, will develop and inter-calibrate equivalent spatial-state reference schemes for diatoms, aquatic macrophytes and fish. In developing the fish model, the STAR project will be clustered with another potential Framework V project, FAME, which will concentrate specifically on fish assemblages. The Environment Agency are advisors to the AQEM project and will be full partners of both STAR and AQEM.

Artificial Intelligence

The use of artificial intelligence (AI) approaches for the assessment of the ecological status of rivers and streams in the United Kingdom is being led by Bill Walley and his colleagues at the School of Computing at Staffordshire University. The dual approach adopted by Walley integrates both “probabilistic”, knowledge-based reasoning and pattern definition. Bayesian reasoning and neural networks are used to develop these two concepts. Walley and Fontama (2000) argue that, unlike RIVPACS, AI takes a more holistic view in which all existing data, not exclusively a single, “high quality” reference state are used to evaluate the ecological status of freshwater sites.

The relative merits of AI techniques and multimetric approaches were the subject of an expert group discussion at the Oxford “RIVPACS” workshop (Johnson 2000). The group concluded that, in terms of classification and prediction of aquatic lotic macro-invertebrate assemblages and derived metrics, AI and the RIVPACS-type approach gave similar results. Where they felt that the AI approach currently offered additional benefits was in providing a diagnostic interpretation. The extent to which the diagnostic properties of AI exceed those of the multivariate and multimetric approaches will become clearer when projects such as AQEM attempt to use non-AI analyses for diagnostic purposes. Walley and colleagues are currently developing a River Pollution Diagnostic System (RPDS). Details of the prototype operational system, which is under test by the Environment Agency, are available at the following internet site: http://www.marlab.ac.uk/NMPR/Papers/NMMP%20Workshop%20Summary.pdf

On this site the following is a verbatim quote of the introduction and overview of the system:

"One process used by experts when interpreting biological data is 'pattern recognition', where data is assessed based on experience of past cases; that is, looking at the data sample as a whole and recognising familiar patterns. In terms of an artificial intelligence system, 'pattern recognition' attempts to categorise objects into groups, using the patterns of important features in the data. For our application, the objects are river site samples and the features are the abundance of the various creatures (benthic macroinvertebrates). (Additional information about the type of river site is also used, such as the width, depth, discharge, and so on.) By examining the patterns in this data, the sites can be classified into output groups representing the river quality.

RPDS is based on a pattern recognition system, MIR-Max (Mutual Information and Regression Maximisation), developed at CIES. MIR-Max 'clusters' data so that each cluster contains data samples that are similar; each cluster can be regarded as being representative of a river water quality 'type' or 'class'. The clusters are arranged so that very similar clusters are close together (and very different clusters are far apart) in the output space, giving a 'map' of the data similar to that produced by Kohonen's Self-Organising Map (SOM) algorithm."

Similar explorations of the merits of AI approaches to developing predictive models for the assessment of the ecological status of rivers forms the basis of a EU Framework V project with the acronym PAEQANN. This project is clustered with AQEM and it is anticipated that the two projects will share reference site data. In PAEQANN, the assessment of river ecosystem integrity will be based on the relationships between environmental impacts and organism groups, i.e. community structure as depending upon the environmental variables. However, the research consortium considers that ecosystem analysis and prediction with empirical statistical and analytical methods are often limited by the spatial complexity, the non-linear relationships between variables and the temporal dynamics of complex ecological processes (http://quercus.cemes.fr/~paeqann/project/projet_gb.html).

For these reasons they consider that artificial neural network (ANN) models have the advantage that they can be used as both predictive tools, but also for explaining and for better understanding the complex relationships between variables. They state that these tools can be applied in river networks throughout Europe and that they will be simple, easy to handle and applicable to stream management and stream policy making.

The main scientific objectives of the PAEQANN project are to

i) Set up a standardised methodological approach
ii) Link the environmental characteristics and the community structure at each reference site by using a defined set of parameters and a combination of target groups representing the main functional levels of the ecosystems
iii) Evaluate, at a functional level, the sensitivity of the studied ecosystems and their response to disturbance through implementation of sensitivity indices and modelling
iv) Investigate the effects of human impacts on the functioning of the ecosystem, i.e. on the composition and change in structural and functional organism groups in comparison to nearby natural reference conditions

In order to promote the development of improved spatial-state reference schemes and the role in that process of AI procedures, the group promoted an international seminar held in Namur, Belgium on 15th-16th September 2001 with the specific objective of addressing the problem of selection of ecological parameters to be used as predictors of community structure in running waters. The proceedings of this meeting, if made publicly available, are likely to be of particular benefit to the current interest of the Environment Agency.

Percentile data selection approach
An alternative method of deriving nutrient reference concentrations for rivers is the frequency distribution approach (US EPA, 2000a). This involves estimating the "best attainable" nutrient concentration of drainage water in a particular geographical area from contemporary measurements over a wide range of streams in the same eco-region. There is an underlying assumption that the ‘best’, or lowest, 25th percentile of these contemporary nutrient concentrations approximates to the best stream nutrient concentrations that are attainable in that area through the treatment of waste water and urban runoff, and through improvements in agricultural practices. However, this is true only if at least 25 percent of the streams included in the analyses are relatively unaffected by anthropogenic influences. This situation would be difficult to achieve in a densely populated country such as Britain, especially in intensively farmed, lowland areas. This technique is, therefore, unlikely to be suitable for use here on a national basis.

6.3.4 Transitional and coastal waters

The USEPA recommend that estuarine and coastal marine reference conditions should be established using some combination of four elements including evaluation of historical data, sampling of reference sites, prediction of expected conditions using models, and expert consensus (USEPA 2000b). As with setting reference conditions for lakes and rivers, the strengths of using present-day biology to assess reference conditions include the availability of actual components that best reflect current status of the water body, and any assemblages or communities of organisms deemed important can be used. The disadvantages of using present-day biology are that even best sites are subject to human impacts, and degraded sites might lower subsequent biocriteria. At a minimum, reference conditions should be identified for each of the estuary and coastal marine classification categories developed for a region (USEPA, 2000b). Reference sites in estuaries and coastal marine waters include either sites that are distant from point and non-point sources and may be applied to a variety of test sites in a given area, or sites that occur along gradients of impact; i.e. nearfield/farfield. All monitoring sites, whether reference or test, can vary spatially and temporally due to natural causes. A central measure from several reference sites is used so that natural variability and uncertainty can be accommodated. Statistically, this means that the status of particular estuarine or coastal marine “test” sites are judged by comparing them to a population of reference sites for a particular classification category.

There are three approaches for using reference sites: selected reference sites, percentile data selection from population distributions, and site-specific reference sites.

Selected reference sites approach
In this approach (USEPA 2000b), reference conditions are characterised based on the best available sites for a given physical class of estuarine or coastal marine waters, and

indexes or models are developed by comparing best sites (the reference sites) to a second set of sites that may be impaired. The approach assumes that within the population of sites some are minimally disturbed and therefore comprise a minimally impaired biological condition.

Selection of reference sites must be physical or chemical; for example, minimal instances of hypoxia, no toxic substances or toxicity, a large proportion of natural vegetation in the watershed, little or no industrial point sources, little or no urban runoff, or little or no agricultural non-point source pollution. Impaired ("test") sites for testing response of metrics and model building are selected for the presence of one or more such anthropogenic disturbances.

Prior definition and selection of reference sites has been used successfully in streams for fish and invertebrate indexes and models (e.g. Barbour *et al*. 1995, Ohio EPA 1987, Reynoldson and Zarull 1993, USEPA 1987, Wright *et al*. 1984) and in estuaries for benthic invertebrate indexes (Engle *et al*. 1994, Summers *et al*. 1993, Weisberg *et al*. 1993).

Percentile data selection approach
This approach has been used successfully for fish and invertebrate indexes in streams (e.g. Karr *et al*. 1986, Plafkin *et al*. 1989) and for fish (Jordan *et al*. 1992, Deegan *et al*. 1997) in estuaries. As described above for lakes and rivers, it can equally be applied to transitional and coastal waters by taking a representative sample of the entire population of estuarine or coastal marine sites. Sites that are known to be severely impaired may be excluded from the sample.

In the United Kingdom the implementation of the WFD in transitional and coastal waters is one of the central themes addressed by the Marine Pollution Monitoring Management Group (MPMMG). Current developments in methods to evaluate the ecological status of these waters were recently summarised at a workshop held in May 2001, 'Development of Indicators on [sic] the quality of the marine environment, hazardous substances, eutrophication and litter'. The proceedings of the workshop include information on the development of spatial-state reference schemes by the University of Hull and are available at:
"www.marlab.ac.uk/NMPR/Papers/NMMP%20Workshop%20Summary.pdf" Further information on the approaches is provided by Elliott and Smith (2000). The University of Hull research includes the prediction, by multiple regression of environmental variable values, of the univariate statistics: species richness, species abundance, species abundance/richness ratios and Shannon's Diversity H'. The second approach by these researchers is the development of a model analogous to the freshwater RIVPACS, called COASTPACS. Like its freshwater equivalent, COASTPACS uses Multiple Discriminant Analysis (MDA) and TWo-way INdicator SPecies ANalysis as the analytical framework for model development. In this case, the predictor variables used are current speed (maximum spring tide), median phi grain size, latitude/longitude, substratum silt content and water depth (logged)).

Both the approaches under development by the University of Hull are used to predict the macrobenthos and to compare the expected statistics/community composition with reference state values derived by a prior sampling programme integrated with conceptual knowledge. However, Elliott and Smith (2000) indicate the potential for developing models for assessing estuarine ecological status using fish populations.

Site-specific reference sites

The site-specific approach (USEPA 2000b) is analogous to upstream/downstream comparisons in running water or control-impact designs. It consists of selecting a reference site paired with each site to be assessed. There is no characterisation of reference conditions for a physical class of estuarine or coastal marine waters; each test site and each reference site is a special case with each test site compared to its reference site. Reference sites are selected to be similar to their respective test site, but unimpaired by the perturbations of interest at the test site. This approach may be less costly at the outset because the design and logistics are simpler than the other approaches. However, after several years of sampling and monitoring, costs for this approach are likely to be similar or greater because each new test site requires its own paired reference site.

The site-specific approach has two problems stemming from the fact that there is usually only a single reference site or a single nearby reference area from which reference sites are selected. The first problem is representativeness: Does the reference site represent reference conditions? Although the reference site may lack the specific stressor that is present at the test site, unless carefully evaluated and placed, it may be subject to other stressors that have not been considered.

The second problem with the site-specific approach is the potential for trivial statistical comparison of two sites in that it is almost always possible to demonstrate a significant difference between two sites by pseudo-replication (Hurlbert 1984). Pseudo-replication is the repeated measurement of a single experimental unit or sampling unit, and treating the measurements as if they were independent replicates of the sampling unit. A single reference site does not yield sufficient information to meaningfully judge the biological relevance of a statistical difference at the test site. The judgement that biotic differences between a single test site and its reference site may be due to differences in impacts cannot depend on statistical tests, but requires a careful weight-of evidence evaluation (e.g. Hurlbert 1984, Schindler 1971).

6.4 Mathematical Modelling Approaches

6.4.1 Introduction

The third approach to establishing reference conditions for waterbodies when there are few historical data is to predict, hindcast or extrapolate baseline nutrient concentrations from related datasets using mathematical models. Such models range from relatively simple models that are easy to use and require very few data, to very complex models that need large amounts of data and computing resources. Decisions over the complexity of model to be used are often driven by financial or practical constraints. In general, the more complex the model is, the greater the cost in applying it. In addition, there may also be a loss of predictive ability as complexity increases (Peters, 1991). In all cases, care must be taken in extrapolating any model beyond a set of known data values and relationships, as is implicitly required for the prediction of baseline nutrient concentrations, as this may produce misleading results.

Many models described in the literature are capable of predicting nutrient concentrations in surface waters from information about their catchments (e.g. topography, climate, soil type, land use). These vary widely in their level of complexity. Only those that could have practical application in the context of implementing the WFD are considered here. It should be noted, however, that most of these models focus on the prediction of phosphorus, rather than nitrogen, concentrations in surface waters.

6.4.2 Rivers

The estimation of historical (reference) nutrient concentrations in rivers requires information from one of the following sources:

- Historical records of nutrient concentrations
- Historical information about the catchment (e.g. land use, population density, agricultural practices, stocking densities of farm animals)

The relative merits of using methods based on the above sources of data are discussed below.

Historical records approach

The best way of establishing baseline nutrient concentrations for rivers is to use historical data. However, these are rarely available and, even where they do exist, are unlikely to be sufficiently accurate for use where nutrient concentrations are low. This is because historical analytical methods had much higher limits of detection compared to those of more modern methods. In general, although this approach may be suitable for a few site-specific determinations, it is unlikely that it could be used on a national or inter-national scale due to the lack of data.

Export coefficient approach

Estimating background nutrient concentrations for rivers from historical information about the upstream catchment is another technique that could be used to establish nutrient reference conditions for rivers.

Data availability, costs, and political or practical constraints are likely to become the main limiting factors in choosing suitable period in most cases. For example, the derivation of historical reference conditions from catchment characteristics depends on the availability of reliable, and comprehensive, historical land cover maps. As the publication dates of these vary from country to country, it will be difficult to ensure that similar standards are applied across Europe. Even in Britain, a scheme for determining reference conditions for lake water quality in Scotland has been developed using land cover information from the mid-1800s (Ferrier, 1997) while a similar scheme in England has used maps from the early 1930s - almost 100 years later (Johnes, 1994). Both of these methods are outlined below.

Ferrier (1997) estimated the historical (reference) nutrient concentrations in Scottish surface waters using an export coefficient approach. They obtained historical land cover data from maps of the extent of ancient natural woodlands (ca. 1850) and a re-interpretation of land-cover categories into natural and semi-natural units which was achieved using relationships between mapped soil information, data on Hydrology of Soil Types (Boorman, 1995) and data on the historical land cover of Scotland. Nutrient losses from each catchment were estimated using catchment specific export coefficients that had been validated with contemporary land use and water quality data and then applied to the historical land use data (Fozzard, 1999). The exception to this was ‘arable land’ which was ascribed a value corresponding to the “next most highly managed unit of land cover, namely improved grassland”. In Scotland, this approach has been applied to the export of phosphorus, only.

Historical nutrient losses from catchments in England and Wales were estimated by (Johnes, 1994) using a modification of the export coefficient approach described above. This model not only takes account of land cover types, but also fertiliser usage, and the number and distribution of livestock and human populations in each catchment. The historical land cover data were derived from maps of survey data collected in the 1930s by Dudley Stamp (1941), while the relevant agricultural statistics were obtained from agricultural census returns from 1930 and fertiliser usage records published by the Ministry of Agriculture, Fisheries and Food (MAFF). The model also takes into account nutrient input from nitrogen fixation and atmospheric deposition. The method is described in detail by Johnes (1996) and, in contrast to the approach used by Ferrier (1997), predicts both phosphorus and nitrogen concentrations in rivers.

Although the export coefficient approaches described above vary in detail, they both rely heavily on the accurate determination of historical export coefficients. These values are very difficult to estimate, even for contemporary studies, as they are affected not only by land cover type or land use, but also by climate, soil type, topography, local agricultural practices and erosion patterns. As such, they are rarely transportable from one catchment to another and must often be ‘validated’ to fit a new situation by comparison with measured data. Such ‘validation’ is rarely possible in an historical context where measured data is unlikely to be available. There may also be problems in applying this simple export coefficient modelling approach to rivers that are downstream from a standing waterbody (such as a lake) or a river that may act as a source, or a sink, of nutrients and sediments (Moss, 1988).

Despite its problems, the export coefficient approach – in one form or another – is probably one of the most practical approaches to setting reference conditions for nutrients where there are few historical data.

Backpropagation neural networks

A recent publication by Lek (1996) suggests that backpropagation neural networks (BNN) could be used to construct a model capable of predicting the export of phosphorus from watersheds on the basis of catchment characteristics. The method was developed and tested on data from 927 stream sites from across the USA, half being used to construct the model and the remainder being used to test the model. Despite variations in climate, soil, and land cover types among catchments, the authors report that the BNN procedure gave good predictions across the USA without the use of regional sub-division or non-linear transformations of the data. The input requirements were fairly simple: % forest cover, % agriculture, % other land cover type, precipitation, stream discharge and animal stocking density. This approach might benefit from further investigation in relation to its use in predicting reference conditions in rivers in England and Wales.

6.3.3 Lakes

Mass balance approach

Reference conditions for nutrients in lakes can be estimated from historical nutrient concentrations in their feeder streams, the hydrology of their catchments and certain morphological characteristics of the lakes themselves. There are many in-lake models that could be used for this purpose, ranging from the simpler models given by OECD (1982), to the more complex models such as PROTECH (Reynolds C, pers comm.). However, in practice, the more complex lake models require input data at a temporal resolution (e.g. daily) that is difficult to achieve for historical situations. The simpler, less data intensive lake water quality models (e.g. OECD, 1982) work on annual average values and are probably more appropriate to this approach. Of the data required, the baseline nutrient concentrations of the feeder streams can be estimated by one of the methods described in Section 6.4.2; the hydrology of the catchment can be determined from its area and effective rainfall; and the morphological characteristics of the lake are usually known. This approach has already been used successfully to determine nutrient reference conditions for standing waters by Johnes (1994) and Ferrier (1997).

Morphoedaphic index approach

An alternative method of estimating nutrient reference conditions for lakes is the morphoedaphic index (MEI) approach. The MEI is based on a ratio of the alkalinity or conductivity of the lake water to the mean depth of the lake. Early studies suggested that this index was correlated with fish and phytoplankton productivity in lakes (Rawson 1951, Rawson 1952, Rawson 1955, Ryder 1961, Ryder 1974, Oglesby 1977). Later, the approach was extended to allow the determination of phosphorus concentrations in lakes from natural background loadings in undisturbed watersheds (Vighi, 1985) using the following predictive equations:

$$\log (TP) = 1.48 + 0.33 (\pm 0.09) \log (MEI_{alk}) \quad (r = 0.83)$$
$$\log (TP) = 0.75 + 0.27 (\pm 0.11) \log (MEI_{cond}) \quad (r = 0.71)$$

where:
TP is the mean in-lake phosphorus concentration ($\mu g\ l^{-1}$)
MEI_{alk} is the ratio of mean annual alkalinity (mequiv l^{-1}) and mean depth (m)
MEI_{cond} is the ratio mean annual conductivity value ($\mu s\ cm^{-1}$ at 20°C) to mean depth (m).

Although the MEI approach is very simple, requires relatively few data and seems to have been highly successful for a limited set of cool-temperate lakes, limnologists have largely ignored it as a method of establishing nutrient reference conditions. It still requires testing on a wider variety of lake types (e.g. shallow, naturally eutrophic lakes, or reservoirs) and across more geographical regions within Europe, especially in coastal areas where maritime influences may significantly affect conductivity. However, the initial results suggest that this approach is probably worth further investigation (EPA 2000, Cardoso 2001). There are no records of this approach being used to predict background concentrations of nitrogen.

6.3.4 Transitional and coastal waters

Nutrient reference conditions for transitional and coastal waters must also be a function of the background nutrient (reference) concentrations of the rivers and streams that drain into them. However, there appear to be no published models for determining baseline nutrient conditions for these types of waters.

6.5 Comparison of methods

There have been few direct comparisons of the methods outlined above, except in the case of palaeoecological nutrient reconstructions and export coefficient modelling approaches for lakes. Johnes *et al*. (2000) compared the export coefficient model output from the EA's lake classification scheme both with observed water quality and with diatom model reconstructions of TP for five English lakes: Crose Mere, Esthwaite Water, Windermere South Basin, Slapton Ley and Hornsea Mere. There was a reasonable agreement between the TP trends hindcast by the two approaches. The authors demonstrated that, even at the coarser Land Use Regions scale, the export coefficient modelling approach was robust for the purposes of establishing baseline trophic status of UK lakes. The diatom model also provided valuable information, particularly with regard to ecological response to nutrient enrichment from catchment sources and changes in soluble reactive phosphorus (SRP).

A comparison of baseline TP concentrations derived from the diatom transfer function approach and the PLUS model, employed in the Scottish lochs classification scheme, (Ferrier *et al*. 1996, 1997) was carried out for a set of 23 loch basins (Bennion *et al*. 2001b). At some sites there was a good match between the two sets of values while at others the results differed substantially (Fig 6.1). The coefficient of determination between the two sets of data, therefore, was not particularly high (r^2=0.46). There did not appear to be a systematic bias in the results but, where discrepancies did occur, the diatom model provided the higher TP values on most occasions.

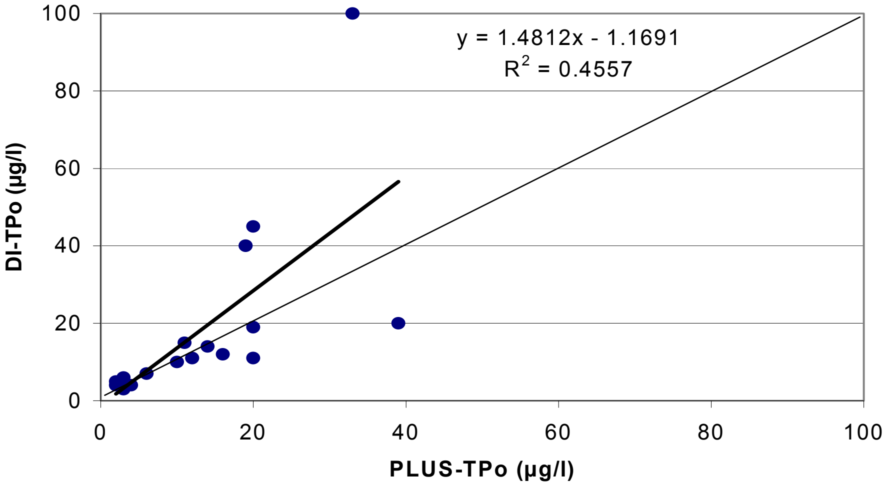


Figure 6.1 Relationship between reference TP concentrations hindcast using the PLUS model (PLUS-TPo) compared to those generated by the diatom transfer function (DI-TPo) for a range of Scottish lakes

(The regression line and its equation are shown along with the 1:1 line)

The greatest discrepancies occurred at the more productive, relatively shallow lochs where the diatom model produced substantially higher baseline values than the PLUS model. Possible explanations for this are i) that the diatom model over-estimated historical TP concentrations owing to the problems of species optima over-estimation, and ii) the PLUS model under-estimated TP because in these enriched, shallow systems there was a significant internal TP load available to the algae which was not being accounted for by the model. PLUS is simply a model that predicts TP delivery from external sources, so internal lake dynamics are not included (Ferrier *et al.* 1996). In contrast, diatoms respond to TP from both internal and external sources and so will also reflect any increase in the internal loading component. The only lochs where the diatom model produced substantially lower baseline TP values than the PLUS model were shallow, macrophyte-dominated systems. This may be because the PLUS model is not accounting for TP sinks within these systems such as aquatic plants or the sediments, resulting in over-estimation of in-lake TP. Similar problems have also been observed when applying the regression equations of OECD (1982) to shallow, macrophyte-dominated lakes. For the large, deep, less productive lochs the models were in extremely good agreement.

Both palaeolimnological reconstructions and export coefficient models have associated errors. Potential error sources in the diatom models include poor estimates of species optima for some taxa, inherent bias in inverse deshrinking techniques, sediment sampling and representativity problems, differential diatom dissolution, and natural high variability in TP concentrations. The PLUS export loss coefficients are based on yearly data, whilst biology responds to seasonal patterns; the determination of point source contributions is rather coarse; there is a lack of internal lake dynamics within the model; and there are problems associated with determining appropriate loss coefficients for individual sites. There is clearly scope for improvement and refinements to both modelling techniques but, given all possible sources of error, and the fact that the models are based on entirely different methodologies there was remarkably good concordance between the baseline TP values generated from the two approaches. The complexity of lake ecosystems must be borne in mind when judging the performance of the models. The reasons for the mismatch varies on a site specific basis and more detailed TP cycling studies and local information on land use and management would be required to assess the causes more fully.

Johnes *et al.* (2000) and Bennion *et al.* (2001b) concluded that the diatom record is an important source of information as it reflects the ecological response to environmental change. However, unless detailed historical catchment data are available, it is not always possible to establish the cause of the observed change from such an investigation. In contrast, the export coefficient approach is able to determine the origin of past nutrient loadings and can differentiate between diffuse and point sources. It cannot, however, give an indication of the ecological response to these inputs or provide an ecological target. Furthermore, the export-coefficient approach is restricted to a single or few estimates of historical TP concentrations whereas the sediment record can be analysed at a resolution sufficient to detect the timing and rate of change. The palaeolimnological approach has a less limited temporal perspective than export coefficient models and may therefore be particularly useful for establishing reference conditions at sites that have been subjected to human impact over long timescales. Johnes *et al.* (2000) and Bennion *et al.* (2001b) recommend that for high priority sites, a combined approach should be taken: the export coefficient model for establishing the origins and changes in nutrient loadings, and the diatom record to reflect the nature and timing of the ecological response.

6.6 Summary

There appears to be no single method of determining nutrient reference conditions that is applicable to all of the types of surface waters covered by the WFD. Some types of waterbody present greater problems than others; for example, there has been little work on determining reference conditions in coastal and transitional waters, and lowland rivers are problematic because very few are unimpacted. Likewise, methods for establishing reference conditions for some biological elements are less well developed than others, e.g. spatial-state schemes are well developed for benthic invertebrates in rivers but poorly developed for fish. This review highlights those techniques that are best suited to determining reference conditions in the different waterbody types in the absence of long term datasets (Table 6.1). However, given the original project brief, the focus is on methods for defining *nutrient* rather than *ecological* reference conditions (see Section 6.1).

There are, basically, two methods of defining reference conditions for surface waters:

i) Site-specific methods that use historical documentary data, palaeoecology or other modelling techniques to derive site-specific data.
ii) Spatial-state methods that use contemporary datasets and can be divided into two approaches:
    a) Selected reference sites approach, where reference sites are selected using expert knowledge, mapped information and existing data.
    b) Percentile data selection of 'best available sites' from a population distribution of sites in a region. This is useful for heavily impacted regions and heavily modified waters such as reservoirs, but it is difficult to choose the cut-off between reference and non-reference sites.

A combination of site-specific and spatial-state methodologies is probably the best approach, as each has advantages and disadvantages. Spatial-state schemes can be scaled up more easily for use at a national level than the site-specific approach while site-specific methods are more resource intensive than spatial-state schemes and are probably of most value for supporting and validating results from the latter, and for providing detailed data for priority sites.

Table 6.1 Methods for defining nutrient reference conditions for each surface water type. Those shown in italics still require some development.

Approach	Lakes	Rivers	Transitional		Coastal
			Estuaries	Lagoons	
Site-specific	Palaeolimnology/ *Analogue-matching* Export-coefficient modelling *MEI*	Export-coefficient modelling		Palaeoecology	*Palaeoecology (MOLTEN)*
Spatial-state	*LAKEPACS?* *PSYM*	RIVPACS AI (RPDS) *AQEM*	*COASTPACS*		*COASTPACS*

The methods outlined in Table 6.1 are summarised briefly, below. Palaeoecology is a powerful technique for defining both nutrient and ecological reference conditions but can only be effectively employed in environments where sediments continually accumulate. Of the surface water types relevant to the WFD, palaeoecological techniques are already available for determining nutrient reference conditions in lakes and lagoons, and under development for coastal waters.

The nutrient transfer function approach is not appropriate for all lakes, especially small, shallow waterbodies where the relationship between nutrients and ecological change is highly complex. In such systems, the derivation of ecological reference conditions using a range of food-web components is recommended. Once the UK lakes inventory (currently in progress) has been developed, a ‘top and bottom’ sediment sampling scheme (with a probability-based sampling design as used by the USEPA) could be adopted in the UK to infer past conditions of large numbers of lakes from a relatively small sub-sample of representative lake types.

Export-coefficient models are already operational in both England and Wales, and Scotland. They do not incorporate any biological elements, but are useful for establishing reference nutrient loads and concentrations in both rivers and lakes. The technique requires further development for application to transitional and coastal waters. An alternative method of estimating nutrient reference conditions for lakes is the morphoedaphic index (MEI). This is a fairly straightforward method that has been used successfully on lakes in the USA and would benefit from further testing on waterbodies in the UK. It is unlikely that this approach could be developed for use in rivers, transitional or coastal waters.

RIVPACS is a well-established, spatial-state reference scheme for evaluating the biological condition of river sites by comparing the observed macro-invertebrate fauna at each site with the fauna predicted from its measured environmental characteristics. The system is widely used by the British water industry, including the EA, and has been shown to be operationally effective. Future research might involve the development of similar schemes for other taxonomic groups (e.g. macrophytes and fish). PSYM integrates the multivariate prediction methods used in RIVPACS and the multimetric approach adopted by the USEPA to link environmental characteristics of ponds to their macrophyte and macroinvertebrate assemblages. However, the scheme is based on very small, shallow waterbodies and would need to be adapted for lakes. Likewise, the ongoing AQEM project employs a combination of these approaches to develop multimetrics for benthic invertebrates in rivers. Artificial Intelligence (AI) methods are also currently being employed to develop a River Pollution Diagnostic System (RPDS). This system is used to diagnose the stressors operating on a site using patterns in benthic macroinvertebrate assemblage data from sites in all states of biological condition. In this approach, all sites act as references for particular states, optimising the full information content of the database. Thus, RPDS differs from conventional spatial-state schemes in which the reference state sites are those representing some form of desirable target. COASTPACS is under development at the University of Hull and aims to generate a model analogous to RIVPACS to predict macrobenthos in transitional and coastal waters.

6.7 Recommendations for Phase II

6.7.1 Rivers

Improving RIVPACS

Reynoldson and Wright (2000) recommended that, where adequate financial resources and expertise were available, and suitable reference sites could be found, RIVPACS would be an appropriate technique for evaluating the biological condition of river sites. An initial one-off investment for each site provides an historical dataset that can be utilised over a long period of time, not only in the prediction system, but also as a source of biological data for species conservation (Wright *et al*. 1996) and for the management of individual sites and rivers. A number of recommendations for improving and refining RIVPACS are given below:

1. The reference condition is currently based on invertebrate community structure; the methodology should be extended to other taxonomic groups (e.g. macrophytes or fish) or functional attributes (e.g. growth rates or functional groups).
2. A more formal process should be adopted for the definition and selection of reference sites; the recognition of high quality sites is not always straightforward, often requiring an iterative process.
3. Subsets of reference sites should be sampled annually to establish the sensitivity of the prediction procedure and document annual variation.
4. The potential benefits of using long-term mean values for prediction, rather than short-term or seasonal measurements of environmental attributes, needs further investigation.
5. Further consideration should be given to possible alternatives to observed over expected ratios when evaluating abundance and functional measures of communities.
6. Nutrient data has been collected at less than half of the 614 RIVPACS reference sites in Great Britain. A new data collation exercise should be undertaken to obtain appropriate nutrient data for all 614 RIVPACS sites so that the relationships between nutrient concentrations and faunal assemblages can be examined.

6.7.2 Lakes

A proposed methodology for lakes

1. Identify potential reference sites using expert knowledge, mapped information and existing data-bases, ensuring that these represent the range of ecotypes for each lake type.
2. Collate existing biological and environmental data for these sites or collect new data where necessary.

3. Perform preliminary data analysis to characterise the biological communities and establish whether the ecotypes are ecologically meaningful; revise typology if necessary.
4. Establish the environmental conditions that support these biological communities based on contemporary datasets.
5. Use existing export coefficient models (where available) to hindcast nutrient concentrations for typical catchments in each ecotype; compare values with current nutrient concentrations to assess whether eutrophication is a key pressure, and to set the environmental conditions that support high ecological status.
6. Collate the above data from a broader spectrum of sites from the modern population for each ecotype; analyse the biological data to establish boundaries between high, good, moderate, poor and bad ecological status.
7. Use palaeoecology, where possible, to confirm identification of reference sites and to establish ecological (for a range of biological elements) and nutrient (and pH?) reference conditions.

Recommendations for palaeoecology based projects for lakes

1. Undertake a desk study to explore the ECRC diatom database to assess the total number of 'top and bottom' core samples already available for UK lakes and determine the number of minimally impacted UK lakes observed to date.
2. Carry out a state-changed assessment for a set of lakes, building on existing assessments and covering the continuum of lakes found in the UK:
    i) Identify a set of potential reference lakes from data held in existing lake databases; information on lake sensitivity and hazard can be used to maximise the chance of selecting minimally impacted sites.
    ii) Review the gaps in our state-changed assessment of lakes with regard to UK lake types (as defined by the WFD system 'B' criteria); for example, few palaeolimnological investigations have been carried out at large, lowland, less productive lakes, mesotrophic lakes or marl lakes.
    iii) Take an open water sediment core from each missing lake type and prepare 'top' and 'bottom' core samples from each (or more samples where resources permit); analyse for a range of proxies representative of the key biological elements, such as diatoms (representing the phytoplankton and phytobenthos; sensitive to lake pH, nutrients and habitat; transfer functions to infer chemical change can be applied), chironomids (representing the benthic invertebrate community; sensitive to oxygen, nutrients, temperature, habitat; transfer functions to infer chemical change can be applied), cladocera (representing the zooplankton and littoral (chydorid) community; can be used to infer changes in fish community structure and shifts in habitat structure (i.e. macrophytes) particularly in shallow lakes) and ostracods (crustacea that preserve well in marl lakes where diatom dissolution may limit the use of the diatom record; good ecological indicators of water quality).
    iv) Perform data analysis using a range of multivariate statistical techniques to describe the deviation of the modern assemblages from the reference assemblages and thus estimate degree of ecological change on a site-by-site basis.
    v) Produce list of minimally impacted sites representing the range of lake types found in the UK to act as high status, reference sites.
    vi) Link palaeo-data with the spatial state datasets to inform the development of a status classification system.

3. Extend analogue matching to lakes beyond the upland, soft waters with particular emphasis on lowland oligotrophic and mesotrophic lakes and to biological elements that are not preserved in the palaeolimnological record (e.g. macrophytes, fish).
4. Assess the degree to which surface sediment samples could be used in place of biological surveys to provide a resource efficient method for characterising present day ecological conditions, as sediments are good integrators of ecological conditions over space and time
5. Explore the potential of the littoral palaeo-record for providing historical information on biological elements that are not transported into the deep water zones (e.g. aquatic plant macrofossils, fish scales, molluscs, bryozoa, and cladocera ephippia); pilot studies on 2-3 small, shallow, productive lakes have shown promising results.
6. Compare reference phosphorus concentrations derived from palaeolimnological and export-coefficient models for a broader spectrum of UK lake types; previous comparisons (see Section 6.5) have suggested that export coefficient models are useful for establishing the origins and changes in nutrient loadings while the diatom record is valuable in establishing the nature and timing of the ecological response.

Other research and development recommendations for lakes

1. Develop a predictive technique for lakes that is similar to the RIVPACS system for rivers but based on modern diatom communities or other aquatic groups; this would be of particular use where there are no historical or palaeolimnological records. The methodology should build upon the PSYM methodology for small standing waters. This requires a protocol for representative sampling of the various biological elements in each lake. Palaeolimnological data could be used to identify appropriate reference sites.
2. Investigate the potential of the morphoedaphic index (MEI) (Section 6.4.3.2) as a simple way of establishing phosphorus reference conditions for lakes; initial results have suggested that this approach should be tested on a wider variety of lake types (e.g. shallow, naturally eutrophic lakes, or reservoirs) and across more geographical regions within Europe, especially in coastal areas where maritime influences may significantly affect conductivity. There are no records of this approach being used to predict background concentrations of nitrogen; this should also be investigated.

6.7.3 Transitional and coastal waters

1. The diatom transfer function developed by Martin (2002) for reconstructing both salinity and nutrient conditions in coastal lagoons indicates that palaeolimnology offers a promising method for establishing reference nutrient concentrations in this water body type. However, the data set contains only 36 lagoons at present. It is recommended that this data set be expanded to encompass a larger suite of coastal lagoons from across the UK.

2. More studies on the autecology of estuarine diatoms are needed before diatom-based models can be generated for defining reference nutrient conditions in estuaries, and there are currently no training sets for inferring nutrient concentrations in coastal wetlands and estuaries. Some basic research would need to be undertaken to explore the potential of quantitative palaeoecology in these systems. Given the range of problems identified in this review it is unlikely that high performance models for

determining nutrient reference conditions for these waters could be produced within the time frame required by the WFD, although development of such techniques should remain an RandD theme.

3. Developments in the EU MOLTEN project and the COASTPACS scheme should be closely followed and any recommendations or methodologies arising from the projects should be incorporated into the UK WFD methodology for deriving site-specific and spatial-state reference conditions, respectively, in coastal waters.

4. The review suggests that there are no published mathematical models for determining baseline nutrient conditions for transitional and coastal waters. The generation of such models would require a large RandD programme which is probably beyond the scope of Phase II.

BIBLIOGRAPHY

Abel P D, 1989 *Water pollution biology*. Ellis Horwood, Chichester, England

Adams C E and Maitland P S, 2002 Invasion and establishment of freshwater fish populations in Scotland - the experience of the past and lessons for the future. *Glasgow Naturalist*, in press

Admiraal W, 1984 The ecology of estuarine sediment inhabiting diatoms. *Progress in Phycological Research*, **3**, 269-322

Ahl T and Wiederholm T, 1977 Svenska vattenkvalitetskritter. Eutrofierand amnen. Stockholm, Sweden: *SNV Report No. 918*. Statens Naturvardsverk

Alabaster J S (ed), 1977 *Biological monitoring of inland fisheries*. Applied Science Publications, London

Alabaster J S and Lloyd R, 1982 *Water quality criteria for freshwater fish*, 2nd edition. Food and Agriculture Organisation of the United Nations, Butterworths, U K

Alabaster J S, Shurben D G and Knowles G, 1979 The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon, *Salmo salar* L. *Journal of Fish Biology*, **15**, 705-712

Alba-Tercedor J and Pujante A M, 2000 *Running water biomonitoring in Spain: opportunities for a predictive approach*. In Assessing the Biological quality of fresh waters: RIVPACS and other techniques (ed. J F Wright, D W Sutcliffe and M T Furse), pp. 207-216, Freshwater Biological Association, Ambleside

Alexandrino J, Ferrand N and Rocha J, 1996 Genetic polymorphism of a haemoglobin chain and adenosine deaminase in European shads: evidence for the existence of two genetic entities with natural hybridisation. *Journal of Fish Biology*, **48**, 447-456

Allen J D, 1995 *Stream ecology: structure and function of running waters*. Chapman and Hall

Allen J I, Howland R M H, Bloomer N and Uncles R J, 1998 Simulating the spring phytoplankton bloom in the Humber plume, UK. *Marine Pollution Bulletin,* **37**, 295-305

Allen J R, Slinn D J, Shammon T M, Hartnoll R G and Hawkins S J, 1998 Evidence for eutrophication of the Irish Sea over four decaded. *Limnology and Oceanography,* **43**, 1970-1974

Allott T E H and Monteith D T, 1999 *Classification of Lakes in Wales for Conservation using Integrated Biological Data* Environmental Change Research Centre Research Report No.53

Allott T E H, Monteith D T, Duigan C A, Bennion H and Birks H J B, 2001 *Conservation classification of lakes in Wales, with implications for the EU Water Framework Directive*. Countryside Council for Wales Contract Science Report No. 426: 44 pp.

Althuis I J A, Gieskes W W C, Villerius L and Colin F, 1994 Interpretation of Fluorometric Chlorophyll Registrations with Algal Pigment Analysis Along a Ferry Transect in the Southern North-Sea. *Netherlands Journal of Sea Research,* **33**, 37-46

American Public Health Association, 1985 *Standard methods for the examination of water and wastewater*. 16th edition American Public Health Association, Washington DC

American Society of Testing and Materials (ASTM), 1995 *Biological effects and environmental fate*. Annual book of Standards: American Society of Testing and Materials, **11.05,** Philadelphia, Pennsylvania

Aminot A, Kirkwood D S and Kérouel R, 1997 Determination of ammonia in seawater by the indophenol-blue method: evaluation of the ICES NUTS I/C5 questionnaire. *Marine Chemistry*, **56**, 59-75

Amoros C and van Urk G, 1989 *Palaeoecological analyses of large rivers: some principles and methods*. In Historical Change of Large Alluvial Rivers: Western Europe (ed. G E Petts, H Möller & A L Roux), pp. 143-165. Wiley, Chichester

Anderson B G, 1944 The toxicity thresholds of various substances found in industrial wastes as determined by the use of *Daphnia magna*. *Sewage Works Journal*, **16**, 1156-1165

Anderson B G, 1946 The toxicity thresholds of various sodium salts determined by the use of *Daphnia magna*. *Sewage Works Journal*, **18**, 82-87

Anderson N J, 1995 Using the past to predict the future: lake sediments and the modelling of limnological disturbance. *Ecological Modelling*, **78**, 149-172

Anderson N J, 1997a *Reconstructing historical phosphorus concentrations in rural lakes using diatom models.* In Phosphorus Loss from Soil to Water (ed. H Tunney, O T Carton, P C Brookes and A E Johnston), pp. 95-118. Cab International

Anderson N J, 1997b Historical changes in epilimnetic phosphorus concentrations in six rural lakes in Northern Ireland. *Freshwater Biology*, **38**, 427-440

Anderson N J and Rippey B, 1994 Monitoring lake recovery from point-source eutrophication: the use of diatom-inferred epilimnetic total phosphorus and sediment chemistry. *Freshwater Biology*, **32**, 625-639

Anderson N J, Rippey B and Gibson C E, 1993 A comparison of sedimentary and diatom-inferred phosphorus profiles: implications for defining pre-disturbance nutrient conditions. *Hydrobiologia,* **253**, 357-366

Anderson N J and Vos P, 1992 Learning from the past: diatoms as palaeoecological indicators of changes in marine environments. *Netherlands Journal of Aquatic Ecology*, **26**, 19-30

Anderson N J, Appleby P G, Patrick S T, Bennion H and Jensen K G, 1999 *Palaeolimnological surveys of selected large lakes in the north of Ireland*. Final report to the DOE (NI) Environment and Heritage Service. ECRC Research Report No. 63: 54 pp.

Andersson G, Graneli W and Stenson J, 1988 The influence of animals on phosphorus cycling in lake ecosystems. *Hydrobiologia*, **170**, 276-284

Anglian_Region, 1995 *Standard Methodologies: Freshwater Phytoplankton*. Peterborough: Anglian Region, National Rivers Authority.

Ankley G T, Katko A and Arthur J W, 1990 Identification of ammonia as an important sediment-associated toxicant in the Lower Fox River and Green Bay, Wisconsin. *Environmental Toxicology and Chemistry*, **9**, 313-322

Apfelbeck R S, 2000 *Development of Biocriteria for Wetlands in Montana*. Draft. www.deq.state.mt.us/ppa/mdm/publications/draft_biocriteria.pdf

Archibald R E M, 1972 Diversity in some South African diatom associations and its relation to water quality. *Water Research,* **6**, 1229-1238

Armitage P D, 1980 The effects of mine drainage and organic enrichment on benthos in the River Nent system, Northern Pennines. *Hydrobiologia,* **74,** 119-128

Armitage P D, 1994 *Prediction of biological responses*. In Rivers Handbook Volume 2 (eds P Calow and G Petts), **2,** 254-275, Blackwell Scientific Publications Ltd

Armitage P D, 2000 *The potential of RIVPACS for predicting the effects of environmental change*. In Assessing the biological quality of freshwaters: RIVPACS and similar techniques (ed. J F Wright, D W Sutcliffe and M T Furse), pp. 93-112, Freshwater Biological Association, Ambleside

Armitage P D and Pardo I, 1995 Impact assessment of regulation at the reach level using macroinvertebrate information from mesohabitats, *Regulated Rivers-Research and Management*, **10**, 147-158

Armitage P D, Moss D, Wright J F and Furse M T, 1983 The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Water Research*, **17**, 333-347

Armitage P D, Pardo I, Furse M T and Wright J F, 1990 Assessment and prediction of biological quality. A demonstration of a British macroinvertebrate-based method in two Spanish rivers. *Limnetica,* **6**, 147-156

Armstrong D A, Chippendale D, Knight A W and Colt J E, 1978 Interaction of ionised and un-ionised ammonia on short term survival and growth of prawn larvae, *Macrobrachium rosenbergii*. *Hydrobiological Bulletin*, **154**, 15-31

Armstrong D A, Stephenson M J and Knight A W, 1976 Acute toxicity of nitrite to larvae of the giant Malaysian prawn, *Macrobrachium rosenbergii*. *Aquaculture*, **9**, 39-46

Arnold K, 1989 *Eutrophication in flowing waters*. Clyde River Purification Board Technical Report, **No. 92**

Asano M and Ito M, 1956 Comparative biochemical studies on aquatic animals. II Phosphorus turnover in freshwater fish and shellfish. *Tohuko Journal of Agricultural Research*, **7**, 291-302

ASTM, 1990 *Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods* (ASTM E 1367-90). American Society for Testing and Materials, Philadelphia, PA, USA.

Aubert M, 1990 Summer time proliferation of toxic plankton. *Recherche*, **21**, 916-919

Auby I, Bachelet G and Labourg P-J, 1994 *Biomass and species composition of macrophytes in Arcachon Bay and Prevost Lagoon, with a compilation of data on primary production in Arcachon Bay*. In Proceedings of the CLEAN Meeting - Coastal Lagoons Eutrophication and Anaerobic Processes (Alicante 1993), edited by P Caumette, 65-72. Commission of the European Communities, Environmental Research Programme, Brussels – Laboratoire d'Oceanographie biologique, Universite Bordeaux I, France.

Axtmann E V and Luoma S N, 1990 *The influence of tributary inputs on the distribution of metals in the bed sediment of the Clark Fork River, Montana*. In Proceedings of 1990 Clark Fork River Symposium. Edited by V J Watson. Montana Academic Sciences

Bachelet G, de Montaudoiun X, Auby I and Labourg P J, 2000 Seasonal changes in macrophyte and macrozoobenthos assemblages in three coastal lagoons under varying degrees of eutrophication. *ICES Journal of Marine Science*, **57**, 1495-1506

Baden S P, Loo L-O, Pihl L and Rosenberg R, 1990 Effects of eutrophication on benthic communities including fish: Swedish west coast. *Ambio*, **19**, 113-122

Bahls L R, Burkantis R and Tralles S, 1992 *Benchmark biology of Montana reference streams.* Department of Health and Environmental Science, Water Quality Bureau, Helena, Montana

Bailey R C, Kennedy M G, Dervish M Z and Taylor R M, 1998 Biological assessment of freshwater ecosystems using a reference condition approach: comparing predicted and actual benthic invertebrate communities in Yukon streams. *Freshwater Biology*, **39,** 765-774

Bailey R G, 1976 *Ecoregions of the United States (Map scale 1:7,500,000.* US Dept of Agriculture (USDA), Forest Service Oden, Utah

Bailey-Watts A E and Maitland P S, 1984 Eutrophication and fisheries in Loch Leven, Kinross, Scotland. *Proceedings of the Institute of Fisheries Management Annual Study Course*, **15**, 170-190

Bailey-Watts A E, Kirika A, May L and Jones D H, 1990 Changes in phytoplankton over various time scales in a shallow eutrophic lake: the Loch Leven experience with special reference to the influence of flushing rate. *Freshwater Biology*, **23**, 85-111

Bailey-Watts A E, Lyle A A, Kirika A and Wise E J, 1987 Coldingham Loch, south-east Scotland: I. Physical and chemical features with special reference to the seasonal patterns in nutrients. *Freshwater Biology*, **17**, 405-418

Balbi D M, 2000 Suspended chlorophyll in the River Nene, a small nutrient-rich river in eastern England: long-term and spatial trends. *The Science of the Total Environment*, **251/252**, 401-421

Ball J, 1982 *Stream Classification Guidelines for Wisconsin.* Wisconsin Department of Natural Resources Technical Bulletin, Wisconsin Department of Natural Resources, Madison, Wisconsin

Ballesteros E, Pérez M and Zabala M, 1984 Approximación al conocimiento de las comunidades algales de la zona infralitoral superior de la costa catalana. *Collectanea Botanica*, **15**, 69-100

Balloch D, Davies C E and Jones F H, 1976 Biological assessment of water quality in three British rivers: the North Esk (Scotland), the Ivel (England) and the Taf (Wales). *Water Pollution Control*, **75**, 92-114

Balls P, 1992 Nutrient behaviour in two contrasting Scottish Estuaries, the Forth and Tay. *Oceanologica Acta*, **15**, 261-277

Balls P W, 1994 Nutrient Inputs to Estuaries from 9 Scottish East-Coast Rivers - Influence of Estuarine Processes on Inputs to the North-Sea. *Estuarine Coastal and Shelf Science*, **39**, 329-352

Balls P W, Macdonald A, Pugh K and Edwards A C, 1995 Long-Term Nutrient Enrichment of an Estuarine System - Ythan, Scotland (1958-1993). *Environmental Pollution,* **90**, 311-321

Balls P W, Macdonald A, Pugh K B and Edwards A C, 1997 Rainfall events and their influence on nutrient distributions in the Ythan estuary (Scotland). *Estuarine Coastal and Shelf Science*, **44**, 73-81

Barbour M T, 1997 The re-invention of biological assessment in the US. *Human and ecological Risk Assessment*, **3**, 933-940

Barbour M T and Yoder C O, 2000 *The multimetric approach to bioasessment, as used in the United States of America*. In Assessing the biological quality of fresh waters (ed. J F Wright, D W Sutcliffe & M T Furse), Ambleside: Freshwater Biological Association

Barbour M T, Bowman M L and White J S, 1994 *Evaluation of the biological condition of streams in the Middle Rockies - Central ecoregion.* Prepared for Wyoming Department of Environmental Quality

Barbour M T, Diamond J M and Yoder C O, 1996 *Biological assessment strategies: Applications and Limitations*, In Whole effluent toxicity testing: An evaluation of methods and prediction of receiving system impacts (ed. D R Grothe, K L Dickson and D K Reed-Judkind), pp.245-270. SETAC Press, Pensacola, Florida

Barbour M T, Gerritsen J and White J S, 1996 *Development of the stream condition index (SCI) for Florida.* Prepared for Florida Department of Environmental Protection, Tallahassee, Florida

Barbour M T, Gerritsen J, Griffith G E, Frydenborg R, McCarron E, White G S and Bastian M L, 1996 A framework for biological criteria for Florida streams using benthic macroinvertebrates. *Journal of the North American Benthological Society*, **15,** 185-211

Barbour M T, Gerritsen J, Snyder B D and Stribling J B, 1999 *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish.* US Environment Protection Agency EPA 841-B-99-002, EPA Office of Water, Washington DC

Barbour M T, Plafkin J L, Bradley B P, Graves C G and Wisseman R W, 1992 Evaluation of EPA's rapid bioassessment benthic metrics: Metric redundancy and variability among reference stream sites. *Environmental Toxicology and Chemistry*, **11,** 437-449

Barbour M T, Stribling J B and Karr J R, 1995 *Multimetric approach for establishing biocriteria and measuring biological condition*. In Biological assessment and criteria. Tools for water resource planning and decision making. (ed. W S Davis and T P Simon), pp.63-77. Lewis Publishers, Boca Raton, Florida

Barbour M T, Swietlik W F, Jackson S K, Courtemanch D L, Davies S P and Yoder C O, 2000 Measuring the attainment of biological integrity in the USA: a critical element of ecological integrity, *Hydrobiologia*, **422,** 453-464

Bargos T, Mesanza J M, Basaguren A and Orive E, 1990 Assessing river quality by means of multifactorial methods using macroinvertebrates. A comparative study of main watercourse of Biscay. *Water Research*, **24,** 1-10

Battarbee R W, 1984 Diatom analysis and the acidification of lakes. *Philosophical Transactions of the Royal Society of London Series B*, **305,** 451-477

Battarbee R W, 1986 *Diatom analysis*. In Handbook of Holocene Palaeoecology and Palaeohydrology (ed. B.E. Berglund), pp. 527-570. Wiley, Chichester

Battarbee R W, 1997 *Freshwater quality, naturalness and palaeolimnology*. In Freshwater Quality: Defining the Undefinable? (ed. P J Boon and D L Howell), pp. 155-171. HMSO, Edinburgh

Battarbee R W, 1999 The importance of paleolimnology to lake restoration. *Hydrobiologia*, **395/396**, 149-159

Beardall J, Berman T, Heraud P, Kadiri M O, Light B R, Patterson G, Roberts S, Sulzberger B, Sahan E, Uehlinger U and Wood B, 2001 A comparison of methods for detection of phosphate limitation in microalgae. *Aquatic Sciences,* **63**, 107-121

Beardall J, Young E and Roberts S, 2001 Approaches for determining phytoplankton nutrient limitation. *Aquatic Sciences,* **63**, 44-69

BEC, 1993 *Water quality of estuaries and coastal waters.* Report to English Nature by BEC Consultants

Beisel J N, Usseglio-Polatera P, Thomas S and Moreteau J C, 1998 Effects of mesohabitat sampling strategy on the assessment of stream quality with benthic invertebrate assemblages. *Archiv fur Hydrobiologie Ergebnisse der Limnologie*, **142,** 493-510

Belcher H and Swale E, 1979 *An Illustrated Guide to River Phytoplankton*. London: Her Majesty's Stationery Office.

Belegratis M R, Bitis I, Economou-Amilli A and Ott J A, 1999 Epiphytic patterns of macroalgal assemblages on *Cystoseira* species (Fucales, Phaeophyta) in the east coast of Attica (Aegean Sea, Greece). *Hydrobiologia*, **412**, 67-80

Bellamy D J, John D M, Jones D J, Starke A and Whittick A, 1972 *The place of ecological monitoring in the study of pollution in the marine environment.* In

Marine pollution and sea life (ed. M Ruivo), pp. 421-425. Fishing News (Books), Surrey

Belliard J, Thomas R B D and Monnier D, 1999 Fish communities and river alteration in the Seine basin and nearby coastal streams. *Hydrobiologia*, **400**, 155-166

Belpaire C, Smolders R, *et al.* 2000 An index of biotic integrity characterising fish populations and the ecological quality of Flandrian water bodies. *Hydrobiologia,* **434**, 17-33

Bennion H, 1993 *A diatom-phosphorus transfer function for eutrophic ponds in south-east England*. Unpublished PhD. University College London

Bennion H, 1994 A Diatom-Phosphorus Transfer-Function for Shallow, Eutrophic Ponds in Southeast England. *Hydrobiologia,* **276**, 391-410

Bennion H, 1995 Surface sediment diatom assemblages in shallow, artificial, enriched ponds, and implications for reconstructing trophic status. *Diatom Research,* **10**, 1-19

Bennion H and Appleby P G, 1999 An assessment of recent environmental change in Llangorse Lake using palaeolimnology. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **9**, 361-375

Bennion H and Smith M A, 2000 Variability in the water chemistry of shallow ponds in southeast England, with special reference to the seasonality of nutrients and implications for modelling trophic status. *Hydrobiologia*, **436**, 145-158

Bennion H, Allott T E H and Shilland E, 1998 *Investigation of environmental change in two mesotrophic lakes in Mid-Wales: Llyn Eiddwen and Llyn Fanod*. Final report to Countryside Council for Wales by ENSIS Ltd. Environmental Change Research Centre, Research Report No. 46, University College London

Bennion H, Allott T E H, Appleby P G, Hunt M, Oliver E and Patrick S T, 1997a *A study of recent environmental change within selected standing waters proposed as Special Areas of Conservation (SAC) in Wales - Llyn Idwal, Llyn Cwellyn and Llyn Safadden (Llangorse Lake) Phase II*. Countryside Council for Wales Contract Science Report No. 187

Bennion H, Appleby P, Boyle J, Carvalho L, Luckes S and Henderson A, 2000a *Water quality investigation of Loweswater, Cumbria*. Final Report to the Environment Agency by the Environmental Change Research Centre, University College London: pp 80

Bennion H, Appleby P G and Phillips G L 2001a. Reconstructing nutrient histories in the Norfolk Broads, UK: implications for the role of diatom-total phosphorus transfer functions in shallow lake management. *Journal of Paleolimnology*, **26**, 181-204

Bennion H, Battarbee R W, Juggins S, Gasse F, Anderson N J and Cameron N G, 1999 *European Diatom Database (EDDI): an information system for palaeoenvironmental reconstruction*. In Lake '99 Abstracts Volume - 8th International Conference of the Conservation and Management of Lakes, 17-21 May, Copenhagen, Denmark

Bennion H, Duigan C A, Haworth E Y, Allott T E H, Anderson N J, Juggins S and Monteith D M, 1996b The Anglesey lakes, Wales, UK - changes in trophic status of three standing waters as inferred from diatom transfer functions and their implications for conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems,* **6**, 81-92

Bennion H, Fluin J, Appleby P and Ferrier B, 2001b *Palaeolimnological Investigation of Scottish Freshwater Lochs*. Final report to SNIFFER SR(00)02F by ENSIS Ltd, University College London: 424 pp

Bennion H, Juggins S and Anderson N J, 1996a Predicting epilimnetic phosphorus concentrations using an improved diatom-based transfer function and its application to lake eutrophication management. *Environmental Science and Technology,* **30**, 2004-2007

Bennion H, Monteith D T and Appleby P G, 1997b *Nutrient reconstructions in standing waters*. Final report to English Nature by ENSIS Ltd. Environmental Change Research Centre, Research Report No. 37, University College London

Bennion H, Monteith D T and Appleby P G, 2000b Temporal and geographical variation in lake trophic status in the English Lake District: evidence from (sub)fossil diatoms and aquatic macrophytes. *Freshwater Biology,* **45**, 394-412

Bennion H, Shilland E and Appleby P G (in press). *An assessment of recent environmental change in Llyn Tegid using the sediment record.* Proceedings of the Llyn Tegid Symposium

Bennion H, Wunsam S and Schmidt R, 1995 The validation of diatom-phosphorus transfer functions: an example from Mondsee, Austria. *Freshwater Biology,* **34**, 271-283

Berg K, 1948 Biological studies on the River Susaa. *Folia Limnologica Scandinavica*, **4**, 1-318

Berman-Frank I, Erez J and Kaplan A, 1998 Changes in inorganic carbon uptake during the progression of a dinoflagellate bloom in a lake ecosystem. *Canadian Journal of Botany-Revue Canadienne De Botanique,* **76**, 1043-1051

Berry C, 2000 Marine health check: A report to gauge the health of the UK's sea-life, pp. 1-50: WWF UK.

Bianchi T S, Johansson B, Elmgren R (2000) Breakdown of phytoplankton pigments in Baltic sediments: effects of anoxia and loss of deposit-feeding macrofauna. *J Exp Mar Biol Ecol,* **251,** 161-183

Bigelow H B, Lillick L C and Sears M, 1940 Phytoplankton and planktonic protozoain the offshore waters of the Gulf of Maine. Part 1. Numerical distribution. *Transactions of the American Philosophical Society,* **21**, 149-191

Biggs B J F and Price G M, 1987 A survey of filamentous algal proliferations in New Zealand rivers. *New Zealand Journal of Marine and Freshwater Research,* **21**, 175-191

Biggs B J F, 1995 The Contribution of Flood Disturbance, Catchment Geology and Land-Use to the Habitat Template of Periphyton in Stream Ecosystems. *Freshwater Biology,* **33**, 419-438

Biggs B J F, 1996 *Patterns in Benthic Algae of Streams*. In Algal ecology: freshwater benthic ecosystems (ed. R J Stevenson, M L Bothwell & R L Lowe), pp.31-56. San Diego: Academic Press

Biggs B J F, Francoeur S N, Huryn A D, Young R, Arbuckle C J and Townsend C R, 2000 Trophic cascades in streams: effects of nutrient enrichment on autotrophic and consumer benthic communities under two different fish predation regimes. *Canadian Journal of Freshwater (Fisheries) and Aquatic Sciences,* **57,** 1380-1394

Biggs J, Williams P, Whitfield M, Fox G and Nicolet P, 2000 *Biological Techniques of Still Water Quality Assessment.* Phase 3. Method Development. R&D Technical Report E110. Bristol: Environment Agency.

Birks H J B, 1998 Numerical tools in palaeolimnology - progress, potentialities, and problems. *Journal of Paleolimnology,* **20**, 307-332

Bjorkland R, Pringle C M and Newton B, 2001 A stream visual assessment protocol (SVAP) for riparian landowners. *Environmental Monitoring and Assessment*, **68,** 99-125

Blackburn T H, Henriksen K (1983) Nitrogen cycling in different types of sediments from Danish waters. *Limnol Oceanogr,* **28,** 477-493

Blackburn T H, Lund B A, Krom M D (1988) C- and N- mineralisation in the sediments of earthen marine fishponds. *Mar Ecol Prog Ser,* **44,** 221-227

Blumenshine S C, Vadeboncoeur Y, Lodge D M, Cottingham K L and Knight S E, 1997 Benthic-pelagic links: responses of benthos to water column nutrient enrichment. *Journ*al *of the North American Benthological Society*, **16,** 466-479

Bock M T, Miller B S and Bowman A W, 1999 Assessment of eutrophication in the Firth of Clyde: Analysis of coastal water data from 1982 to 1996. *Marine Pollution Bulletin,* **38**, 222-231

Bode R W and Novak M A, 1995 *Development and application of biological impairment criteria for rivers and streams in New York State.* In Biological assessment and criteria. Tools for water resource planning and decision making. (ed. W S Davis and T P Simon), pp. 97-107. Lewis Publishers, Boca Raton, Florida

Boisneau P, Mennesson-Boisneau C and Guyomard R, 1992 Electrophoretic identity between Allis Shad, *Alosa alosa* L. and Twaite Shad, *A. fallax* Lacepede. *Journal of Fish Biology*, **40**, 731-738

Bokn T and Lein T E, 1978 Long-term changes in association of the inner Oslofjord, Norway. *Norwegian Journal of Botany*, **25,** 9-14

Bombelli V and Lenzi M, 1996 *Italy – the Orbetello lagoon and the Tuscan coast*. In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (ed. W Schramm and P Neinhuis), pp. 331-338. Springer-Verlag, Berlin

Bonsdorff E, Blomquist E M, Mattila J and Norkko A, 1997 Coastal eutrophication: causes, consequences and prespectives in the Archipelago areas of the northern Baltic Sea. *Estuarine Coastal and Shelf Science*, **44** (Suppl A), 63-72

Boon P J, Holmes N T H, Maitland P S and Rowell T A, 1996 *System for evaluating rivers for conservation. Version 1 Manual.* Scottish Natural Heritage Research, Survey and Monitoring Report No 61. Edinburgh: Scottish Natural Heritage

Boon P J, Holmes N T H, Maitland P S, Rowell T A and Davies J, 1997. *A system for evaluating rivers for conservation 'SERCON': development structure and function*. In Freshwater quality: defining the indefinable? (ed. P J Boon and D L Howell), pp. 24-38. Edinburgh, HMSO

Boorman D B, Hollis J M and Lilly A, 1995 *The Hydrology of Soil Types*; a Hydrologically Based Classification of the Soils of the UK: Institute of Hydrology, Wallingford.

Borchardt M A, 1996 *Nutrients*. In Algal Ecology: freshwater benthic ecosystems (ed. R J Stevenson, M L Bothwell & R L Lowe), pp. 184-227. San Diego: Academic Press

Borgmann U, 1994 Chronic toxicity of ammonia to the amphipod *Hyalella azteca*; importance of ammonium ion and water hardness. *Environ Pollut,* **86**, 329-335

Borum J, 1996 *Shallow waters and land / sea boundaries*. Eutrophication in coastal marine ecosystems. Washington, DC: American Geophysical Union

Borum J, Geertz-Hansen O, Sand-Jensen K and Vium-Andersen S, 1990 *Eutrofiering-effekter på marine primærproducenter*. NPO-forskning fra Miljøstyrelsen, Nr. C3, Miljøminiteriet, København

Boudou A, Ribeyre F 1989 *Aquatic Ecotoxicology: Fundamental Concepts and Methodologies*. CRC Press, Boca Raton, Florida, USA

Bournaud M and Keck G, 1980 Species diversity and structure of macrobenthos communities along a stream: the River Furans (Ain, France). *Acta Oecologica, Oecologica Generale*, **1,** 131-150

Bournaud M, Keck G, Richoux P, 1980 Macroinvertebrate samples and how they reveal the physiognomy of a river. *Annales de Limnologie*, **16 (1),** 55-75

Bowburn Consultancy, 1996 *The Trophic Diatom Index: A User's Manual*, pp.

148. Bristol: Environmental Agency.

Boynton W R, Hagy J D, Murray L, Stokes C and Kemp W M, 1996 A comparative analysis of eutrophication patterns in a temperate coastal lagoon. *Estuaries,* **19**, 408-421

Boynton W R, Kemp W M, Keefe C W, 1982 *A comparative analysis of nutrients and other factors influencing estuarine phytoplankton production*. In Estuarine comparisons. International estuarine research conference 6 (ed. V S Kennedy) pp. 69-90. Academic Press, New York

Breitburg D L, Sanders J G, Gilmour C C, Hatfield C A, Osman R W, Riedel G F, Seitzinger S B, Sellner K G, 1999 Variability in responses to nutrients and trace elements, and transmission of stressor effects through an estuarine food web. *Limnology and Oceanography,* **44,** 837-863

Breukelaar A W, Lammens E H R R, Breteler J G P and Tatrai I, 1994 Effects of benthivorous bream Abramis brama and carp Cyprinus carpio on sediment resuspension and concentrations of nutrients and chlorophyll *a*. *Freshwater Biology*, **32**, 113-121

Breur G and Schramm W, 1988 *Changes in macroalgal vegetation of Kiel Bight (western Baltic Sea) during the past 20 years*. Kieler Meeresforchungen Sonderheft, **6**, 241-255

Briand X, 1989 *Prolifération de l'algue vert* Ulva *sp. En baie de Lanninon (France): étude d'une nuisance et de son traitement par fermentation anaérobie.* Unpublished PhD thesis. University of Lille, France

Briand X and Morand P, 1987 Ulva*, stranded algae: a way of depollution through methanization.* In Biomass for Energy and Industry pp. 834-839. Elsevier Applied Science, London

Brinkhurst R O, 1974 *The benthos of lakes*. St Martin's Press, New York

Brinkhurst R O, 1993 *Future directions in freshwater biomonitoring using benthic macroinvertebrates*. In Freshwater biomonitoring and benthic macroinvertebrates (ed. Rosenberg and Resh)**,** pp. 442-460

Brodersen K P and Lindegaard C, 1999 Classification, assessment and trophic reconstruction of Danish lakes using chironomids. *Freshwater Biology,* **42**, 143-157

Brodersen K P, Dall P C and Lindegaard C, 1998 The fauna in the upper stony littoral of Danish lakes: macroinvertebrates as trophic indicators. *Freshwater Biology,* **39**, 577-592

Brodersen K P, Odgaard B V, Vestergaard O and Anderson N J, 2001 Chironomid stratigraphy in the shallow and eutrophic Lake Søbygaard, Denmark: chironomid-macrophyte co-occurrence. *Freshwater Biology*, **46**, 253-267

Brodin Y Y, 1982 Paleoecological studies of the recent developments of the Lake Vaxjosjon: interpretation of the eutrophication process through analysis of sub-fossil chironomids. *Archiv Fur Hydrobiologie,* **93**, 313-326

Bronmark C and Weisner S E B, 1992 Indirect effects of fish community structure on submerged vegetation in shallow eutrophic lakes: an alternative mechanism. *Hydrobiologia*, **243/244**, 293-301

Brooks S J, Bennion H and Birks H J B, 2001 *Tracing lake trophic history with a chironomid-total phosphorus inference model.* Freshwater Biology*,* **46**, 513-533

Brown G, 1995 *Water quality in the Thredbo River: Impacts of nutrients on periphyton growth and macroinvertebrates*. Water - Melbourne then Artarmon-, **22,** 30pp, 0310-0367, Australian Water and Wastewater Inc

Brown L R, 2000 Fish communities and their associations with environmental variables, lower San Joaquin River drainage, California. *Environmental Biology of Fishes*, **57**, 251-269

Brundin L, 1949 Chironomiden und andere Bodentiere der sud-schwedischen Urgebirgsseen. *Report of the Institute of Freshwater Research, Drottningholm,* **30**, 1-914

Brundin L, 1958 The bottom faunistical lake type system and its application to the southern hemishpere. Moreover a theory of glacial erosion as a factor of productivity in lakes and streams. *Internationale Vereingung fur Theoretische und Angewandte Limnologie Verhandlungen,* **13**, 288-297

Brush G S and Davis F W 1984 Stratigraphic evidence of human disturbance in an estuary. *Quaternary Research*, **22**, 91-108

Bryant R and Wilhm J, *Species Diversity of benthic macroinvertebrates in Salt Creek, Oklahoma*. Dept of Zoology, Oklahoma State University

Bryant R, 1988 *Effect of nutrient enrichment from agricultural runoff on macroinvertebrates in Salt Creek, a Prairie stream in North Central Oklahoma*. PhD Dissertation, **155,** Oklahoma State University, Stillwater, OK

Bunker F, Rostron D and Perrins J, 1995 *An assessment of the impact of eutrophication by domestic sewage effluent in Whiteness Voe Marine Consultation Area.* Report to SNH North East Region SNH NE/94/006

Burgess A, 1998 *A palaeolimnological assessment of eutrophication-related changes in the ecological status of Felbrigg Lake, Norfolk*. Unpublished M.Res thesis. University College London

Burk C, Usleber E and Martlbauer E, 1998 Intoxications through marine algae toxins - a review. *Archiv Fur Lebensmittelhygiene,* **49**, 16-20

Burn A, 1993 *Water quality of estuaries and coastal waters – a summary and analysis of the BEC report for English Nature*. English Nature 33pp.

Butcher RW, 1933 Studies on the ecology of rivers. I. On the distribution of macrophytic vegetation in the rivers of Britain. *Journal of Ecology*, **21**, 59-91

Cabecadas G, Nogueira M and Brogueira M J, 1999 Nutrient dynamics and productivity in three European estuaries. *Marine Pollution Bulletin,* **38**, 1092-1096

Cadee G C, 1992 Phytoplankton variability in the Marsdiep, the Netherlands. *Ices Journal of Marine Science,* **195**

Caffery J, 1987 *Macrophytes as indicators of organic pollution in Irish rivers*. In Biological Indicators of Pollution (ed. D Richardson), pp. 77-87. Royal Irish Academy, Dublin

Cahn A R, 1929 The effect of carp on a small lake: the carp as a dominant. *Ecology*, **10**, 271-274

Cairns J, 1974 Indicator species vs. the concept of community structure as an index of pollution. *Water Resources Bulletin,* **10,** 338-347

Cairns J, 1985 *Multispecies toxicity testing*. Pergamon Press, New York. pp 253

Cairns, J and Dickson K L, 1971 A simple method for the biological assessment of the effects of waste discharges on aquatic bottom-dwelling organisms. *Journal of the Water Pollution Control Federation*, **43,** 755-772

Cairns J and Dickson K L, 1973 *Biological methods for the assessment of water quality*. American Society for Testing and Materials special technical publication 528, Philadelphia, PA

Cairns J and Pratt J R, 1993 *A History of Biological Monitoring Using Benthic Macroinvertebrates*. In Freshwater Biomonitoring and Benthic Macroinvertebrates (ed. D M Rosenberg and V Resh), pp. 10-25. London: Chapman and Hall

Camargo J A, 1993 Macrobenthic surveys as a valuable tool for assessing freshwater quality in the Iberian Peninsula. *Environmental Monitoring and Assessment,* **24,** 71-90

Camargo J A, Ward J V, 1992 Short-term toxicity of sodium nitrate ($NaNO_3$) to non-target freshwater invertebrates. *Chemosphere,* **24**, 23-28

Camargo J A, Ward J V, 1995 Nitrate (NO_3^- -N) toxicity to aquatic life: a proposal of safe concentrations for two species of nearctic freshwater invertebrates. Chemosphere, **31,** 3211-3216

Cambridge M L and McComb A J, 1984 The loss of seagrass from Cockburn Sound, Western Australia. I. The time course and magnitude of seagrass decline in relation to industrial development. *Aquatic Botany*, **20**, 229-243

Canfield D E Jnr, Jones J R, 1984 *Assessing the Trophic Status of Lakes with Aquatic Macrophytes.* In Proceedings of 3RD Annual Conference North American Lake Management Society 18-20 Oct

Cardoso A C, Duchemin J, Magoarou P and Premazzi G, 2001 *Criteria for the Identification of Freshwaters Subject to Eutrophication: Their use for the implementation of the "Nitrates" and Urban Waste Water Treament Directives*. Luxembourg: Office for Official Publications of the European Communities.

Carline R F, 1986 *Indices as predictors of fish community traits*. Pages 46-56 in: Hall, G E, Van Den Avyle, M J Eds Reservoir fisheries management strategies for the 80s. Bethesda: American Fisheries Society.

Carlson R E, 1977 A trophic state index for lakes. *Limnology and Oceanography,* **22**, 361-369

Carpenter K E, 1927 Faunistic ecology of some Cardiganshire streams. *Journal of Ecology*, **15**, 33-54

Carpenter K E, 1928 *Life in inland waters.* London: Sidgwick and Jackson.

Carpenter S R and Kitchell J F, 1993 *The trophic cascade in lakes*. Cambridge: Cambridge University Press.

Carrick H J, Lowe R L and Rotenberry J T, 1988 Functional associations of benthic algae along experimentally manipulated nutrient gradients: relationships with algal community diversity. *Journal of the North American Benthological Society,* **7**, 117-128

Carvalho L and Moss B, 1995 The current status of a sample of English Sites of Special Scientific Interest subject to eutrophication. *Aquatic Conservation: Marine and Freshwater Ecosystems,* **5**, 191-204

Carvalho L, Bingham N and Martin A, 2000 Lagoonal charophyte conservation: a palaeoecological approach. *Internationale Vereinigung für theoretische und angewandte Limnologie, Verhandlungen*, **27**, 884-886

Casabianca-Chassany M-L, 1983 Relations entre la production algale macrophytique et le degré deutroehication du milieu dans une lagune Medirerraneenne. *Rapport Commission Internationale pour la Mer Mediterranee*, **28**, 359-363

Casabianca-Chassany M-L, 1989 Dégradation des ulves *(*Ulva rotundata*,* Lagune de Prevost*,* France*). CR Academy of Science, Paris*, **308**, 155-160

Castel J, Caumette P and Herbert R, 1996 Eutrophication gradients in coastal lagoons as exemplified by the Bassin d'Arcachon and the Etang du Prevost. *Hydrobiologia*, **329**, 9-28

Cattaneo A, 1978 The microdistribution of epiphytes on the leaves of natural and artificial macrophytes. *British Phycological Journal,* **13**, 183-188

Ceccherelli V U, Ferrari I and Viaroli P, 1994 Ecological Research on the Animal Communities of the Po River Delta Lagoons. *Bollettino Di Zoologia,* **61**, 425-436

Chadd S & Sharp L, 1995 Assessment of freshwater riverine environments using macrophytes (revised July 1996, draft)

Chadd S & Sharp L (1996) Methodology for the Assessment of freshwater riverine macrophytes for the purposes of the Urban Waste Water Treatment Directive (draft)

Chambers P A, 1996 *Nutrient Enrichment in the Peace, Athabasca and Slave Rivers: Assessment of Present Conditions and Future Trends*. Northern River Basins Study Synthesis Report No. 4

Chandler J R, 1970 A biological approach to water quality management. *Water Pollution Control,* **69,** 415-421

Charles D F, *Smol J P and Engstrom D R, 1984 Palaeolimnological approaches to biological* monitoring. In Biological Monitoring of Aquatic Systems (ed. S L Loeb and A Spacie), pp. 233-293. Florida: CRC Press

Chauvaud L, Jean F, Ragueneau O and Thouzeau G, 2000 Long term variation of the Bay of Brest ecosystem: benthic-pelagic coupling revisited. *Marine Ecology-Progress Series,* **200**, 35-48

Chen J C and Chen S F, 1992 Effects of nitrite on growth and moulting of *Penaeus monodon* juveniles. *Comp Biochem and Physiol*, **101**, 453-458

Chen J C, Lee Y, 1997 Effects of nitrite on mortality, ion regulation and acid-base balance of *Macrobrachium rosenbergii* at different external chloride concentrations. *Aquat Toxicol,* **39**, 291-305

Chen J C, Lin C Y, 1991 Lethal effects of ammonia and nitrite on *Penaeus penicillatus* juveniles at two salinity levels. *Comp Biochem and Physiol,* **100**, 477-482

Chen J C, Lin C Y, 1992 Lethal effects of ammonia on *Penaeus chinensis* Osbeck juveniles at different salinity levels. *J Exp Mar Biol Ecol*, **156**, 139-148

Chen J C, Nan F H, 1991 Lethal effect of nitrite on *Metapenaeus ensis* larvae. *J World Aquacult Soc,* **22**, 51-56

Chessmen B C, 1994 *The use of macroinvertebrates for the rapid biological assessment of streams in the Sydney region, New South Wales, Australia*. Water Research Commission Report TT. Conference: Classification of rivers, and environmental health indicators, **63/94,** 235-246, 1868450473

Chessman B C, 1995 Rapid assessment of rivers using macroinvertebrates: A procedure based on habitat-specific sampling, family level identification and a biotic index. *Australian Journal of Ecology,* **20,** 122-129

Chessman B C and McEvoy P K, 1998 Towards diagnostic biotic indices for river macroinvertebrates. *Hydrobiologia*, **364,** 169-182

Chessman B, Growns I, Currey J and Plunkett-Cole N, 1999 Predicting diatom communities at the genus level for the rapid bioassessment of rivers. *Freshwater Biology*, **41,** 317-331

Chipman W A, 1934 *The role of pH in determining the toxicity of ammonia compounds*. PhD Thesis, University of Missouri, Columbia, MO

Chovanec S R, Jager P, *et al.*, 2000 The Austrian way of assessing the ecological integrity of running waters: a contribution to the EU Water Framework Directive. *Hydrobiologia*, **422**, 445-452

Chutter F M, 1972 An empirical biotic index of the quality of water in South African streams and rivers. *Water Research*, **6,** 19-30

Clarke R, T, 2000 *Uncertainty in estimates of biological quality based on RIVPACS*. In Assessing the biological quality of freshwaters: RIVPACS and similar techniques (ed. J F Wright, D W Sutcliffe and M T Furse), pp.39-54. Freshwater Biological Association, Ambleside

Clarke R T, Furse M T, Wright J F and Moss D, 1996 Derivation of a biological quality index for river sites: comparison of the observed with the expected fauna. *Journal of Applied Statistics*, **23,** 311-332

Clarke R T, Wright J F and Davy-Bowker J, 2001 *Testing and further development of RIVPACS. Package 8. An appraisal of RIVPACS for evaluating trophic structure*. Draft report to the Environment Agency*,* 48pp

Clendenning K A and North W J, 1959 *Effects of waste on the giant kelp* Macrocystis pyrifera. In Proceedings of the 1st International Conference, pp. 82-91. Berkley.

Clerk S, Hall R I, Quinlan R and Smol J P, 2000 Quantitative inferences of past hypolimnetic anoxia and nutrient levels from a Canadian Precambrian Shield lake. *Journal of Paleolimnology*, **23**, 319-336

Clesceri L S, Greenberg A E and Eaton A D, 1998 *Standard methods for the examination of water and wastewater*. Washington: American Public Health Association.

Cloern J E, 2001 Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology-Progress Series,* **210**, 223-253

Clokie J J P and Boney A D, 1979 David Lansborough (1779-1854): an assessment of his contribution to algal studies in the Firth of Clyde. *Glasgow Naturalist*, **19,** 443-462

Codispoti L A, 1989 Phosphorus vs nitrogen limitations of new and export production. In Productivity of the ocean: past and present (ed. W H Berger et al). Wiley

Cole J J, Lane J M, Marino R and Howarth R W, 1993 Molybdenum Assimilation by Cyanobacteria and Phytoplankton in Fresh-Water and Salt-Water. *Limnology and Oceanography,* **38**, 25-35

Cole R A, 1973 Stream community response to nutrient enrichment. *Journal of Water Pollution Control Federation*, **45**, 1874-1888

Coleman, N V & Stewart W D P, 1979 Enteromorpha prolifera in a polyeutrophic loch in Scotland*. British Phycological Journal*, **14,** 121

Colinj F and Dijkema K S, 1981 Species composition of benthic diatoms and distribution of chlorophyll a on an intertidal flat in the Dutch Wadden Sea. *Marine Ecology Progress Series*, **4**, 9-21

Collingwood, R W, 1977 *A survey of eutrophication in Britain and its effects on water supplies*, Water Research Centre Technical Report TR40

Collos Y, 1986 Time-Lag Algal Growth Dynamics - Biological Constraints on Primary Production in Aquatic Environments. *Marine Ecology-Progress Series,* **33**, 193-206

Collos Y, Vaquer A, Bibent B, Slawyk G, Garcia N and Souchu P, 1997 Variability in nitrate uptake kinetics of phytoplankton communities in a Mediterranean coastal lagoon. *Estuarine Coastal and Shelf Science,* **44**, 369-375

Colt J, Tchobanoglous G 1976 Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. *Aquacult,* **8,** 209-224

Conley D J and Malone T C, 1992 Annual Cycle of Dissolved Silicate in Chesapeake Bay - Implications for the Production and Fate of Phytoplankton Biomass. *Marine Ecology-Progress Series,* **81**, 121-128

Constanzo S D, O'Donohue M J and Dennison W C, 2000 Gracilaria edulis *(Rhodophyta)* as a biological indicator of pulsed nutrients in oligotrophic waters. *Journal of Phycology*, **36**, 680-685

Cooper E L, 1967 Annual production of brook trout *Salvelinus fontinalis* in fertile and infertile streams of Pennsylvania. *Proceedings of the Pennsylvania Academy of Science*, **41**, 65-70

Cooper S D, Kratz K, Diehl S, Wiseman S and Jenkins T M, 1995-96 *Effects of predators (Trout, stoneflies) and nutrients on the mean levels, overall variability, and spatial heterogeneity of invertebrates and algae in streams*. Research Reports, Sierra Nevada Aquatic Research Laboratory, University of California Natural Reserve System

Cooper S D, Roll S, Dominguez A, Diehl S, Kratz K, and Wiseman S, 1996-97 *Effects of consumers and nutrients on the densities and spatial heterogeneity of organisms in streams*, http://nrs.edu/nrs2/reserves/snarl/Rsch96_97.html

Cooper S R, 1995 Chesapeake Bay watershed historical land use: impact on water quality and diatom communities. *Ecological Applications,* **5**, 703-723

Cooper S R, 1999 *Estuarine paleoenvironmental reconstructions using diatoms*. In The Diatoms: Applications for the Environmental and Earth Sciences (ed. E F Stoermer and J P Smol), pp 352-373. CambridgeUniversity Press, Cambridge

Cooper S R and Brush G S, 1991 Long-Term History of Chesapeake Bay Anoxia. *Science,* **254**, 992-996

Cooper S R and Brush G S, 1993 A 2,500-Year History of Anoxia and Eutrophication in Chesapeake Bay. *Estuaries,* **16**, 617-626

Coring E, 1996 *Situation and developments of algal (diatom)-based techniques for monitoring rivers in Germany*. In Use of Algae for Monitoring Rivers III (ed. J Prygiel, B A Whitton and J Bukowska), pp. 122-127. Douai, France: Agence de l'Eau Artois-Picardie

Corkum L D and Ciborowski J J H, 1988 Use of alternative classification in studying broad scale distributional patterns of lotic invertebrates. *Journal of the North American Benthological Society*, **7,** 167, 179

Corkum L D and Ciborowski J J H, 1989 Patterns of benthic invertebrate assemblages in rivers of northwestern North America. *Freshwater Biology*, **21,** 191-205

Coste M, Bosca C and Dauta A, 1991 Use of algae for monitoring rivers in France. In Use of Algae for Monitoring Rivers (ed. B A Whitton, E Rott and G Friedrich) Innsbruck

Cottingham K L and Schindler D E, 2000 Effects of grazer community structure on phytoplankton response to nutrient pulses. *Ecology,* **81**, 183-200

Cotton A D, 1910 On the growth of *Ulva latissima* in water polluted by sewage. Bulletin of Miscellaneous Information, Royal Botanic Gardens Kew, 15-19

Cotton A D, 1911 *On the growth of* Ulva latissima *in excessive quantity, with special reference to the* Ulva *nuisance in Belfast Laigh.* Royal Commission on Sewage Disposal, 7th Report II (Appendix IV), pp. 121-142. London: HMSO

Council of the European Communities, 2000 Council Directive of 23 October 2000 establishing a framework for Community action in the field of water policy (2000/60/EC). *Official Journal of the European Communities,* **L 327/1** (43), 1-72

Courtemanch D L, 1995 *Merging the science of biological monitoring with water resource management policy: Criteria development*. In Biological assessment and criteria. Tools for water resource planning and decision making. (ed. W S Davis and T P Simon), pp. 315-325

Craig J F, 1980 Growth and production of the 1955 to 1972 cohorts of Perch, *Perca fluviatilis* L, in Windermere. *Journal of Animal Ecology*, **49**, 291-315

Crawford R E, Allen G H, 1977 Seawater inhibition of nitrite toxicity to Chinook salmon. *Trans Am Fish Soc,* **106**, 105-109

CRC for Freshwater Ecology 2000 *'Dirty water' models. Predicting community composition for streams in disturbed landscapes*. Summary Report. September 2000. Scoping Study ScD1.

Crivelli A, 1983 The destruction of aquatic vegetation by carp. *Hydrobiologia,* **106**, 37-41

Crouzet P, Leonard J, Nixon S, Rees Y, Parr W, Laffon L, Bøgestrand J, Kristensen P, Lallana C, Izzo G, Bokn T, Bak J and Lack T J, 1999 *Nutrients in European ecosystems*. Environmental Assessment Report No 4, European Environment Agency, Copenhagen.

Crozier R, 1994 *Kootenay Lake*. In The book of Canadian lakes (ed. R J Allan, M Dickman, C B Gray and V Cromie), pp. 459-473 Burlington: Canadian Association on Water Quality

Cullen J J, 1990 On models of growth and photosynthesis in phytoplankton. *Deep-Sea Research Part A – Oceanographic Research Papers*, **37**, 667-683

Culp J M and Podemski C L, 1996 *Impacts of contaminants and nutrients in bleached kraft mill effluent on benthic insect and periphyton communities: Assessments using artificial streams, Athabasca River, 1993 and 1994.* Northern River Basins Study Technical Report No 92

Culp J M, Podemski C L and Casey C, 1996 *Design and application of a transportable experimental stream system for assessing effluent impacts on riverine biota*. Northern River Basins Study Technical Report No 128

Cumming B F, Smol J P, Kingston J C, Charles DF, Birks H J B, Camburn K E, Dixit S S, Uutala A J and Selle A R, 1992 How much acidification has occurred in Adirondack region lakes (New York, USA) since preindustrial times? *Canadian Journal of Fisheries and Aquatic Sciences*, **49**, 128-141

Cumming B F, Wilson S E and Smol J P, 1993 Palaeolimnological potential of chrysophyte cysts and scales, and sponge spicules as indicators of lake salinity. *International Journal of Salt Lake Research*, **2**, 87-92

Cyr H and Pace M L, 1992 Grazing by zooplankton and its relationship to community structure. *Canadian Journal of Fisheries and Aquatic Sciences,* **49**, 1455-1465

Daldorph P W G and Thomas J D, 1995 Factors influencing the stability of nutrient-enriched freshwater macrophyte communities - the role of sticklebacks *Pungitius pungitius* and freshwater snails. *Freshwater Biology*, **33**, 271-289

Dale B and Nordberg K, 1993 *Evidence for prehistoric and historic "blooms" of the toxic dinoflagellate Gymnodinium catenatum in the Kattegat-Skagerrak region of Scandinavia.* In Toxic phytoplankton blooms in the sea (ed. T J Smayda and Y Shimuza), pp. 53-57. Amsterdam: Elsevier

Dance K W and Hynes H B N, 1980 Some effects of agricultural land use on stream insect communities. *Environmental Pollution*, **22**, 19-28

Davidson N C, Laffoley D d' A, Doody J P, Way L S, Gordon J, Key R, Drake C M, Pienkowski M W, Mitchell R M and Duff K L, 1991 Nature conservation and estuaries in Great Britain. Peterborough, Nature Conservancy Council

Davies B R and Walker K F, 1986 The Ecology of River Systems. *Monographiae Biologicae,* **60,** Dr W Junk, The Hague

Davies J,1991 *Benthic marine ecosystems in Great Britain: a review of current knowledge. Western Channel and Bristol Channel and approaches (MNCR coastal sectors 8 and 9).* Nature Conservancy Council. CSD report No 1173, Marine Nature Conservation Review report No MNCR/OR/009

Davies P.E. 2000 *Development of a national river bioassessment system (AUSRIVAS) in Australia*. In Assessing the biological quality of fresh waters. RIVPACS and other techniques. Proceedings of an International Workshop, Oxford, September 1998, (ed. Wright J F, Sutcliffe D W and Furse M T), pp. 113-124. Freshwater Biological Association, Ambleside

Davies S P, Tsomides L, Courtemanch D L and Drummond F, 1993 *Maine Biological Monitoring and Biocriteria Development Program*. Maine Department of Environmental Protection, Bureau of Water Quality, Division of Environmental Evaluation and Lake Studies. Augusta, Maine

Davies T T, Davis D and Elmore J, 1993 *Technical panel recommendations concerning use of acute amphipod tests in evaluation of dredged material*. Technical Report. US Environmental Protection Agency, Office of Water, Washington, DC.

Davis J C, 1975 Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. *J Fish Res Board Can,* **32,** 2295-2332

Davis W S and Simon T P, 1995 *Biological assessment and criteria: tools for water resource planning and decision making*. Boca Raton: Lewis Publishers

Davis W S, Snyder B D, Stribling J B and Stoughton C, 1996 *Summary of State biological assessment programs for streams and rivers,* EPA 230-R-96-007, US Environmental Protection Agency, Office of Planning, Policy, and Evaluation, Washington, DC

Dawson F H and Newman J R, 1996 *Mean Trophic Rank Survey*. Progress to September 1996. R&D Progress Report E1/i694/02 to Environment Agency.

Dawson F H and Newman J R, 1996 *Mean Trophic Rank Macrophyte Survey Interim current best-available guidance on the use/application of the MTR System for the Assessment of Trophic Status of Rivers and the designation of Sensitive Areas (Eutrophic) for Urban Waste Water Treatment Directive.* R & D Interim Report 694/NW/03 to Environment Agency, 40pp

Dawson F H, Newman J R and Gravelle M J, 1996 *Mean Trophic Rank Macrophyte Survey: An Assessment of the Trophic Status of Rivers using Macrophytes*. R & D Interim Report 694/NW/01 to Environment Agency

Dawson F H, Newman J R and Gravelle M J, 1999 *Mean Trophic Rank Macrophyte Survey: An Assessment of the Trophic Status of Rivers using Macrophytes*. Project Record E1-i694, Environment Agency

De Cassabianca M-L, 1996 *France – the Mediterranean Lagoons*. In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (ed. W Schramm and P Neinhuis), pp. 307-330. Springer-Verlag, Berlin

De Jonge V N, 1990 Response of the Dutch Wadden Sea ecosystem to phosphorus discharge from the Rhine River. *Hydrobiologia,* **195**, 49-62

De Jonge V N, 1997 High remaining productivity in the Dutch western Wadden Sea despite decreasing nutrient inputs from riverine sources. *Marine Pollution Bulletin,* **34**, 427-436

De Pauw N and Vanhooren G, 1983 Method for biological quality assessment of water courses in Belgium. *Hydrobiologia,* **100,** 153-168

Deegan L A, Finn J T, Ayvazian S G, Ryder-Kieffer C A and Buonaccorsi J, 1997 Development and Validation of an Estuarine Biotic Integrity Index. *Estuaries,* **20**, 601-617

Delong M D and Brusven M A, 1998 Macroinvertebrate community structure along the longitudinal gradient of an agriculturally impacted stream. *Environmental Management*, **22,** 445-457

Den Hartog C, 1994 Suffocation of a littoral Zostera bed by Enteromorpha radiata. *Aquatic Botany*, **47,** 21-28

Den Hartog C and Polderman P J G, 1975 Changes in the seagrass populations of the Dutch Wadden Sea. *Aquatic Botany*, **1**, 141-147

Department of Ecology Publications, PO Box 47600, Olympia, WA 98504-7600, 1997, *Using invertebrates to assess the quality of Washington streams and to describe biological expectations*, **Pub no. 97-332**

Department of Environmental Protection Florida, Division of Water Resource Management, Bioassessment Program, Biocriteria Committee Meeting Summary, September 27-29, 2000.

Department of the Environment (DoE), 1987 *Methods for the use of Aquatic Macrophytes for assessing water quality 1985-86*. Methods for the examination of water and associated materials, 176pp

Department of the Environment, Ministry of Agriculture & Welsh Office, 1992 *Criteria and procedures for identifying sensitive areas and less sensitive areas (Urban Waste Water Treatment Directive) and Criteria and procedures for identifying 'polluted waters' (Nitrates Directive) in England and Wales*. Consultation Paper

Department of the Environment, Ministry of Agriculture & Welsh Office (DoE), 1993 *Methodology for identifying sensitive areas (Urban Waste Water Treatment Directive) and Methodology for designating vulnerable zones (Nitrates Directive) in England and Wales*. Consultation Paper

Descy J P and Ector L, 1996 *Use of diatoms for monitoring rivers in Belgium and Luxemburg.* In Use of Algae for Monitoring Rivers III (ed. J Prygiel, B A Whitton and J Bukowska), pp. 128-137. Douai, France: Agence de l'Eau Artois-Picardie

DeShon J E, 1995 *Development and application of the invertebrate community index (ICI).* In Biological assessment and criteria. Tools for water resource planning and decision making. (ed. W S Davis and T P Simon), pp. 217-243, Lewis Publishers, Boca Raton, Florida

Devai G and Moldovan J, 1983 An attempt to trace eutrophication in a shallow lake (Balaton, Hungary) using chironomids. *Hydrobiologia,* **103**, 169-175

Devillers P, Devillers-Terschuren J and Ledant J P, 1991 *CORINE biotopes manual. Habitats of the European Community.* Luxembourg: Commission of the European Community.

Devinny J S and Volse L A, 1978 Effects of sediments on the development of *Macrocystis pyrifera gametophytes*. *Marine Biology*, **48**, 343-348

DeVries I, Philippart C J M, De Groot E G and van der Toll M W M, 1996 *Coastal eutrophication and marine benthic vegetation: a model analysis*. In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (ed. W Schramm and P Neinhuis), pp. 79-114. Springer-Verlag, Berlin

Diaz J R and Rosenburg R, 1995 Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. A review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanogr Mar Biol*, **33**, 245-303

Dines R A and Murray-Bligh J A D, 2000 *Quality assurance and RIVPACS*. In Assessing the biological quality of freshwaters: RIVPACS and similar techniques, (ed.Wright J F, Sutcliffe D W and Furse M T), pp71-78. Freshwater Biological Association, Ambleside

Dion P and Le Bozec S, 1996 *The French Atlantic coasts*. In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (ed. W Schramm and P Neinhuis), pp. 251-264. Springer-Verlag, Berlin

Dixit S S, Smol J P, Charles D F, Hughes R M, Paulsen S G and Collins G B, 1999 Assessing water quality changes in the lakes of the northeastern United States using sediment diatoms. *Canadian Journal of Fisheries and Aquatic Sciences,* **56**, 131-152

Dodds W K, Jones J R and Welch E B, 1998 Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen and phosphorus. *Water Research,* **32**, 1455-1462

Dodge, 1989 Proceedings of the International Large River Symposium. *Canadian Special Publications of Fisheries and Aquatic Sciences*, **106**

DOE/CDEP, 1986 *Nitrate in water*. Pollution Paper 26. HMSO, London

Doering P H, Oviatt C A, Nowicki B L, Klos E G and Reed L W, 1995 Phosphorus and Nitrogen Limitation of Primary Production in a Simulated Estuarine Gradient. *Marine Ecology-Progress Series,* **124**, 271-287

Domburg P, Edwards A C, Sinclair A H, Wright G G and Ferrier R C, 1998 Changes in fertilizer and manurial practices during 1960-1990: Implications for N and P inputs to the Ythan catchment, N.E. Scotland. *Nutrient Cycling in Agroecosystems,* **52**, 19-29

Dong L F, Nedwell D B, Underwood G J C and Sage A, 2000 Environmental limitations of phytoplankton in estuaries, pp. 1-51: DETR

Dong L F, Thornton D C O, Nedwell D B and Underwood G J C, 2000 Denitrification in sediments of the River Colne estuary, England. *Marine Ecology-Progress Series,* **203**, 109-122

Doughty C R and Maitland P S, 1994 The ecology of the River Endrick: present status and changes since 1960. *Hydrobiologia*, **290**, 139-151

Dowden B F and Bennet H J, 1965 Toxicity of selected chemicals to certain animals. *J Wat Poll Cont Fed,* **37**, 1308-1316

Downing J A and Plante C, 1993 Production of fish populations in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **50**, 110-120

Downing J A, Plante C and Lalonde S, 1990 Fish production correlated with primary productivity, not morphoedaphic index. *Canadian Journal of Fisheries and Aquatic Sciences*, **47**, 1929-1936

Duarte C M, 1995 Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia*, **41**, 87-112

Dubina L V, Hejny S, Hroudovai Z, Husak S, Jerabkova O, Otahelova H, Stojko S M, Sytnik K M, Seljag-sosonko J R and Tasenkevich L A, 1993 Macrophytes as indicators of change in natural conditions.

Duce R A, Liss P S, Merrill J T, Atlas E L, Buat-Menard P, Hicks B B, Miller J M, Prospero J M, Arimoto R, Church T M, Ellis W, Galloway J N, Hansen L, Jickells T D, Knap A H, Reinhardt K H, Schneider B, Soudine A, Tokos J J, Tsunogai S, Wollast R and Zhou M, 1991 The atmospheric input of trace species to the world ocean. *Globl Biogeochem Cycles*, **5**, 193-259

Dudley Stamp L, 1941 *The Land of Great Britain*. London: HMSO

Dunn J, 1972 *A general survey of Langstone Harbour with particular reference to the effects of sewage*. Report to Hampshire River Authority and Hampshire County Council, pp. 79. Portsmouth Polytechnic, Portsmouth

Dyer K R, 1973 *Estuaries: A physical introduction*. John Wiley and Sons Ltd, London

EA Engineering, Science and Technology Inc, 1986 *Proposed modified effluent limitations for ammonia*. EA Report BET54E. Prepared for Bethlehem Steel Corporation, Sparrow Point, MD

Eaton L E, 1997 *Development and testing of biocriteria using benthic invertebrates for North Carolina Estuaries*. Poster Session: 14th Biennial Estuarine Research Federation International Conference

Eaton L E, 2001 Development and validation of Biocriteria Using Benthic Macroinvertebrates for North Carolina Estuarine Waters. *Marine Pollution Bulletin*, **42,** 23-30

Edgar G J, Barret N S, Last P R, 1999 The distribution of macroinvertebrates and fishes in Tasmanian estuaries. *J Biogeog*, **26**, 1169-1189

Edmonson W T and Lehman J T, 1981 The effect of changes in the nutrient income on the condition of Lake Washington. *Limnology and Oceanography*, **26**, 1-29

Edwards P, 1972 Cultured red alga to measure pollution. *Marine Pollution Bulletin*, **3**, 184-188

Edwards P, 1973 The benthic marine algae of polluted estuaries on County Durham. *Proceedings of the Challenger Society*, **4**, 161-162

Eklov A G, Greenberg L A, Bornmark C, Larsson P and Berglund O, 1998 Response of stream fish to improved water quality: a comparison between the 1960s and 1990s. *Freshwater Biology*, **40**, 771-782

Elliott M and Smith T F, 2000 *Defining reference ecological conditions for transitional and coastal waters*. SNIFFER Conference November 20-22 2000, Edinburgh

Elliot, M., Griffiths, A.H. & Taylor, C.J.L. 1988. The role of fish studies in estuarine pollution assessment. *Journal of Fish Biology*, **33A**, 51-61

Elliott J A, Reynolds C S and Irish A E, 2001 An investigation of dominance in phytoplankton using the PROTECH model. *Freshwater Biology,* **46**, 99-108

Elliott J M, Fletcher J M, Elliott J A, Cubby P R and Baroudy E, 1996 Changes in the population density of pelagic salmonids in relation to changes in lake enrichment in Windermere northwest England. *Ecology of Freshwater Fish*, **5**, 153-162

Emmerth P and Webber E C, 1993 Comparison of reference streams between ecoregions using rapid bioassessment protocols. *Journal of Alabama Academic Sciences,* **64,** 99

Energy, Mines and Resources Canada, 1986 *Canada Wetland regions (map scale 1:7,500,000), MCR 4108,* Canada Map Office, Energy, Mines, and Resources Canada, Ottawa, Ontario

Engle V D, Summers J K and Gaston G R, 1994 *A benthic index of environmental condition of Gulf of Mexico estuaries*. Estuaries, **17**, 372-384

English Nature, 1992 *River, lake and canal SSSIs in England subject to eutrophication*. English Nature Report, English Nature, Peterborough, June 1992

English Nature, 1993 *Comments by English Nature on 'River Quality: the Government's proposals, English Nature Report*. English Nature, Peterborough, February 1999

Engstrom D R and Wright Jr H E, 1985 *Chemical stratigraphy of lake sediments as a record of environmental change.* In Lake sediments and environmental history (ed. J W G Lund and E Y Haworth), pp. 11-67. University of Leicester Press, Leicester

Entek UK Ltd, 1996 *National strategy for eutrophication control – targets for eutrophication control*. Technical Report 898/30/13, National Rivers Authority – Anglian Region, pp. 88

Environment Agency, 1996 *Methodology for the Assessment of Freshwater Riverine Macrophytes for the Purposes of the Urban Waste Water Treatment Directive.* May 1996 Version 2

Environment Agency, 1996 *The Environment Agency's Strategy on Eutrophication*, 58pp

Environment Agency, 1996 *The quality of rivers in England and Wales*. A Report by the Environment Agency, November 1996, 106pp

Environment Agency, 1998a *Aquatic eutrophication in England and Wales; a proposed management strategy*. Environmental Issues Series. Environment Agency, Bristol, UK, pp. 36

Environmental Change Network http://www.ecn.ac.uk/protocols

Environmental Change Network, 2001 *Indicators of Freshwater Quality*, http://www.ecn.ac.uk/freshwater/index.htm

Environmental Health Service, Northern Ireland, 2000 *Eutrophication in Northern Ireland*

EPA, 2000 *Nutrient Criteria - Lakes and Reservoirs*. Washington DC: United States Environmental Protection Agency, 232pp

Erickson R J, 1985 An evaluation of mathematical models for the effects of pH and temperature on ammonia toxicity to aquatic organisms. *Wat Res,* **19**, 1047-1058

European Union, 2000 *Establishing a framework for community action in the field of water policy.* Directive of the European Parliament and of the Council 2000/60/EC. PE-CONS 3639/1/00 REV 1, Luxembourg

Facher E and Schmidt R, 1996 A siliceous chrysophean cyst-based pH transfer function for Central European lakes. *Journal of Paleolimnology***,** **16**, 275-321

Fahy E, Goodwillie R, Rochford J and Kelly D, 1975 Eutrophication of a partially enclosed estuarine mudflat. *Marine Pollution Bulletin*, **6,** 29-31

Fairchild G W, Lowe R L and Richardson W B, 1985 Algal Periphyton Growth on Nutrient-Diffusing Substrates - an *In situ* Bioassay. *Ecology,* **66**, 465-472

Farnham W F, 1986 Marine algae of Poole Harbour. *Estuarine Brackish-water Science Association Newsletter*, **44**, 9-10

Fasham M J R, Ducklow H W and McKelvie S M, 1990 A nitrogen-based model of plankton dynamics in the oceanic mixed layer. *Journal of Marine Research*, **48**, 591-639

Fenchel T, Blackburn T H, 1979 *Bacteria and mineral cycling*. Academic Press, New York

Fennessy S, 1993 *The effectiveness of riparian buffer strips for the control of agricultural run-off*. Report to the World Wide Fund for Nature. 56pp

Ferretti J A, Calesso D F, Hermon T R, 2000 *Evaluation of methods to remove ammonia interference in marine sediment toxicity tests.*

Ferrier R C, Owen R, Edwards A C, Malcolm A and Morrice J G, 1997 *Hindcasting of phosphorus concentrations in Scottish standing waters*. In Freshwater Quality: Defining the indefinable (ed. P J Boon), pp. 144-148. Edinburgh: The Stationary Office

Ferrier R C, Malcolm A, McAlister E, Edwards T, Morrice J and Owen R, 1996 *Hindcasting of in-loch phosphorus concentrations based on land cover classification*. Report for the Scotland and Northern Ireland Forum for Environmental Research

Fichez R, Jickells T D and Edmunds H M, 1992 Algal Blooms in High Turbidity, a Result of the Conflicting Consequences of Turbulence on Nutrient Cycling in a Shallow- Water Estuary. *Estuarine Coastal and Shelf Science,* **35**, 577-592

Finney N, 1998 *A nutrient history of the Lunan Burn lochs, central Scotland.* Unpublished MRes thesis. University College London

Fish G R, 1963 Limnological conditions and growth of trout in three lakes near Rotorua. *Proceedings of the New Zealand Ecological Society*, **10**, 1-7

Fisher T R, Peele E R, Ammerman J W and Harding L W, 1992 Nutrient Limitation of Phytoplankton in Chesapeake Bay. *Marine Ecology-Progress Series,* **82**, 51-63

Fleischer S, Rydberg L, Stibe L and Sundberg J, 1985 Temporal variations in nutrient transport to the Laholm Bay. *Vatten*, **41,** 29-35

Fletcher R L, 1974 *Ulva* problem in Kent. *Marine Pollution Bulletin*, **5,** 21

Fletcher R L, 1996a *The occurrence of 'Green Tides' - a review*. In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (ed. W Schramm and P H Neinhuis), pp. 7-43. Springer-Verlag, Berlin

Fletcher R L, 1996b *The British Isles*. In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (ed. W Schramm and P Neinhuis), pp. 223-249. Springer-Verlag, Berlin

Flindt M R, Pardal M A, Lillebø A I, Martins I and Marques J C, 1999 Nitrogen cycling and plant dynamics in estuaries: a brief review. *Acta Oecologica – International Journal of Ecology*, **20**, 237-248

Flower R J, 2001 Change, stress, sustainability and aquatic ecosystem resilience in North African wetland lakes during the 20th century: an introduction to integrated biodiversity studies within the CASSARINA Project. *Aquatic Ecology*, **36**: in press

Flower R J, Juggins S J and Battarbee R W, 1997 Matching diatom assemblages in lake sediment cores and modern surface sediment samples: the implications for conservation and restoration with special reference to acidified systems. *Hydrobiologia*, **344**, 27-40

Fong P, Boyer K E and Zedler J B, 1998 Developing an indicator of nutrient enrichment in coastal estuaries and lagoons using tissue nitrogen content of the opportunistic alga Enteromorpha intestinalis *(L. Link). Journal of Experimental Marine Biology and Ecology*, **231**, 63-79

Fong P, Zedler J B and Donohoe R M, 1993 Nitrogen vs. Phosphorus limitation of algal biomass in shallow coastal lagoons. *Limnology and Oceanography*, **38**, 906-923

Fore L S, Karr J R and Wisseman R W, 1996 Assessing invertebrate responses to human activities: evaluating alternative approaches. *Journal of the North American Benthological Society*, **15,** 212-231

Forel F A, 1904 *Le Leman*. Lausanne: Monographie Limnologique

Forsberg C, Ryding S, 1980 Eutrophication parameters and trophic state indices in 30 Swedish waste-receiving lakes. *Arch. Hydrobiol*. **80**, 189-207

Forsgren G, Jansson M, Nilsson P, 1996 Aggregation and sedimentation of iron, phosphorous and organic carbon in experimental mixtures of freshwater and estuarine water. *Estuar Coastal Shelf Sci*, 43, 259-268

Forth River Purification Board. 1993. Annual report. Edinburgh, Forth River Purification Board.

Foster I D L, Owens P N and Walling D E, 1996 Sediment yields and sediment delivery in the catchment of Slapton Lower Ley, south Devon, UK. *Field Studies*, **8**, 629-661

Fozzard I R, Doughty C R, Ferrier R C, Leatherland T M and Owen R, 1999 A quality classification for management of Scottish standing waters. *Hydrobiologia*, **395/396**, 433-453

Frazier et al, 1996 Temporal and vertical distributions of total ammonia nitrogen and un-ionised ammonia nitrogen in sediment pore water form the upper Mississippi River. *Environ Toxicol Chem* **15**, 92-99

Frederiksen O T, 1987 The fight against eutrophication in the inlet of "Odense fjord" by reaping of sea lettuce (*Ulva lactuca*). *Water Science and Technology*, **19,** 81-87

Fuentes J M, 1986 *Dinámica, estructura y producción de una comunidad fitobentónica intermareal (horizonte de Fucus vesiculosus) en las Rias gallegas.* Unpublished PhD thesis, Universite Santiago de Compostela

Fulton R S, 1984 Predation, production and the organisation of an estuarine copepod community. *Journal of Plankton Research*, ***6***, 399-415

Funen Island Council, 1991 *Eutrophication of coastal waters. Coastal water quality management in the county of Funen, Denmark 1976-1990.* Funen Island Council, Denmark

Furse M T, 1998 *The Faunal Richness of Headwater Streams,* PhD Dissertation, University of Reading

Furse M T, 2000 *The application of RIVPACS procedures in headwater streams - an extensive and important national resource*. In Assessing the biological quality of freshwaters: RIVPACS and similar techniques (ed. J F Wright, D W Sutcliffe and M T Furse), pp. 79-92. Freshwater Biological Association, Ambleside

Furse M T, Fernandes J N, Wright J F and Armitage P D, 1990 *The use of aquatic macro-invertebrate assemblages for assessment of the environmental quality of rivers.* In Water Quality, Assessment and Management, Proceedings of an International Seminar, Lisbon 17-19 May 1989, pp. 171-181. ONESCO

Furse M T, Moss D, Wright J F and Armitage P D, 1984 The influence of seasonal and taxonomic factors on the ordination and classification of running-water sites in Great Britain and on the prediction of their macroinvertebrate communities. *Freshwater Biology*, **17,** 257-280

Furse M T, Moss D, Wright J F and Armitage P D, 1987 *Freshwater site assessment using multivariate techniques*. In The use of invertebrates in site assessment for conservation (ed M L Luff). Proceedings of a meeting held University of Newcastle-upon-Tyne, 7 Jan 1987, Agricultural Research Group. University Newcastle-upon-Tyne

Furse M T, Wright J F, Armitage P D and Moss D, 1981 An appraisal of pond-net samples for biological monitoring of lotic macroinvertebrates. *Water Research*, **15**, 679 – 689

Gallegos C L, Jordan T E and Correll D L, 1992 Event-Scale Response of Phytoplankton to Watershed Inputs in a Subestuary - Timing, Magnitude, and Location of Blooms. *Limnology and Oceanography,* **37**, 813-828

Gallegos C L, Jordan T E and Correll D L, 1997 Interannual variability in spring bloom timing and magnitude in the Rhode River, Maryland, USA: Observations and modeling. *Marine Ecology-Progress Series,* **154**, 27-40

Galloway J N, Schlesinger W H, Levy H, Michaels A, Schnoor J L, 1995 Nitrogen fixation: anthropogenic enhancement-environmental response. *Globl Biogeochem Cycles,* **9,** 235-252

Gardner W S, Seitzinger S P, Malczyk J M, 1991 The effects of sea salts on the forms of nitrogen released from estuarine and freshwater sediments: does ion paring affect ammonium flux? *Estuaries,* **14**, 157-166

Gaufin A R, 1958 The effects of pollution on a midwestern stream. *Ohio Journal of Science*, **58,** 197-208

Gaufin A R, 1973 *Use of aquatic invertebrates in the assessment of water quality.* In Biological methods for the assessment of water quality (ed. Cairns and Dickson)**,** pp**.** 96-116

Geertz-Hansen O and Sand-Jensen K, 1990 *Nitrogen limitation of growth, and grazing control of abundance of the marine macroalga* Ulva lactuca *L. in a eutrophic Danish estuary.* Presentation made at the 25th European Marine Biology Symposium, Sept. 10-15, 1990, Ferrara, Italy

Geertz-Hansen O, Sand-Jensen K, Hansen D F and Christiansen A, 1993 Growth and grazing control of abundance of the marine macroalgae *Ulva lactuca L.* in a eutrophic Danish estuary. *Aquatic Botany*, **46**, 101-109

Genkal S I, 1997 *Problems in identifying centric diatoms for monitoring the water quality of large rivers*. In Use of Algae for Monitoring Rivers III (ed. J Prygiel, B A Whitton & J Bukowska), pp. 182-187. Douai, France: Agence de l'Eau Artois-Picardie

Gerritsen J, 1995 Additive biological indices for resource management. *Journal of the North American Benthological Society*, **14,** 451-457

Gerritsen J, Jessup B, Leppo E W and White J, 2000 *Development of Lake Condition Indexes (LCI) for Florida*. Florida Department of Environmental Protection, Under Contracts WM 565 and WM 655, July 2000

Gerstmeier R, 1989 Lake typology and indicator organisms in application to the profubdal chironomid fauna of Starnberger See (Diptera, Chironomidae). *Archiv Fur Hydrobiologie,* **116**, 227-234

Giannakou U, Kamarianos A, Kousouris T and Kilikidis S, 1997 The distribution of benthic macroinvertebrates in relation to physico-chemical parameters as a tool for assessing river water pollution in a lowland stream in North Greece. *Fresenius Environmental Bulletin*, **6**, 560-567

Gibson C E, Wu Y, Smith S J, Wolfe-Murphy S A, 1995 Synoptic limnology of a diverse geological region: catchment and water chemsitry. *Hydrobiology*, **306**, 213-227

Gibson C E, Foy R H and Bailey-Watts A E, 1996 An analysis of the total phosphorus cycle in some temperate lakes: the response to enrichment. *Freshwater Biology,* **35**, 525-532

Gibson G R, Barbour M T, Stribling J B, Gerritsen J and Karr J R, 1996 *Biological criteria: Technical guidance for streams and small rivers (revised edition).* EPA 822-B-96-001, US Environmental Protection Agency, Office of Water, Washington DC

Gieskes W W C, Kraay G W, 1977 Continous plankton records: changes in the plankton of the North Sea and its eutrophic Southern Bight from 1949 to 1975. *Neth J Sea Res,* **11**, 334-364

Glasgow H B and Burkholder J M, 2000 Water quality trends and management implications from a five- year study of a eutrophic estuary. *Ecological Applications,* **10**, 1024-1046

Glasgow H B, Burkholder J M, Schmechel D E, Tester P A, Rublee P A, 1995 Insidious effects of a toxic estuarine dinoflagellate on fish survival and human health. *J Toxic Environ Health,* **46**, 501-522

Godrey P J, 1978 Diversity as a measure of benthic macroinvertebrate community response to water pollution. *Hydrobiologia,* **57,** 111-122

Goericke R and Montoya J P, 1998 Estimating the contribution of microalgal taxa to chlorophyll a in the field - variations of pigment ratios under nutrient- and light-limited growth. *Marine Ecology-Progress Series,* **169**, 97-112

Goldman C R, 1984 *Oceanic nutrient cycles*. In Flows of energy and materials in marine ecosystems. Theory and practice. (ed. M J R Fasham), pp. 137-170: Plenum Press

Goldsmith B, 2001 Diatoms and water quality in lowland streams. In Geography. London: London (University College)

Golubic S, 1970 Effects of organic pollution on benthic communities. *Marine Pollution Bulletin*, **1**, 56-57

Gorman M L and Raffaelli D G, 1993 The Ythan Estuary. *Biologist,* **40,** 10-13

Government of Alberta, Canada, Alberta Environment. Nutrients in Northern River Basins, http://www.gov.ab.ca/env/water/nrbs/sect3/sect37.html

Gowen R J, Mills D K, Trimmer M, Nedwell D B, 2000 Production and its fate in two coastal regions of the Irish Sea: the influence of anthropogenic nutrients. *Mar Ecol Prog Ser,* **208**, 51-64

Graca M A S and Coimbra C N, 1998 The elaboration of indices to assess biological water quality. *Water Research*, **32,** 380-392

Gray J S, 1989 Effects of environmental stress on species rich assemblages. *Biological Journal of the Linnean Society,* **37,** 19-32

Greenberg A E, Clesceri L S and Eaton A D (Eds), 1992 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, American Water Works Association and Water Environment Federation, Washington, pp 78 –115.

Grenager B, 1957 Algological observations from the polluted area of the Oslofjord. *Nytt Magasin foer Botanikk*, **5**, 41-60

Grimes B H and Hubbard J C E, 1971 A comparison of film type and the importance of season for interpretation of coastal marshland vegetation. *Photogrammetric Record*, **7**, 213-222

Grönland T, 1993 Diatoms in surface sediments of the Gotland Basin in the Baltic Sea. *Hydrobiologia*, **269/270**, 235-242

Guhl W, 1987 Aquatic ecosystem characterization by biotic indices. *Internationale Revue der Gesamten Hydrobiologie*, **72,** 431-455

Guillard R R L, Keller M D, O'Kelly C J and Floyd G L, 1991 *Pycnococcus provasolii* gen. et sp. nov., a coccoid prasinoxanthin-containing phytoplankter from the western North Atlantic and Gulf of Mexico. *Journal of Phycology,* **27**, 39-47

Gulson J, Parr W and Mainstone C P, 1993 *Phosphates in freshwaters: standards for nature conservation.* WRC unpublished report to English Nature, English Nature, Peterborough

Haathela I and Letho J, 1982 The occurrence of bladder-wrack (*Fucus vesiculosus*) in 1975-1980 in the Seile area, Archipelago Sea. *Memoranda Societatis pro Fauna et Flora Fennica*, **58**, 1-5

Haines T A, 1973 Effect of nutrient enrichment and a rough fish population carp on a game fish population smallmouth bass. *Transactions of the American Fisheries Society*, **102**, 346-354

Hall M O and Bell S S, 1988 Response of small mobile epifauna to complexity of epiphytic algae on seagrass blades. *Journal of Marine Research*, **46**, 613-630

Hall R K, Wolinsky G A, Husby P, Harrington J, Spindler P, Vargus K and Smith G, 2000 Status of aquatic bioassessment in US EPA region IX. *Environmental Monitoring and Assessment*, **64,** 17-30

Hallegraeff G M, 1993 A Review of Harmful Algal Blooms and Their Apparent Global Increase. *Phycologia,* **32**, 79-99

Hannewijk A, 1988 *De verspreiding en biomassa van macrofyten in het Veerse Meer, 1987.* Delta Instituut voor Hydrobiologisch Onderzoek, Yerseke. Rappporten en Verslagen 1988-92. 25pp.

Hansson L A, 1992 Factors regulating periphytic algal biomass. *Limnology and Oceanography,* **33**, 121-128

Harader R R, Allen G H, 1983 Ammonia toxicity to chinook salmon parr: reduction in saline waters. *Trans Am Fish Soc*, **112**, 834-837

Harding J P C, 1981 *Macrophytes as Monitors of River Quality in the Southern N.W.W.A. Area*. North West Waters, Rivers Division, Warrington, 54pp

Harding J P C & Hawley G R W, 1991 *Use of algae for monitoring rivers in the United Kingdom*. In Use of Algae for Monitoring Rivers. (ed. B A Whitton, E Rott & G Friedrich), pp. 183-193. Institut fur Botanik, Innsbruck

Harding J P C and Kelly M G, 1996 *Recent developments in algal-based monitoring in the United Kingdom*. In Use of Algae for Monitoring Rivers III (ed. J Prygiel, B A Whitton & J Bukowska), pp. 26-34. Douai, France: Agence de l'Eau Artois-Picardie

Harding J S, Young R G, Hayes J W, Shearer K A and Stark J D, 1999 Changes in agricultural intensity and river health along a river continuum. *Freshwater Biology*, **42,** 345-357

Harding L W and Perry E S, 1997 Long-term increase of phytoplankton biomass in Chesapeake Bay, 1950-1994. *Marine Ecology-Progress Series,* **157**, 39-52

Haritonidis S, 1996 *Greece.* In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (eds. W Schramm and P Neinhuis), pp. 403-420. Springer-Verlag, Berlin

Harlin M M and Thorne-Miller B, 1981 Nutrient enrichment of seagrass beds in a Rhode Island coastal lagoon. *Marine Biology*, **65**, 221-229

Harper D, 1992 *Eutrophication of freshwaters - principles, problems and restoration,* Chapman and Hall, London

Harper D, 1996 *Nutrient management in rivers: the ecological problems and the economic possibilities.* Report of a workshop, Environment Agency and English Nature, Peterborough, October 1996, 30pp

Harris G P, 1999 Comparison of the biogeochemistry of lakes and estuaries: ecosystem processes, functional groups, hysteresis effects and interactions between macro- and microbiology. *Marine and Freshwater Research,* **50**, 791-811

Harris J H, 1995 The use of fish in ecological assessments. *Australian Journal of Ecology*, **20**, 65-80

Harrison A D and Elsworth J F, 1958 Hydrobiological studies on the Great Berg River, Western cape Province. *Transactions of the Royal Society of South Africa*, **35**, 125-329

Harrison P J, Parslow J S and Conway H L, 1989 Determination of Nutrient-Uptake Kinetic-Parameters - a Comparison of Methods. *Marine Ecology-Progress Series,* **52**, 301-312

Harrison P J, Waters R E and Taylor F J R, 1980 A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *Journal of Phycology,* **16**, 28-35

Hart C W and Fuller S L H (eds), 1974, *Pollution ecology of freshwater invertebrates*. Academic Press, New York

Hartmann J, 1984 *The charrs* Salvelinus alpinus *of Lake Constance, a lake undergoing cultural eutrophication.* In Biology of the Arctic Charr. (ed. L Johnson and B L Burns), pp. 471-486. Winnipeg: University of Manitoba Press

Haslam S M, 1978 *River Plants*, Cambridge University Press, Cambridge.

Haslam S M 1981 *Vegetation in British Rivers. Volume 1 text & Volume 2 River maps* Nature Conservancy Council, pp. 120 & 200

Haslam S M, 1987 *River Plants of Western Europe*, Cambridge University Press, Cambridge

Haslam S M & Wolseley P A, 1981 *River vegetation: its identification, assessment and management.* Cambridge University Press, Cambridge, 154pp

Haury J, Peltre M-C, Muller S, Tremolieres M, Barbe J, Dutartre A & Guerlesquin, 1995 Macrophyte indices to assess stream water quality: preliminary proposals from the group of scientific interest 'Macrophytes of inland waters'. ANPP-Colloque International marqueurs biologiques de pollution, Chinon, September 1995, 243-253

Hawkes H A, 1975 *River zonation and classification*. In River ecology (ed. R B Whitton), pp. 312-374. Blackwell: London

Hawkes H A, 1979 *Invertebrates as indicators of river quality*. In Biological Indicators of Water Quality (ed. A James and L Evison), Chap. 2. John Wiley, Chichester, England

Hawkins C P and Norris R H, 1997 Abstract - Comparison of the ability of multimetric and multivariate assessment techniques to detect biological impairment in mountainous streams of California. *Bulletin of the North American Benthological Society*, **14,** 96

Hawkins, Murphy and Anderson, 1982 Effects of canopy, substrate composition, and gradient on the structure of macroinvertebrate communities in Cascade Range streams of Oregon. *Ecology,* **63,** 1840-1856

Heegaard E, Birks H H, Gibson C E, Smith S J, Wolfe-Murphy S, 2001 Species-environmental relationships of aquatic macrophytes in Northern Island. *Aquatic Botany*, **70**, 175-223

Heilmann J P, Richardson K and Aertebjerg G, 1994 Annual Distribution and Activity of Phytoplankton in the Skagerrak Kattegat Frontal Region. *Marine Ecology-Progress Series,* **112**, 213-223

Heinis F and Davids C, 1993 Factors governing the spatial and temporal distribution of chironmid larvae in the Maarsseveen lakes with special emphasis on the role of oxygen conditions. *Netherlands Journal of Aquatic Ecology,* **27**, 21-34

Hellawell J M, 1977 *Biological surveillance and water quality monitoring.* In Biological Monitoring of Inland Fisheries (ed J S Alabaster), pp. 69-88, Applied Science Pubs, London

Hellawell J M, 1978 *Biological Surveillance of Rivers: a Biological Monitoring Handbook*. Water Research Centre, Medmenham, England

Hellawell J M, 1986 *Biological Indicators of Freshwater Pollution and Environmental Management,* Elsevier Applied Science, London and New York

Hellenthal R A, 1982 *Using aquatic insects for the evaluation of freshwater communities*. In Acquisition and Utilization of Aquatic Habitat Inventory Information. Proceedings of a Symposium, Portland, OR Oct 28-30, 1981, (ed. N B Armantrout), pp. 347-354, Western Division, American Fisheries Society, Bethesda, MD

Hemminga M, Duarte C M, 2000 Seagrass Ecology. Cambridge University Press

Hemsley-Flint B, 2000 *Classification of the biological quality of rivers in England and Wales*. In Assessing the Biological quality of fresh waters: RIVPACS and other techniques (ed. J F Wright, D W Sutcliffe and M T Furse), pp. 55-59

Henley W J and Ramus J, 1989 Time course of physiological reesponse to *Ulva rotundata* to growth irradiance transitions. *Marine Ecology Progress Series*, **54**, 171-177

Herricks E E and Cairns J, 1982 Biological monitoring Part III - Receiving system methodology based on community structure. *Water Research*, **16,** 141-153

Hessen D O, 1999 *Catchment properties and the transport of major elements to estuaries*. In Advances in ecological research 29, (ed. D B Nedwell and D G Raffaelli), pp. 1-34. Academic Press, London

Hickel W, Mangelsdorf P, Berg J, 1993 The human impact in the German Bight: eutrophication through three decades. *Helgolander Meeresuntersuchungen,* **47**, 243-263

Hickey C W and Vickers M L, 1994 Toxicity of ammonia to nine native New Zealand freshwater invertebrate species. *Arch Environ Contam Toxicol,* **26**, 292-298

Hilmy A M, El-Domiaty N A, Wershana K, 1987 Acute and chronic toxicity of nitrite to *Clarias lazera*. *Comp Biochem Physiol,* **86**, 247-253

Hilsenhoff W, 1982 *Using a Biotic Index to Evaluate Water Quality in Streams.* Technical Bulletin No 132 Wisconsin Department of Natural Resources, Madison, WI

Hilsenhoff W L, 1987 An improved biotic index of organic stream pollution. *Great Lakes Entomologist,* **20,** 31-39

Hilsenhoff W L, 1988 Rapid field assessment of organic pollution with a family level biotic index. *Journal of the North American Benthological Society*, **7,** 65-68

Hiltner A L and Hershey A E, 1992 Blackfly *(Diptera:Simuliidae*) responses to phosphorus enrichment of an arctic tundra stream. *Hydrobiologia,* **240,** 259-266

Hiscock K, 1981 *South west Britain sublittoral survey. Final report.* Report from Oil Pollution Research Unit, Field Studies Council to nature Conservancy Council, CSD report No 327

Ho K T, Kuhn M, Pelletier M, McGee F, Burgess R M, Serbst J, 2000 Sediment toxicity assessment: a comparison of standard and new testing designs. *Arch Environ Contam Toxicol,* **39**, 462-468

Ho Y B, 1979 Inorgranic mineral nutrient level studies on *Potamogeton pectinatus L.* and *Enteromorpha prolifera* in Forfar Loch, Scotland. *Hydrobiologia,* **62**, 7-15

Ho Y B, 1987 Ulva lactuca *(Chlorophyta, Ulvales)* in Hong Kong intertidal waters – its nitrogen and phosphorus content and its use as a bioindicator of eutrophication. *Asian Marine Biology*, **4**, 97-102

Hofmann W, 1988 The significance of chironomid analysis *(Insecta: Diptera)* for paleolimnological research. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **62**, 501-509

Holligan P M, 1979 *Dinoflagellate blooms associated with tidal fronts around the British Isles*. In Toxic dinoflagellate blooms (ed. D L Taylor and H H Seliger), pp. 249-256. New Youk: Elsevier. North Holland

Holmboe N, Jensen H S and Andersen F O, 1999 Nutrient addition bioassays as indicators of nutrient limitation of phytoplankton in an eutrophic estuary. *Marine Ecology-Progress Series,* **186**, 95-104

Holmes N T H, 1983 *Typing Rivers according to their flora.* Focus on Nature Conservation No. 4., Nature Conservancy Council, 200pp. (ISSN 0264-8474)

Holmes N T H, 1995 *Macrophytes for Water and Other River Quality Assessments*. A report to the National Rivers Authority. March 1995. National Rivers Authority, Anglian Region, Peterborough

Holmes N T H, 1996 The Use of Riverine Macrophytes for the Assessment of Trophic Status: Review of 1994/95 data and refinements for future use. A report to the National Rivers Authority, Peterborough

Holmes N T H and Newbold C, 1984 *River Plant Communities- Reflectors of Water and Substrate Chemistry*. Focus on Nature Conservation No. 9. Nature Conservancy Council, Peterborough

Holmes N T H and Whitton B A, 1977 Macrophyte vegetation of the River Swale, Yorkshire. *Freshwater Biology*, **7**, 545-558

Holmes N T H, Boon P J and Rowell, 1998 A revised classification system for British rivers based on their aquatic plant communities. *Aquatic Conservation: marine and freshwater ecosystems,* **8**, 555-578

Holmes N T H, Whitton B A and Hargreaves J W, 1978 A coded list of freshwater macrophytes of the British Isles. Water Archive Manual Series No 4, Department of the Environment Water Data Unit, 201 pp

Holmes N T H, Newman J R, Chadd S, Rouen K J, Sharp L and Dawson F H, 1999 Mean Trophic Rank: A users manual. R & D Technical Report No. E38, Environment Agency, 129pp

Holopainen I J, Tonn W M and Paszkowski C A, 1992 Effects of fish density on planktonic communities and water quality in a manipulated forest pond. *Hydrobiologia*, **243/244**, 311-321

Honjo T, 1993 *Overview on bloom dynamics and physiological ecology of Heterosigma akashiwo*. In Toxic phytoplankton blooms in the sea. (ed. T J Smayda and I Shimuza), pp. 33-42. Amsterdam: Elsevier

Hooda P S, Moynagh M, Svoboda I F and Miller A, 2000 Macroinvertebrates as Bioindicators of Water Pollution in Streams Draining Dairy Farming Catchments. *Chemistry and Ecology*, **17**, 17-30

Hopkinson C S, 1987 Nutrient regeneration in shallow-water sediments of the estuarine plume region of the nearshore Georgia Bight, U.S.A. *Mar Biol,* **94**, 127-142

Horner R R, Welch E B and Veenstra R B, 1983 *Development of nuisance periphytic algae in laboratory streams in relation to enrichment and velocity*. In Periphyton of Freshwater Ecosystems (ed. R G Wetzel), pp. 121-134. The Hague: Dr W. Junk Publishers

Horner R R, Welch E B, Seeley M R and Jacoby J M, 1990 Responses of periphyton to changes in current velocity, suspended sediment and phosphorus concentration. *Freshwater Biology,* **24**, 215-232

Hourigan T, 1999 *Reconstructing stream water quality on the Sussex Downs: a new technique*. Unpublished undergraduate dissertation, Department of Geography, University College London.

Howarth R W, Billen G, Swaney D, Townsend A, Jaworski N, Lajtha K, Downing J A, Elmgren R, Caraco N, Jordan T, Berendse F, Freney J, Kudeyarov V, Murdoch P and Zhu Z L, 1996 Regional nitrogen budgets and riverine N&P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. *Biogeochemistry,* **35**, 75-139

Howarth R W, Billen G, Swaney D, Townsend A, Jaworski N, Lajtha K, Downing J A, Elmgren R, Caraco N, Jordan T, Berendse F, Freney J, Kudeyarov V, Murdoch P and Zhaoliang Z, 1996 Regional nitrogen budgets and riverine N and P fluxes for the drainage to the North Atlantic Ocean: natural and human influences. *Biogeochem,* **35**, 75-139

Howarth R W, Chan F and Marino R, 1999 Do top-down and bottom-up controls interact to exclude nitrogen-fixing cyanobacteria from the plankton of estuaries? An exploration with a simulation model. *Biogeochemistry,* **46**, 203-231

Howarth R W, Swaney D P, Butler T J and Marino R, 2000 Climatic control on eutrophication of the Hudson River estuary. *Ecosystems,* **3**, 210-215

Hoyer M V and Canfield D E, 1991 A phosphorus-fish standing crop relationship for streams? *Lake Reservoir Management*, **7**, 25-32

Hruby T, 1987 Using similarity measures in benthic impact assessments. *Environmental Monitoring and Assessment*, **8,** 163-180

http://enterprise.canberra.edu.au/WWW/www-directreps.nsf/996f439750a23457ca2567fc001c75ae/1902141bd6a4b974ca256a010004036b?OpenDocument

http://es.epa.gov/ncer/final/grants/94/bio/deegan.html

http://www.benthos.org/database/allnabstracts.cfm/db/lacrosse2001abstracts/id/345

http://www.ces.fau.edu/library/index.ihtml

http://www.ces.fau.edu/search.html

http://www.dep.state.fl.us/water/bioassess/docs/techdox/Florida%20LCI.pdf

http://www.environment-agency.gov.uk/commondata/105385/eutrophication.pdf

Huet M, 1946 Note preliminaire sur les relations entre la pente et les populations piscicoles des eaux courantes. *Dodonaea*, **13,** 232-243

Huet M, 1959 Profiles and biology of western European streams related to fish management. *Transactions of the American Fisheries Society*, **88**, 155-163

Huey D W, Beitinger T L, 1980 Toxicity of nitrite to larvae of the salamander *Ambystoma texanum*. *Bull Environ Contam Toxicol,* **25,** 909-912

Hughes B D, 1978 The influence of factors other than pollution on the value of Shannon's diversity index for benthic macroinvertebrates in streams. *Water Research,* **12,** 359-364

Hughes N, 2000 *Benthic Diatoms as Monitors of Water Quality in Shallow Lowland Lakes*, Report submitted for transfer of registration from M.Phil to PhD, Department of Geography, University College London, November 2000

Hughes R G, 2001 Biological and physical processes that affect saltmarsh erosion and saltmarsh restoration: development of hypotheses. *Ecol Stud,* **151**, 173-192

Hughes R G, Lloyd D, Ball L, Emson D, 2000 The effects of the polychaete *Nereis diversicolor* on the distribution and transplanting success of *Zostera noltii. Helgol Mar Res,* **54**, 129-136

Hughes R M, 1985 Use of watershed characteristics to select control streams for estimating effects of metal mining wastes on extensively disturbed streams. *Environmental Management*, **9,** 253-262

Hughes R M and Larsen D P, 1988 Ecoregions: An approach to surface water protection. *Journal of Water Pollution Control Federation*, **60,** 486-493

Hughes R M and Larsen D P, 1995 *Defining acceptable biological status by comparing with reference conditions.* In Biological assessment and criteria. Tools for water resource planning and decision making. (ed. W S Davis and T P Simon), pp. 31-47, Lewis Publishers, Boca Raton, Florida

Hughes R M, Larsen D P and Omernik J M, 1986 Regional reference sites: A method for assessing stream potentials. *Environmental Management,* **10,** 629-635

Hughes R M, Paulson S G and Stoddard J L, 2000 EMAP-Surface Waters: a multiassemblage, probability survey of ecological integrity in the USA. *Hydrobiologia*, **422,** 429-443

Hughes R M, Gakstatter H, Shirazi M A and Omernik J M, 1982 *An approach for determining biological integrity in flowing waters.* In Resource Inventories: Principles and Practices, Proceedings of a National Workshop. (ed. T B Brann), pp. 877-888, Society of American Foresters, Bethesda, Maryland

Hughes R M, Whittier T R, Thiele S A, Pollard J E, Peck D V, Paulsen S G, McMullen D, Lazorchak J, Larsen D P, Kinney W L, Kaufmann P R, Hedtke S, Dixit S S, Collins G B and Baker J R, 1992 *Lake and stream indicators for the United States Environmental Protection Agency's Environmental Monitoring and Assessment Program.* In Ecological Indicators, **1,** pp. 305-335, Elsevier Applied Science

Hull S, 1987 Macroalgal mats and species abundance: a field experiment. *Estuarine Coastal and Shelf Science*, **25**, 519-532

Humphrey C I, Storey A W and Thurtell L 2000 *AUSRIVAS: operator sample processing errors and temporal variability – implications for model sensitivity*. In Assessing the biological quality of fresh waters: RIVPACS and other techniques (ed. J F Wright, D W Sutcliffe and M T Furse**)**, pp.143-164, Proceedings of an International Workshop, Oxford, September 1998. Freshwater Biological Association, Ambleside

Hurlbert S H 1984 Pseudoreplication and the design of ecological field experiments. *Ecological Monographs,* **54**

Hutchinson G E and Loffler H, 1956 The thermal classification of lakes. *Proceedings of the National Academy of Science, Washington,* **42**, 84-86

Hutchinson G E, 1957 *A Treatise on Limnology Volume I: Geography, physics and chemistry*. Wiley, New York

Hutchinson G E, 1967 *A Treatise on Limnology Volume 2: Introduction to Lake Biology and the Limnoplankton*. New York: John Wiley & Sons

Hyatt K D and Stockner J G, 1985 Responses of Sockeye Salmon *Oncorhynchus nerka* to fertilisation of British Columbia coastal lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 320-331

Hyne R V, Everett D A, 1998 Application of a benthic euryhaline amphipod, *Corophium* sp., as a sediment toxicity test organism for both freshwater and estuarine systems. *Arch Environ Contam Toxicol*. **34**, 26-33

Hynes H B N, 1960 *The Biology of polluted waters*. Liverpool University Press

Hynes H B N, 1970 *The Ecology of Running Waters.* University of Toronto Press

Ibelings B, Admiraal W, Bijkerk R, Ietswaart T and Prins H, 1998 Monitoring of algae in Dutch rivers: does it meet its goals? *Journal of Applied Phycology,* **10**, 171-181

Illies J (ed) 1978 Limnofauna Europaea – a checklist of the animals inhabiting European Inland Waters, with accounts of their distribution and ecology (except Protozoa). Gustav Fischer Verlag, Stuttgart, New York. Swets & Zeitlinger BV Amsterdam

Ingman G L and Kerr M A, 1990 *Clark Fork River Basin nutrient pollution source assessment.* In Proceedings of 1990 Clark Fork River Symposium edited by V J Watson

Irmer U, 2000 The new EC Framework Water Directive: Assessment of the chemical and ecological status of surface waters. *Acta Hydrochimica et Hydrobiologica*, **28**, 7-14

Irvine K, Moss B and Balls H, 1989 The loss of submerged plants with eutrophication. II. Relationships between fish and zooplankton in a set of experimental ponds, and conclusions. *Freshwater Biology*, **22**, 89-107

Irving R, 1993 *Too much of a good thing – Nutrient enrichment in the UK's inland and coastal waters*. Report to World Wide Fund for Nature. 99pp

Irving R, 1993 *Too much of a good thing: nutrient enrichment in the UK's inland and coastal waters*. Report to the WWF (UK)

Isle of Man Government Laboratory, Freshwater Quality, www.gov.im/govLabs/fresh_water.html

ISO (International Organisation for Standardization), 1979 *Assessment of the biological quality of rivers by a macroinvertebrate 'score'.* ISO/TC 147/SC5/ WG 6 N5, Draft proposal

Jacobi G Z, Smolka L R and Jacobi M D, 1995 The use of biological assessment criteria in the evaluation of a high mountain stream, the Rio Hondo, New Mexico, USA. Proceedings of Congress in Sao Pualo 1995. *Internationale Association of Theoretical and Applied Limnology*, **26,** 1227-1234

James A and Evison L (eds), 1979 *Biological Indicators of Water Quality,* John Wiley, Chichester, England

Jana B B, Kundu G, Sarka G, 1986 Pattern of oxygen and phosphate uptake by Indian carp (Cirrhinus mrigala) eggs during development in the Chinese hatchery system. *Prog Fish Cult,* **48**, 284-287

Jeffrey D W, 1993 Sources of nitrogen for nuisance macroalgal growths in Dublin Bay, Republic of Ireland. *The Phycologist*, **34**, 30

Jeffrey D W, Madden B, Rafferty B, Dwyer R and Wilson J G, 1991 *Indicator organisms as a guide to estuarine management* In Bioindicators and Environmental Management (ed. D W Jeffrey and B Madden), pp. 55-64. Academic Press, London

Jeffrey D W, Madden B, Rafferty B, Dwyer R, Wilson J and Allott N, 1992 *Dublin Bay - Water quality management plan.* Technical Report No. 7. Algal growths and foreshore quality. 168pp. Environmental Research Unit. Dublin

Jenkins R M, 1982 The morphoedaphic index and reservoir fish production. *Transactions of the American Fisheries Society*, **111**, 133-140

Jensen M H, Lomstein E, Sørensen J, 1990 Benthic ammonium and nitrate flux following sedimentation of a spring phytoplankton bloom in Aarhus Bight, Denmark. *Mar Ecol Prog Ser*, **61**, 87-96

Jeppesen E, Jensen J P, Kristensen P, Sondergaard M, Mortensen E, Sortkjaer O and Olrik K, 1990 Fish manipulation as a lake restoration tool in shallow, eutrophic temperate lakes 2: threshold levels, long-term stability and conclusions. *Hydrobiologia,* **200/201**, 219-227

Jeppesen E, Jensen J P, Sondergaard M, Lauridsen T and Landkildehus F, 2000 Trophic structure, species richness and biodiversity in Danish lakes: changes along a phosphorus gradient. *Freshwater Biology,* **45**, 201-218

Jeppesen E, Jensen J P, Sondergaard M, Lauridsen T, Pedersen L J and Jensen L, 1997 Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia,* **342/343**, 151-164

Jeppesen E, Sondergaard M, Kanstrup E, Petersen B, Eriksen R B, Hammershojm M, Mortensen E, Jensen J P and Have A, 1994 Does the impact of nutrients on the biological structure and function of brackish and freshwater lakes differ? *Hydrobiologia*, **276**, 15-30

Jeppesen E, Søndergaard M, Søndergaard M and Christoffersen K (ed.), 1998 *The structuring role of submerged macrophytes in lakes*. Ecological Studies: Springer Verlag.

Jeppesen E, Madsen E A, Jensen J P and Anderson N J, 1996 Reconstructing the past density of planktivorous fish and trophic structure from sedimentary zooplankton fossils: a surface sediment calibration data set from shallow lakes. *Freshwater Biology*, **36**, 115-127

Jickells T D, 1998 Nutrient biogeochemistry of the coastal zone. *Science,* **281**, 217-222

Jickells T, Andrews J, Samways G, Sanders R, Malcolm S, Sivyer D, Parker R, Nedwell D, Trimmer M and Ridgway J, 2000 Nutrient fluxes through the Humber estuary - Past, present and future. *Ambio,* **29**, 130-135

John D M, Brook A J and Whitton B A, 2002 *The Freshwater Algal Flora of the British Isles*: Cambridge University Press

Johnes P J, 1996 Evaluation and management of the impact of land use change on the nitrogen and phosphorus load delivered to surface waters: The export coefficient modelling approach. *Journal of Hydrology,* **183**, 323-349

Johnes P, Moss B and Phillips G, 1994 *Lakes – Classification and Monitoring*. NRA Research and Development Note 253. National Rivers Authority, Bristol

Johnes P J, Bennion H, Curtis C, Moss B, Whitehead P and Patrick S, 2000 *Trial classification of lake water quality in England and Wales*. Research and Development Project Record E2-i721/5. Environment Agency, Bristol: 241 pp

Johnson R K, 1989 Classification of profundal choronmid communities in oligotrophic/humic lakes of Sweden using environmental data. *Acta Biol. Debrecina Oceol. Hungarica,* **3**, 167-175

Johnson R K 2000 *RIVPACS and alternative statistical modelling techniques: accuracy and soundness of principles*. In "Assessing the biological quality of fresh waters. RIVPACS and other techniques". Proceedings of an International Workshop, Oxford, September 1998 (Ed. J F Wright, D W Sutcliffe and M T Furse), pp.323-332. Freshwater Biological Association, Ambleside

Johnson R K, Wiederholm T and Rosenberg D M, 1993 *Freshwater Biomonitoring using individual organisms, populations, and species assemblages of benthic macroinvertebrates*. In "Freshwater biomonitoring and benthic macroinvertebrates" (ed. Rosenberg and Resh)**,** 40-158

Johnston A M, Scrimgeour C M, Henry M O and Handley L L, 1999 Isolation of NO_3-N as 1-phenylazo-2-naphthol (Sudan-1) for measurement of δ15N. *Rapid Communications in Mass Spectrometry*, **13**, 1531-1534

Johnston C S, 1971/1972 Macroalgae and their environment. *Proceedings of the Royal Society Edinburgh*, **71**, 195-207

Johnston N T, Perrin C J, Slaney P A and Ward B R, 1990 Increased juvenile growth by whole-river fertilisation. *Canadian Journal of Fisheries and Aquatic Sciences*, **47**, 862-872

Jones J R, Smart M M and Burroughs J N, 1984 Factors related to algal biomass in Missouri Ozard streams. *Verhandlingen der Internationalen Vereinigung fur theorestische und angewandte Limnolologie*, **22,** 1867-1875

Jones R A and Lee G F, 1978 *Evaluation of the elutriate test as a method of predicting contaminant release during open water disposal of dredged sediment and environmental impact of open water dredged material disposal (Vol 1: Discussion).* Technical report D-78-45. US Army Engineer, Waterways Experiment Station, Vicksburg, MS, USA

Jones V J, Battarbee R W, Rose N L, Curtis C, Appleby P G, Harriman R and Shine A, 1997 Evidence for pollution of Loch Ness from the analysis of its recent sediments. *Science of the Total Environment,* **203**, 37-49

Jonker R, Groben R, Tarran G, Medlin L, Wilkins M, Garcia L, Zabala L and Boddy L, 2000 Automated identification and characterisation of microbial populations using flow cytometry: the AIMS project. *Scientia Marina,* **64**, 225-234

Jordan P, Rippey B and Anderson N J, 2001 Modeling diffuse phosphorus loads from land to freshwater using the sedimentary record. *Environmental Science and Technology*, **35**, 815-819

Jordan S, Carmichael J and Richardson B, 1992 *Habitat measurements and index of biotic integrity based on fish sampling in northern Chesapeake Bay*. A Proceedings: Estuarine and Near Coastal Bioassessment and Biocriteria Workshop, Annapolis, Maryland, USEPA, Office of Science and Technology, Washington DC

Jordan T E, Correll D L, Miklas J and Weller D E, 1991 Nutrients and Chlorophyll at the Interface of a Watershed and an Estuary. *Limnology and Oceanography,* **36**, 251-267

Jørgensen B B, 1982 Mineralisation of organic matter in the sea bed - the role of sulphate reduction. *Nature,* **296**, 643-645

Jorgensen G F, 1978 Use of bioindices as a tool for water quality analysis in rivers. Internationale Vereinigung fur Theorestische und Angewantdte Limnologie Verhandlungen, **20,** 1772-1778

Josselyn M N and West J A, 1985 The distribution and temporal dynamics of the estuarine macroalgal community of San Francisco Bay. *Hydrobiologia*, **129,** 139-152

Juggins S, 1992 Diatoms in the Thames estuary, England: ecology, paleoecology and salinity transfer function. *Bibliotheca Diatomologica*, **25**, 216 pp

Kamermans P, Malta E-J, Verschuure J M, Lentz L F and Schrijvers L, 1998 Role of cold resistance and burial for winter survival and spring initiation of an Ulva spp. (Chlorophyta) bloom in a eutrophic lagoon (Veerse Meer lagoon, The Netherlands*).* *Marine Biology***, 131**, 45-51

Kamermans P, Verschuure J M and Rijstenbil J W, 1996 *De kwaliteit van het Veerse Meer en de zeesla-bloci.* Raporten en verslagen. Vol. 2. Nederlands Instituut voor Oecologisch onderzoek centrum voor Estuariene en Mariene Oecologie (NIOO-CEMO), Yerseke

Kangas P and Niemi A, 1985 Observations on recolonisation by the bladder wrack *Fucus vesiculosus* on the southern coasts of Finland. *Aqua Fennica*, **15**, 133-141

Kangas P, Autio H, Hallfors G, Luther H, Niemi A and Salemaa H, 1982 A general model of the decline of *Fucus vesiculosus* at Trarminne, south coast of Finland, in 1977-81. *Acta Botanica Fennica*, **118**, 1-27

Kansanen P H, Aho J and Paasivirta L, 1984 Testing the benthic lake type concept based on chironomid associations in some Finnish lakes using multivariate statistical methods. *Annales Zoologici Fennici,* **21**, 55-76

Kansanen P H, Paasivirta L and Vayrynen T, 1990 Ordination analysis and bioindices based on zoobenthos communities used to assess pollution of a lake in southern Finland. *Hydrobiologia,* **202**, 153-170

Karr J R and Chu E W, 1997 Biological monitoring: Essential foundation for ecological risk assessment. *Human and Ecological Risk Assessment*, **3,** 933-1004

Karr J R and Chu E W, 1999 *Restoring life in running waters: Better biological monitoring*. Island Press, Washington, DC

Karr J R and Dudley D R, 1981 Ecological perspectives on water quality goals. *Environmental Management,* **5,** 55-68

Karr J R, Fausch K D, Angermeier P L, Yant P R and Schlosser I J, 1986 A*ssessing Biological Integrity in Running Waters: a Method and Its Rationale.* Illinois Natural History Survey Special Publication 5, Champaign, IL

Karr J R, Fausch K D, Angermeier P L, Yant P R and Schlosser I J, 1991 Biological integrity: A long-neglected aspect of water resource management. *Ecological Applications*, **1,** 66-84

Kautsky H, Kautsky L, Kautsky N, Kautsky V and Lindblad C, 1992 Studies on the *Fucus vesiculosus* community in the Baltic Sea. Acta Phytogeographica Suecica, **78**, 33-48

Kautsky N, Kautsky H, Kautsky U and Waern M, 1986 Decreased depth penetration of *Fucus vesiculosus L.* since the 1940s indicates eutrophication of the Baltic Sea. *Marine Ecology Progress Series*, **28**, 1-8

Keller A A, Doering P H, Kelly S P, Sullivan B K, 1990 Growth of juvenile Atlantic menhaden, *Brevortia tyrannus* (Pieces: Clupeidae) in MERL mesocosms: effects of eutrophication. *Limnol Oceanogr,* **35,** 109-122

Kelly M 1989 Monitering water pollution: the role of plants. *Plants Today* (May June), 96-100

Kelly M G, 1996a *Diatoms as tools for water quality managers*. Environment Agency, R&D Technical Report E3. 21 pp

Kelly M G, 1996b *The Trophic Diatom Index. A Users Manual*. Environment Agency, R&D Technical Report E2. 148 pp

Kelly M G, 1996c *Use of Diatoms to Monitor Nutrients in Rivers*. Environment Agency, R&D project record E1/i681/5 (or 431). 52 pp

Kelly M G, 1996d *The Trophic Diatom Index*. EA R&D Technical Report E3

Kelly M G, 1998a Use of community-based indices to monitor eutrophication in European rivers. *Environmental Conservation,* **25**, 22-29

Kelly M G, 1998b Use of the trophic diatom index to monitor eutrophication in rivers. *Water Research,* **32**, 236-242

Kelly, M and Whitton B A, 1993 *Survey Methodology for algae and other phototrophs in small rivers*. National Rivers Authority, R&D Note 278.(abst.)

Kelly M G and Whitton B A, 1995a *Plants for monitoring rivers: report of a workshop held at Hatfield College, University of Durham, September 1994*. National Rivers Authority R&D Note 366

Kelly M G and Whitton B A, 1995b The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. *Journal of Applied Phycology*, **7**, 433-444

Kelly M G & Whitton B A, 1996 *Use of Diatoms to Monitor Nutrients in Rivers*. Environment Agency, R&D Note 431. 52 pp

Kelly M G and Whitton B A, 1998 Biological monitoring of eutrophication in rivers. *Hydrobiologia*, **384,** 55-67

Kelly M G, Whitton B A and Lewis A, 1996 *Use of algae to monitor eutrophication in UK* rivers. In Use of Algae for Monitoring Rivers, edited by Whitton, BA, Rott, E and Friedrich G, Institut für Botanik, Universität Innsbruck, 79-87.

Kelly M G, Adams C, Graves A C, Jamieson J, Krokowski J, Lycett E B, Murray-Bligh J, Pritchard S and Wilkins C, 2001 *The Trophic Diatom Index*: *A Users Manual.* Revised Edition. R&D Technical Report E2/TR2, Bristol: Environment Agency

Kelly M G, Cazaubon A, Coring E, Dell' Uomo A, Ector L, Goldsmith B, Guasch H, Hurlimann J, Jarlman A, Kawecka B, Kwandrans J, Laugaste R, Lindstrom E A, Leitao M, Marvan P, Padisak J, Pipp E, Prygiel J, Rott E, Sabater S, van Dam H and Vizinet J, 1998 Recommendations for the routine sampling of diatoms for water quality assessments in Europe. *Journal of Applied Phycology,* **10**, 215-224

Kemp W M, Boynton W R, Twilley R R, Stevenson J C and Means J C, 1983 The decline of submerged vascular plants in Upper Chesapeake Bay: summary of the results concerning possible causes. *Marine Society Technology Journal*, **17**, 78-89

Kennison G C B, Dunsford D S, Schutten J, 1998 Stable or Changing Lakes? A classification of aquatic macrophyte assemblages from a eutrophic shallow lake system in the United Kingdom. *Aquatic Conservation and Freshwater Ecosystems*, **8**, 669-684

Kentucky Department of Environmental Protection (KDEP), 1993 *Methods for assessing biological integrity of surface waters*. Kentucky Department of Environmental Protection, Division of Water, Frankfort, Kentucky

Kerans B L and Karr J R, 1994 A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee Valley. *Ecological Applications*, **4,** 768-785

Kerans B L, Karr J R and Ahlstedt S A, 1992 Aquatic invertebrate assemblages: Spatial and temporal differences among sampling protocols. *Journal of the North American Benthological Society*, **11,** 377-390

Kerner M, Gramm H, 1995 Changes in oxygen consumption at the sediment-water interface formed by settling seston from the Elbe estuary. *Limnol Oceanogr,* **40**, 544-555

Kern-Hansen U and Dawson F H, 1978 *The standing crop of aquatic plants of lowland streams in Denmark and the inter-relationships of nutrients in plant, sediment and water*. Proceedings of the 5th European Weed Research Council Symposium on Aquatic Weeds 143-150.

Kesminas V and Virbickas T, 1995a *The biotest for estimation of rivers fish community state. In: Fish and Land/Inland Ecotones*. UNESCO/MAB, University of Lodz International Symposium

Kesminas V and Virbickas T, 1995b *Fish production in ecotones of three Lithuanian rivers of different pollution level.* In Fish and Land/Inland Ecotones. UNESCO/MAB: University of Lodz International Symposium

Khedr A H A and El-Demerdash M A, 1997 Distribution of aquatic plants in relation to environmental factors in the Nile Delta. *Aquatic Botany*, **56**, 75-86

Khoo K H, Culberson C H, Bates R G, 1977 Thermodynamics of the dissociation of ammonium ion in seawater from 5 to 40°C. *J Soln Chem,* **6**, 281-290

Khoruzhaya T A, 1989 Prospects for the use of biochemical indicators in biomonitoring of natural waters. *Hydrobiological Journal,* **25,** 48-52

Kiffney P M and Richardson J S, In press *Effects of nutrient enrichment on periphyton and vertebrate (Ascaphus truei) and invertebrate grazers in experimental channels.* Copeia. North West Fisheries Science Center Environmental Conservation Division - Watershed Program

Kiirikki M and Blomster J, 1996 Wind induced upwelling as a possible explanation for mass occurrences of epiphytic Ectocarpus siliculosus *(Phaeophyta)* in the northern Baltic Proper. *Marine Biology*, **127**, 353-358

Klavestad N, 1978 The marine algae of the polluted inner part of the Oslo-fjord. A survey carried out 1962-1966. *Botanica Marina*, **21**, 71-97

Klein A W O and van Buuren J T, 1992 *Eutrophication of the North Sea in the Dutch coastal zone.* Report to Directorate-Generaal for Public Works and Water Management, Tidal Waters Division, The Hague. Report No DGW-92.003

Klemm D J and Lazorchak J M, 1994 *Environmental monitoring and assessment program - surface waters and Region 3 regional environmental monitoring and assessment program. Pilot field operation and methods manual for streams.*

EPA/620/R-94/004, Environmental Monitoring Systems Lab, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio

Klemm D J, Lewis P A, Fulk F, Lazorchak J M, 1990 *Macroinvertebrate field and laboratory methods for evaluating the biological integrity of surface waters.* EPA-600-4-90-030, US Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio

Kleynhans C J, 1999 The development of a fish index to assess the biological integrity of South African rivers. *Water South Africa*, **25**, 265-278

Kliffmuller R, 1962 Deer Anstieg des Phosphat-Phosphors als Ausdruck fortschreitender Eutrophierung im Bodensee. *Internationale Revue der Gesamten Hydrobiologie und Hydrographie*, **47**, 118-122

Knepp G L, Arkin G F, 1973 Ammonia toxicity levels and nitrate tolerance of channel catfish. *Prog Fish Cult,* **35**, 221-224

Kohler A, Wonneberger R and Zeltner G, 1973 Chemical data and aquatic vascular plants as indicators for pollution in the Moosach river system near Munich. *Archiv fur Hydrobiologia,* **72**, 533-549

Kohn N P, Word J Q, Niyogi D K, Ross L T, Dillon T and Moore D W, 1994 Acute toxicity of ammonia to four species of marine amphipod. *Mar Environ Res*, **38**, 1-15

Kolkwitz R and Marsson M, 1909 Okologie des tierischen Saprobien. Beitrage zur Lehre von des biologischen Gewasserberurteilung. *Internationale Revue der Gesamten Hydrobiologie und Hydrographie,* **2**, 126-152

Korhola A and Blom T, 1996 Marked early 20th century pollution and the subsequent recovery of Toolo Bay, central Helsinki, as indicated by subfossil diatom assemblage changes. *Hydrobiologia*, **341**, 169-179

Kosmala A, Charvet S, Roger M C and Faessel B, 1999 Impact assessment of a waste water treatment plant effluent using instream invertebrates and the Ceriodaphnia dubia chronic toxicity test. *Water Research*, **33,** 266-278

Kratzer C R, Brezonik P L, 1981 A Carlson-type trophic state index for nitrogen in Florida lakes. *Water Res. Bull.*, **17**, 713-15

Krienitz L, Kasprzak P and Koschel R, 1996 Long term study on the influence of eutrophication, restoration and biomanipulation on the structure and development of phytoplankton communities in Feldberger Haussee Baltic Lake District, Germany. *Hydrobiologia*, **330**, 89-110

Kross B C, Ayebo A D, and Fourtes L J, 1992 Methemoglobinemia: nitrate toxicity in rural America. *Am Fam Physician*, **46**, 183-188

Lachavanne J B, Juge R, Noetzlin A and Perfetta J, 1985 Ecological and chorological study of Swiss lake aquatic plants: a basic method to determine the bioindicator value of species. *Verhandlungen der internationale Vereinigung fur theoretische und angewandte Limnologie*, **24**, 943-948

Ladle M, House W A, Armitage P D and Farr I S, 1989 Faunal characteristics of a site subject to sewage plant discharge. *Tenside Detergents*, **2,** 159-168

Ladson A R, White L J, Doolan J A, Hart B T, Lake P S and Tilleard J W, 1999 Development and testing of an Index of Stream Condition for waterway management in Australia. *Freshwater Biology*, **41,** 453-468

Lagler K F, 1949 *Studies in freshwater fisheries biology*. Ann Arbor: University of Michigan

Lamarra V A, 1975 Digestive activities of carp as a major contributor to the nutrient loading of lakes. *Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie*, **19**, 2461-2468

Lampert W and Sommer U, 1997 *Limnoecology: the ecology of lakes and streams*. New York: Oxford University Press

Lang C, 1985 Eutrophication of Lake-Geneva Indicated by the Oligochaete Communities of the Profundal. *Hydrobiologia,* **126**, 237-243

Lang C, 1998 Using oligochaetes to monitor the decrease of eutrophication: the 1982-1996 trend in Lake Geneva. *Archiv Fur Hydrobiologie,* **141**, 447-458

Lang C and Lang-Dobler, 1980 *Structure of tubificid and lumbriculid worm communities, and three indices of trophy based upon these communities, as descriptors of eutrophication level of Lake Geneva (Switzerland).* In Aquatic Oligochaete Biology (ed. R Brinkhurst and D G Cook). New York: Plenum Press

Lang C and Reymond O, 1992 Reversal of Eutrophication in Lake Geneva - Evidence from the Oligochaete Communities. *Freshwater Biology,* **28**, 145-148

Lang C and Reymond O, 1996 Empirical relationships between oligochaetes, phosphorus and organic deposition during the recovery of Lake Geneva from eutrophication. *Archiv Fur Hydrobiologie,* **136**, 237-245

Lange C R and Lambert K E, 1995 *Biomonitoring*. Water Environment Research, **67,** 738-749

Lange-Bertalot H, 1979 Pollution tolerance of diatoms as a criterion for water quality estimation. *Nova Hedwidia, Beiheft,* **64**, 285-303

Lapointe B E, Tomasko D A and Matzie W R, 1994 Eutrophication and trophic state classification of seagrass communities in the Florida Keys. *Bulletin of Marine Science*, **54**, 696-717

Larsen D P, Stevens D L, Selle A R and Paulsen S G, 1991 Environmental Monitoring and Assessment Program-Surface Waters: a northeast lake pilot. *Lake and Reservoir Management*, **7**, 1-11

Lavery P S and McComb A J, 1991 Macroalgal-sediment nutrient interactions and their importance to macroalgal nutrition in a eutrophic estuary. *Estuarine Coastal and Shelf Science*, **32**, 281-295

Lazaridou E, Orfanidis S, Haritonidis S and Seferlis M, 1997 Impact of eutrophication on species composition and diversity of macrophytes in the Gulf of Thessaloniki, Macedonia, Greece: First evaluation of the results of a one year study. *Fresenius Environmental Bulletin*, **6**, 54-59

Le Bozec S, 1993 Green tides on the Brittany shore: The case of the Bay of Saint-Brieuc. *The Phycologist*, **34**, 23

Le Cren E D, Kipling C and McCormack J C, 1967 A study of the numbers, biomass and year class strengths of Perch (*Perca fluviatilis L.*) in Windermere from 1941-1966. *Journal of Animal Ecology,* **46**, 281-307

Le Cren E D, Kipling C and McCormack J C, 1974 Windermere: effects of exploitation and eutrophication on the salmonid community. *Journal of the Fisheries Research Board of Canada*, **29**, 819-832

Le Pape O, Del Amo Y, Menesguen A, Aminot A, Queguiner B and Treguer P, 1996 Resistance of a coastal ecosystem to increasing eutrophic conditions: Bay of Brest (France), a semi-enclosed zone of Western Europe. *Continental Shelf Research,* **16**, 1885-1907

Leach J H, Johnson M G, Kelso J R M, Hartmann J, Numann W and Entz B, 1977 Responses of percid fish and their habitats to eutrophication. *Journal of the Fisheries Research Board of Canada*, **34**, 1964-1971

Leaf S S and Chatterjee R, 1999 Developing a strategy on eutrophication. *Water Science and Technology*, **39,** 307-314

Leclercq L, Rosillon F and VanderBorght P, 1996 Chemical and biological quality of the Belgian Semois Basin. *Bulletin Francais de la peche et de la pisciculture*, **341-342,** 81-108

Lee G F, Jones R A, Saleh F Y, Mariani G M, Homer D H, Butler J S and Bandyopadhyay P, 1978 *Evaluation of the elutriate test as a method of predicting contaminant release during open water disposal of dredged sediment and environmental impact of open water dredged material disposal (Vol II: Data Report)*. Technical report D-78-45. US Army Engineer, Waterways Experiment Station, Vicksburg, MS, USA

Lehmann A, Lachavanne B, 1999 Changes in the water quality of Lake Geneva indicated by submerged macrophytes. *Freshwater Biology*, **42**, 457-466

Lek S, Dimopoulos I and Fabre A, 1996 Predicting phosphorus concentration and phosphorus load from watershed characteristics using backpropagation neural networks. *Acta Oecologica-International Journal of Ecology,* **17**, 43-53

Lemley D A, 1982 Modification of benthic insect communities in polluted streams: combined effects of sedimentation and nutrient enrichment, *Hydrobiologia*, **87**, 229-245

Lenat D R, Smock L A and Penrose D L, 1980 *Use of benthic macroinvertebrates as indicators of environmental quality*. In Biological Monitoring for Environmental Effects, (ed. DL Worf)**,** pp. 97-112. DC Heath, Lexington, MA

Lenat D R, Smock L A and Penrose D L, 1993 A biotic index for the southeastern United States: Derivation and list of tolerance values, with criteria for assigning water-quality ratings. *Journal of the North American Benthological Society*, **12,** 279-290

Leonard P M and Oth D J, 1986 Application and testing of an index of biotic integrity in small, coolwater streams. *Transactions of the American Fisheries Society*, **115,** 401-414

Letts E A and Richards E H, 1911 *Report on green seaweeds (and especially* Ulva latissima*) in relation to the pollution of the waters in which they occur.* Royal Commission on sewage disposal 7th Report, Vol 11, Appendix III, HMSO, London

Leuven R S E W, van der Velde G, Kersten H L M, 1992 Interrelations between Ph and other physico-chemical factors of Dutch soft waters. *Arch. Hydrobiol.* **126**, 27-51

Levine H R and Wilce R T, 1980 *Ulva lactuca as a bioindicator of coastal water quality.* Water Resources Research Centre, University of Massachusetts, Amherst, MA. 83pp.

Lewis J W and Hanna N S, 1995 Responses of electric fish (family Mormyridae) to inorganic nutrients and tributyltin oxide. *Chemosphere,* **31**, 3753-3769

Lewis M, 1996 *Palaeolimnology of the Roman Wall Loughs*. Unpublished BSc Hons Geography Dissertation, University of Newcastle

Lewis W M and Morris D P, 1986 Toxicity of nitrite to fish: a review. *Trans Am Fish Soc,* **115**, 183-199

Likens G E, 1975 *Primary production of inland aquatic ecosystems.* In Primary Production of the Biosphere (ed. H Leith and R R Whittaker), pp. 185-202. Springer-Verlag, New York

Lin H P, Thuet P, Trilles J P, Mounet-guillaume R and Charmantier G, 1993 Effects of ammonia on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus*. *Mar Biol*, **117**, 591-598

Lin J and Hines A H, 1994 Effects of suspended food availability on the feeding mode and burial depth of the Baltic clam *Macoma balthica*. *Oikos*, **69**, 28-36

Lindegaard C, 1995 *Classification of water-bodies and pollution*. In The Chironomidae: Biology and Ecology of Non-Biting Midges (ed. P D Armitage, P S Cranston and L C V Pinder), pp. 385-404. London: Chapman and Hall

Line J M, ter Braak C J F and Birks H J B, 1994 WACALIB version 3.3 - a computer program to reconstruct environmental variables from fossil diatom assemblages by weighted averaging and to derive sample-specific errors of prediction. *Journal of Paleolimnology,* **10**, 147-152

Lippemeier S, Hintze R, Vanselow K H, Hartig P and Colijn F, 2001 In-line recording of PAM fluorescence of phytoplankton cultures as a new tool for studying effects of fluctuating nutrient supply on photosynthesis. *European Journal of Phycology,* **36**, 89-100

Little C and Smith L P, 1980 Vertical zonation on rocky shores in the Severn Estuary, UK. *Estuarine Coastal and Marine Science*, **11**, 651-670

Little J L, Hall R I, Quinlan R and Smol J P, 2000 Past trophic staus and hypolimnetic anoxia during eutrophication and remediation of Gravenhurst Bay, Ontario: comparison of diatoms, chironomids, and historical records. *Canadian Journal of Fisheries and Aquatic Sciences,* **57**, 333-341

Loeb S L and Spacie A, 1994 *Biological monitoring of aquatic systems*. 400, Boca Raton: Lewis Publishers

Loftus K H and Regier H A, 1972 Eds Symposium on salmonid communities in oligotrophic lakes. *Journal of the Fisheries Research Board of Canada*, **29**, 613-986

Lopes R J, Pardal M A and Marques J C, 2000 Impact of macroalgal blooms and wader predation on intertidal macroinvertebrates: experimental evidence from the Mondego estuary (Portugal). *J Exp Mar Biol Ecol*, **249**, 165-179

Lotter A F, Birks H J B, Hofmann W and Marchetto A, 1998 Modern diatom, cladocera, chironomid, and chrysophyte cyst assemblages as quantitative indicators for the reconstruction of past environmental conditions in the Alps. II. Nutrients. *Journal of Paleolimnology*, **19**, 443-463

Lotze H K, Schramm W, Schories D and Worm B, 1999 Control of macroalgal blooms at early developmental stages*: Pilayella littoralis* versus *Enteromorpha* spp. *Oecologia*, **119**, 46-54

Lowthion D, Soulsby P G and Houston M C M, 1985 Investigation of a eutrophic tidal basin. 1. Factors affecting the distribution and biomass of macroalgae. *Marine Environmental Research*, **15**, 263-284

Lucas L V, Koseff J R, Monismith S G, Cloern J E and Thompson J K, 1999 Processes governing phytoplankton blooms in estuaries. II: The role of horizontal transport. *Marine Ecology-Progress Series,* **187**, 17-30

Lucas M C and Marmulla G, 2000 *An assessment of anthropogenic activities on and rehabilitation of river fisheries: current state and future direction*. In Management and ecology of river fisheries (ed. I G Cowx), pp. 261-278. Oxford: Fishing News Books

Lund J W G, 1950 Studies on *Asterionella formosa* Hass. II. Nutrient depletion and the spring maximum. *Journal of Ecology,* **38**, 1-35

Lund J W G, 1961 The algae of the Malham Tarn district. *Field Studies,* **1**, 85-115

Lycett E, 1997 *Assessment of the effects of Marlborough sewage treatment works on the river Kennet using the Trophic Diatom Index*. Unpublished undergraduate training report, Department of Zoology, University of Wales, Cardiff, 78pp

Lyngby J E, Mortensen S and Ahrensberg N, 1999 Bioassessment techniques for monitoring of eutrophication and nutrient limitation in coastal ecoststems. *Marine Pollution Bulletin*, **39**, 212-223

Lyons J, 1992 *Using the index of biotic integrity (IBI) to measure environmental quality in warm water streams of Wisconsin.* General Technical Report NC-149, US Department of Agriculture, Forest Service, St Paul, Minnesota

Lyons J, Wang L and Simonson T D, 1996 Development and Validation of an Index of Biotic Integrity for Coldwater Streams in Wisconsin. *North American Journal of Fisheries Management*, **16,** 241-256

Macdonald A M, Edwards A C, Pugh K and Balls P W, 1994 *The impact of land use on nutrient transport into and through three rivers in the north east of Scotland*. In Integrated River Basin Development (ed. C Kirby and W R White), pp. 201-214. Wiley

MacGarvin M, 1995 *Marine eutrophication in the UK: A discussion document*. Report to the World Wide Fund for Nature UK. 23pp

MacGarvin M, 2000 *Scotland's Secret?* Aquaculture, nutrient pollution eutrophication and toxic blooms, pp. 1-21: WWF Scotland

Mackay D W, Holmes P J and Redshaw C J, 1989 The application of bioassay techniques to water pollution problems- the United Kingdom experience. *Hydrobiol*, **188/189**, 77-86

Madsen T V and Sand-Jensen K, 1991 Photosynthetic carbon assimilation in aquatic macrophytes. *Aquat. Bot*, **41** 5-40

MAFF, 1992 *The EC Nitrates Directive*. Information note produced by the Environmental Protection Division, as part of NSA Pilot Scheme Information Pack, January 1992

Maidstone C, Gulson J and Parr W, undated *Phosphates in Freshwater – Standards for n*ature *conservation*. English Nature Research Reports No. 73, 130pp

Mainstone C P, Parr W and Day M, 2000 *Phosphorus and river ecology*, Report to English Nature and the Environment Agency, 1 85716 505 5, English Nature

Maitland P S, 1962 The fauna of the River Endrick in relation to local water use. *Water and Waste Treatment*, **1962**, 78-86

Maitland P S, 1966 *The fauna of the River Endrick*. Glasgow: Blackie

Maitland P S, 1972 Loch Lomond: man's effects on the salmonid community. *Journal of the Fisheries Research Board of Canada*, **29**, 849-860

Maitland P S, 1984 The effects of eutrophication on aquatic wildlife. *Institute of Terrestrial Ecology Symposium*, **13**, 101-108

Maitland P S, 1987 Fish in the Clyde and Leven systems - a changing scenario. *Proceedings of the Institute of Fisheries Management Conference,* **1987**, 13-20

Maitland P S, 1990 *The biology of fresh waters*. Glasgow: Blackie

Maitland P S, 1992 The status of Arctic Charr, *Salvelinus alpinus* L., in southern Scotland: a cause for concern. *Freshwater Forum*, **2**, 212-227

Maitland P S and Campbell R N, 1992 *Freshwater fishes of the British Isles*. London: HarperCollins

Maitland P S and Hudspith, P M G, 1974 The zoobenthos of Loch Leven, Kinross, and estimates of its production in the sandy littoral area during 1970 and 1971. *Proceedings of the Royal Society of Edinburgh,* **74**, 219-239

Maitland P S and Lyle A A, 1991 Conservation of freshwater fish in the British Isles: the status and biology of threatened species. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **1**, 25-51

Maitland P S and Lyle A A, 1996 The smelt in Scotland. *Freshwater Forum,* **6**, 57-68

Maitland P S and Morgan N C, 1997 *Conservation management of freshwater habitats - Lakes, rivers and wetlands.* London: Chapman and Hall

Malone T C, Conley D J, Fisher T R, Glibert P M, Harding L W and Sellner K G, 1996 Scales of nutrient-limited phytoplankton productivity in Chesapeake Bay. *Estuaries,* **19**, 371-385

Marchese M and de Drago IE, 1999 Use of benthic macroinvertebrates as organic pollution indicators in lotic environments of the Parana River drainage basin. *Polskie Archiwum Hydrobiologii*, **46,** 233-256

Maristo L, 1941 Die Seetypen Finnlands auf floristischer und vegetationsphysiognomischer Grundlage. *Ann. Bot. Soc. Zool. – Bot. Fenn. Vanamo*, **15**(5), 1-312

Marshall K E, 1993 *The literature of biomonitoring*. In Freshwater biomonitoring and benthic macroinvertebrates (ed. Rosenberg and Resh), 28-39

Marshall H G and Alden R W, 1990 A Comparison of Phytoplankton Assemblages and Environmental Relationships in 3 Estuarine Rivers of the Lower Chesapeake Bay. *Estuaries,* **13**, 287-300

Marshall H G and Alden R W, 1993 A Comparison of Phytoplankton Assemblages in the Chesapeake and Delaware Estuaries (USA), with Emphasis on Diatoms. *Hydrobiologia,* **269**, 251-261

Martens H and Naes T, 1989 *Methods for calibration*. In Multivariate Calibration (ed. H Martens and T Naes), pp. 73-85. Wiley, Chichester

Martin A, 2002 *The ecology and palaeoecology of the charophyte Lamprothamnium papulosum in UK coastal lagoons*. Unpublished PhD thesis, University of London.

Martin A and Carvalho L, submitted *Conservation of the rare charophyte Lamprothamnium papulosum*. Aquatic Conservation: Marine and Freshwater Ecosystems

Martinez-Arroyo A and Romero J, 1990 *Some features of* Ulva *spp. : Photosynthesis and nutrient uptake in a semi estuarine embankment.* Presentation made at the 25th European Marine Biology Symposium, Sept. 10-15, 1990, Ferrara, Italy

Mason C F, 1981 *Biology of freshwater pollution,* Longmans, London

Massachusetts Coastal Zone Management. The Coastal Wetlands Ecosystem Protection Project. www.state.ma.us/czm/wastart.htm

Matthews R A, Buikema A L, Cairns J and Rodgers J H, 1982 Biological monitoring. Part IIA. Receiving system functional methods, relationships and indices. *Water Research*, **16,** 129-139

Matthews W J, Hough D J and Robison H W, 1992 Similarities in fish distribution and water-quality patterns on streams of Arkansas - congruence of multivariate analyses. *Copeia*, **2**, 296-305

Maude S H and Di Maio J, 1999 Benthic macroinvertebrate communities and water quality of headwater streams of the Oak Ridges Moraine, southern Ontario. *Canadian Field-Naturalist*, **113,** 585-597

Maxwell H, 1897 *Memories of the months. First series*. London: Maclehose

Maybeck, 1982 Carbon, nitrogen and phosphorus transport by world rivers. *American Journal of Science*, **282,** 401-450

McBride G B, 1985 *The role of monitoring in the management of water resources.* In Biological Monitoring in Freshwaters: Proceedings of a Seminar, Hamilton, November 21-23, 1984 Part I, (ed. R D Pridmore and A B Cooper) Water & Soil Miscellaneous Publications No 82, p7-16, National Water and Soil Conservation Authority, Wellington, NZ

McCarthy J J and Goldman C R, 1979 Nitrogenous nutrition of marine phytoplankton in nutrient-depleted waters. *Science,* **203**, 670-672

McComb A J and Davis J A, 1993 Eutrophic waters of southwestern Australia. *Fertiliser Research*, **36**, 105-114

McComb A J, Atkins R P, Birch P B, Gordon D M and Lukatelich R J, 1981 *Eutrophication in the Peel-Harvey Estuarine System, Western Australia*. In Estuaries and Nutrients. (ed. B J Neilson and L E Cronin), Humana Press, New Jersey

McComb A J, Qiu S, Lukatelich R J and McAuliffe T F, 1998 Spatial and temporal heterogeneity of sediment phosphorus in the Peel-Harvey Estuarine System. *Estuarine Coastal and Shelf Science*, **47**, 561-577

McElravy E P, 1988 *Temporal variability in abundance of aquatic insects: a comparison of temperate and tropical environments*. PhD Dissertation, University of California, Berkley, CA

McElravy E P, Lamberti G A and Resh V H, 1989 Year-to-year variation in the aquatic macroinvertebrate fauna of a northern California stream. *Journal of the North American Benthological Society,* **8,** 51-53

McGowan S, Britton G, Haworth E and Moss B, 1999 Ancient blue-green blooms. *Limnology and Oceanography,* **44**, 436-439

McGuire D L, 1990 *Aquatic Macroinvertebrate Surveys in the Clark Fork River.* 1986 60 19888, ibscore.dbs.umt.edu/clarkfork/Past_Proceedings/1990_proceedings/mcguire/McGuire.htm

McGuire D L, 1990 *Aquatic Macroinvertebrate Surveys in the Clark Fork River*. 1986 to 1988, http://ibscore.dbs.umt.edu/clarkfork/Past_Proceedings/1990_proceedings/mcguire/McGuire.htm

Mead M E and Watts S A, 1995 Toxicity of ammonia, nitrite and nitrate to juvenile Australian crayfish, *Cherax quadricarinatus*. *J Shellfish Res*, 14, 341-346

Meadows P S and Reid A, 1966 The behaviour of *Corophium votulator* (Crustacea: Amphipoda). *J Zool (Lond),* **150**, 387-399

Mee L D, 1992 The Black Sea in crisis: a need for concerted international action. *Ambio*, **21**, 278-286

Meeuwig J J, 1999 Predicting coastal eutrophication from land-use: an empirical approach to small non-stratified estuaries. *Marine Ecology-Progress Series,* **176**, 231-241

Meeuwig J J, Kauppila P and Pitkanen H, 2000 Predicting coastal eutrophication in the Baltic: a limnological approach. *Canadian Journal of Fisheries and Aquatic Sciences,* **57**, 844-855

Meeuwig J J, Rasmussen J B and Peters R H, 1998 Turbid waters and clarifying mussels: their moderation of empirical chl:nutrient relations in estuaries in Prince Edward Island, Canada. *Marine Ecology-Progress Series,* **171**, 139-150

Meijer M L, De Haan M W, Breukelaar A W and Buiteveld H, 1990a Is reduction of the benthivorous fish an important cause of light transparency following biomanipulation in shallow lakes? *Hydrobiologia,* **200/201**, 303-315

Meijer M L, Lammens E H R R, Raat A J P, Grimm M P and Hosper S H, 1990b Impact of cyprinids on zooplankton and algae in ten drainable ponds. *Hydrobiologia*, **191**, 275-284

Melzer A, 1988 *Die Gewasserbeurteilung bayerischer Seen mit Hilfe makrophytischer Wasserpflanzen Gefahrdung und Schutz Von Gewassern,* (Ed. Verlag Eugen Ulmer) 106-116. Universitat Hohenheim, Stuttgart

Menendez M and Comin F A, 2000 Spring and summer proliferation of floating macroalgae in a Mediterranean coastal lagoon (Tancada Lagoon, Ebro Delta, NE Spain). *Estuarine Coastal and Shelf Science*, **51**, 215-226

Menesguen A and Piriou J-Y, 1995 Nitrogen loadings and macroalgal *(*Ulva *sp.)* mass accumulation in Brittany (France). *Ophelia*, **42**, 227-237

Meriaux J-L, 1982 L'utilisation des macrophytes et des phytocoenoses aquatiques comme indicateurs de la qualite des eaux. *Les Naturalistes Belges*, **63**, 12-28

Merilainen, 1987 The profundal zoobenthos used as an indicator of the biological condition of Lake Paijanne. *Biological Research Report, University of Jvyaskyla,* **10**, 87-94

Metcalfe J L, 1989 Biological water quality assessment of running waters based on macroinvertebrate communities: history and present status in Europe. *Environmental Pollution,* **60,** 101-139

Middelburg J J and Nieuwenhuize J, 2000 Uptake of dissolved nitrogen in turbid, tidal estuaries. *Mar Ecol Prog Ser*, **192**, 79-88

Middleboe A L and Sand-Jensen K, 2000 Long-term changes in macroalgal communities in *a* Danish estuary. *Phycologia*, **39**, 245-257

Millbrink, 1979 Indicator communities of oligochaetes in Scandinavian lawes. *Verh. internat. Verein. Limnol.,* **20**, 2046

Millbrink, 1983 An improved environmental index based on the relative abundance of oligochaete species. *Hydrobiologia,* **102**, 89-97

Miller DC, Poucher S, Cardin J A and Hansen D, 1990 The acute and chronic toxicity of ammonia to marine fish and mysids. *Arch Environ Contam Toxicol*, **19**, 40-48

Mills C A, Heaney S I, Butterwick C, Corry J E and Elliott J M, 1990 Lake enrichment and the status of Windermere charr, Salvelinus alpinus L. *Journal of Fish Biology*, **37A**, 167-174

Mills K H, 1985 Responses of lake whitefish Coregonus clupeaformis to fertilisation of Lake 226, the Experimental Lakes Area. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 129-138

Miltner R J and Rankin E T, 1998 Primary nutrients and the biotic integrity of rivers and streams. *Freshwater Biology*, **40,** 145-158

Moestrup O, 1994 *Economic aspects: "blooms", nuisance species, and toxins*. In The Haptophyte algae (ed. J C Green and B S C Leadbeater), pp. 265-285. Oxford: Clarendon Press

Mol A, 1980 The role of invertebrate fauna in the biological assessment of water quality. *Hydrobiological Bulletin*, **14,** 222

Monbet Y, 1992 Control of Phytoplankton Biomass in Estuaries - a Comparative-Analysis of Microtidal and Macrotidal Estuaries. *Estuaries,* **15**, 563-571

Monitoring and Assessment Research Centre, 1985, *Historical Monitoring,* MARC Report No. 31, Monitoring and Assessment Research Centre, London

Montgomery H A C and Soulsby P G, 1981 Effects of eutrophication on the intertidal ecology of Langstone Harbour, UK and proposed control measures. *Progress in Water Technology*, **13**, 287-294

Montgomery H A, Soulsby P G, Hart I C and Wright S L, 1985 Investigations of a eutrophic tidal basin: Part 2 - nutrients and environmental aspects. *Marine Environmental Research*, **15**, 285-302

Moore D W, Bridges T S, Gray B R and Duke M B, 1997 Risk of ammonia toxicity during sediment bioassays with the estuarine amphipod *Leptocheirus plumulosus*. *Environ Toxicol Chem*, **16**, 1020-1027

Moreau S, Bertru G and Buson C, 1998 Season and spatial trends in nitrogen and phosphorus loads to the upper catchment of the river Vilaine (Brittany): relationships with land use. *Hydrobiologia,* **373/374**, 247-258

Moss B, 1976 The effects of fertilisation and fish on community structure and biomass of aquatic macrophytes and epiphytic algal production. An ecosystem experiment. *Journal of Ecology*, **64**, 313-342

Moss B, 1988 *Ecology of Freshwaters. Man and Medium*. Oxford: Blackwell Scientific

Moss B, 1998 *Ecology of Freshwaters: man and medium, past to future*. Oxford: Blackwell Science Ltd

Moss B, Beklioglu M, Carvalho L, Kilinc S, McGowan S and Stephen D, 1997 Vertically-challenged limnology; Contrasts between deep and shallow lakes. *Hydrobiologia,* **342**, 257-267

Moss B, Johnes P and Phillips G, 1996 The monitoring of ecological quality and the classification of standing waters in temperate regions: A review and proposal based on a worked scheme for British waters. *Biological Reviews of the Cambridge Philosophical Society,* **71**, 301-339

Moss B, McGowan S and Carvalho L, 1994 Determination of phytoplankton crops by top-down and bottom up mechanisms in a group of English lakes, the West Midland Meres. *Limnol. Oceanogr.*, **39**, 1020-1029

Moss B, Madgwick J and Phillips G, 1996b *A guide to the restoration of nutrient-enriched shallow lakes*. W.W. Hawes, UK, 168 pp

Moss D, Furse M T, Wright J F and Armitage P D, 1987 The prediction of the macroinvertebrate fauna of unpolluted running-water sites in Great Britain using environmental data. *Freshwater Biology*, **17,** 41-52

Moss D, Wright J F, Furse M T and Clarke R T, 1999 A comparison of alternative techniques for prediction of the fauna of running waters. *Freshwater Biology*, **41,** 167-181

Moss D, 2000 *Evolution of statistical methods in RIVPACS*. In Assessing the biological quality of freshwaters: RIVPACS and similar techniques (ed. J F Wright, D W Sutcliffe, and M T Furse), pp.25-38. Freshwater Biological Association, Ambleside

Mount D I, 1984 *The role of biological assessment in effluent control*. In Biological testing of effluents and receiving waters. Proceedings of an international workshop, Duluth, Minnesota, USA. pp. 15-30

Mount D I, Thomas N, Barbour M, Norberg T, Roush T and Brandes R, 1984 *Effluent and ambient toxicity testing and in-stream community response on the Ottawa River, Lima, Ohio,* EPA 600/3-84-080, Permits Division, Washington DC and Office of Research and Development, Duluth, Minnesota

Mulholland P J, Steinman A D, Palumbo A V, Elwood J W and Kirschtel D B, 1991 Role of nutrient cycling and herbivory in regulating periphyton communities in laboratory streams. *Ecology,* **72**, 966-982

Muller R, 1992 Trophic state and its implications for natural reproduction of salmonid fish. *Hydrobiologia,* **243**, 261-268

Munda I M, 1993 Changes and degradation of seaweed stands in the northern Adriatic. *Hydrobiologia*, **260/261**, 239-253

Munda I M, 1996 *The Northern Adriatic Sea*. In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (ed. W Schramm and P Neinhuis), pp. 369-402. Springer-Verlag, Berlin

Murphy K J and Ali M M, 1998 *Can functional groups improve on species assemblages as the basis of indicator schemes for trophic assessment of rivers?* Small Ecological Grants Reports, British Ecological Bulletin, 20

Murphy K J and Eaton J W, 1983 Effects of pleasure-boat traffic on macrophyte growth in canals. *Journal of Applied Ecology*, **20**, 713-729

Muscutt A D and Withers P J A, 1996 The phosphorus content of rivers in England and Wales. *Water Research*, **30,** 1258-1268

National Marine Monitoring Programme, 2001 *Proceedings of the NMMP Indicators Workshop, Edinburgh, May 2001.* http://www.marlab.ac.uk/NMPR/NMP.htm

National Rivers Authority, 1990 *Toxic Blue-green Algae*, pp. 125. London: National Rivers Authority

National Technical Information Service, Springfield VA USA, 1992, *Fisheries: Environmental Aspects - Bibliography Dec 1983-Sept 1989.* Selected Water Resources Abstracts Database, 56, Order No PB89-872881/GAR 106

Natural Environment Research Council, 1976 *Biological Surveillance*. (NERC Publication Series B, no. 18) Swindon: NERC. Environmental Change Network

Naumann, 1929 Einige neue Gesichtspunke zur Systematik der Gewassertypen. Mit besonderer Berucksichtigung der Seetypen. *Archiv Fur Hydrobiologie,* **20**, 191-198

Neal C, House W A, Whitton B A and Leeks G J L, 1998 Conclusions to special issue: water quality and biology of United Kingdom rivers entering the North Sea: the Land Ocean Interaction Study (LOIS) and associated work. *Science of the Total Environment*, **210-211,** 585-594

Neal C, Jarvie H P, Wade A J and Whitehead P G, 2001 (in press) Water quality functioning of lowland permeable catchments: inferences from an intensive study of the River Kennet and upper River Thames. *Science of the Total Environment*

Nedwell D B, 1982 Exchange of nitrate, and the end products of bacterial nitrate reduction, between seawater and sediment from a U.K. saltmarsh. *Estuar Coastal Shelf Sci*, **14**, 557-566

Nedwell D B and Trimmer M, 1996 Nitrogen fluxes through the upper estuary of the Great Ouse, England: the role of the bottom sediments. *Mar Ecol Prog Ser*, **142**, 273-286

Nedwell D B, Dong L F, Sage A and Underwood G J C, 2001 Variations of the nutrients loads to the mainland U.K. estuaries: correlation with catchment areas, urbanisation and coastal eutrophication. *Estuarine Coastal and Shelf Science*

Nedwell D B, Jickells T D, Trimmer M and Sanders R, 1999 *Nutrients in estuaries*. In Estuaries (ed. D B Nedwell, D G Raffaelli), pp. 43-92. Advances in ecological research 29. Academic Press, London

Nedwell D B, Walker T R, Ellis-Evans J C and Clarke A, 1993 Measurements of seasonal rates and annual budgets of organic carbon fluxes in an antarctic coastal environment at Signy Island, South Orkney Islands, suggest a broad balance between production and decomposition. *Appl Environ Microbiol*, **59**, 3989-3995

Nehring D, 1992 Eutrophication of the Baltic Sea. *Science Total Environment*, **1992**, 673-682

Neter J, Wasserman W and Kutner M H, 1990 *Applied Linear Statistical Models: Regression, Analysis of Variance, and Experimental Designs*. Irwin, Homewood, Illinois

Neundorfer J V and Kemp W M, 1993 Nitrogen versus phosphorus enrichment of brackish waters – responses of the submersed plant *Potamogeton perfoliatus* and its associated algal community. *Marine Ecology Progress Series*, **94**, 71-82

New Mexico Environment Department Surface Water Quality Bureau, 2000 *Nutrient Assessment Protocol for Streams*
http://www.nmenv.state.nm.us/swqb/NutrientAssessment Protocol.PDF

Newbold C, 1992 *The causes and effects of eutrophication in lakes and rivers - a nature conservation perspective*. Unpublished communication, Joint Nature Conservancy Council

Newbold C and Palmer M A, 1979 *Trophic Adaptations of Aquatic Plants*. CST Notes No. 18. Nature Conservancy Council, Peterborough

Newman P J, 1988 *Classification of Surface Water Quality: Review of Schemes Used in EC Member States*. Oxford: Heinemann Professional Publishing Ltd

Newman P J, Nixon S C and Rees Y J, 1994 Surface-water quality monitoring, classification, biological assessment and standards. *Water Science and Technology*, **30,** 1-10

Newman J R and Dawson F H, 1996 Guidance on Interpretation of the Mean Trophic Rank System for Assessment of Trophic Status of Rivers Using Macrophytes. Use of the MTR for the Purposes of The Urban Waste Water Treatment Directive. October 1996. Environment Agency R&D Progress Report E1/i694/03

Newman J R, Dawson F H and Rouen K J, 1997a *Using diatoms and macrophytes to assess the trophic status of rivers*. A workshop summary, Lancaster, 7-8 March 1996. R&D Progress Report E1/i694/01 to Environment Agency

Newman J R, Dawson F H and Rouen K J, 1997b *Using diatoms and macrophytes to assess the trophic status of rivers*. A report of a workshop, Lancaster, 7-8 March 1996. R&D Progress Report E1/i694/02 to Environment Agency, 12pp

Nicholls D J, Tubbs C R and Haynes F N, 1981 The effect of green algal mats on intertidal macrobenthic communities and their predators. *Kieler Meeresforchungen Sonderheft*, **5**, 511-520

Niell F X, Fernandez C, Figueroa F L, Figueiras F G, Fuentes J M, Perez-Llorens J L, Garcia-Sanchez M J, Hernandez I, Fernandez J A, Espejo M, Buela J, Garcia-Jimenez M C, Clavero V and Jimenez C, 1996 *Spanish Atlantic coasts*. In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (ed. W Schramm and P Neinhuis), pp. 265-281. Springer-Verlag, Berlin

Nielson K and Knudsen L, 1990 *Eutrophic effects on the marine vegetation, southern Kattegat.* Presentation made at the 25th European Marine Biology Symposium, Sept. 10-15, 1990, Ferrara, Italy

Nienhuis P H, 1983 Temporal and spatial patterns of eelgrass (*Zostera marina L.*) in a former estuary in the Netherlands, dominated by human activities. *Marine Technology Society Journal,* **17**, 69-77

Nienhuis P H, 1992 Ecology of coastal lagoons in the Netherlands (Veerse Meer and Grevelingen). *Vie Milieu*, **42**, 59-72

Nienhuis P H, 1996 *The north sea coasts of Denmark, Germany and the Netherlands*. In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (ed. W Schramm and P Neinhuis), pp. 187-222. Springer-Verlag, Berlin.

Nikolaidis G, 1985 *Qualitative and quantitative investigation of the marine plant macrophyceae (Chloro-, Phaeo- and Rhodophyceae) in different polluted areas (Thessaloniki Gulf) and not polluted areas (Chalkidiki).* Unpublished PhD thesis, University of Thessaloniki, Greece

Nikolaidis G and Moustaka-Gouni M, 1990 The structure and dynamics of phytoplankton assemblages from the inner part of Thermaikos Gulf, Greece. I.

Phytoplankton composition and biomass from May 1988 to April 1989. *Helgolander wissenschaftliche Meeresuntersuchungen*, **44**, 487-501

Nixon S W, 1995 Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia*, **41**, 199-219

Nixon S W, 1995 Coastal Marine Eutrophication - a Definition, Social Causes, and Future Concerns. *Ophelia,* **41**, 199-219

Nixon S W, Ammerman J W, Atkinson L P, Berounsky V M, Billen G, Boicourt W C, Boynton W R, Church T M, Ditoro D M, Elmgren R, Garber J H, Giblin A E, Jahnke R A, Owens N J P, Pilson M E Q and Seitzinger S P, 1996 The fate of nitrogen and phosphorus at the land sea margin of the North Atlantic Ocean. *Biogeochemistry,* **35**, 141-180

Nordin R N, 1985 *Water Quality Criteria for Nutrients and Algae (Technical Appendix)*. Victoria: Water Quality Unit, British Columbia Ministry for the Environment

Norris R H and Georges A, 1993 *Analysis and interpretation of benthic macroinvertebrates.* In Freshwater biomonitoring and benthic macroinvertebrates (ed. Rosenberg and Resh), pp. 234-286

Norris R H and Norris K R, 1995 The need for biological assessment of water-quality - Australian perspective. *Australian Journal of Ecology*, **20,** 1-6

Norris R H, Liston P, Whitington J and Linke S, 2001 *Assessment of River Condition at the National Scale: auditing to meet the need of managers and politicians*. Presented at the NABS Annual Meeting, La Crosse, Wisconsin, 2001. North American Benthological Society

North Sea Task Force, 1994 *North Sea Subregion 5 Assessment Report* (ed. H Karup), Ministry of the Environment / Danish Environmental Protection, Copenhagen. pp. 33-35

Northcote T G, 1972 Some effects of mysid introduction and nutrient enrichment on a large oligotrophic lake and its salmonids. *Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie*, **18**, 1096-1106

Northcote T G, 1979 Fish in the structure and function of freshwater ecosystems: a 'top-down' view. *Canadian Journal of Fisheries and Aquatic Sciences*, **45**, 361-379

Norton S B, 2000 Using biological monitoring data to distinguish among types of stress in streams of the Eastern Corn Belt Plains Ecoregion. *Dissertation Abstracts International Part B: Science and Engineering*, **60,** 4996

Norton S B, Cormier S M, Smith M and Jones R C, 2000 Can biological assessments discriminate among types of stress? A case study from the Eastern Corn Belt Plains ecoregion. *Environmental Toxicology and Chemistry*, **19,** 1113-1119

NRA, 1992 *Water Quality 2000.* A Strategy for the Water Quality Function. Version 8

NRA, 1994a *Standard Methodologies*. Assessment of Freshwater Riverine Environments using Macrophytes. NRA Anglian Region. Final Draft April 1994.

NRA, 1994b *Urban Waste Water Treatment Directive: Sensitive Areas (eutrophic) and Polluted Waters (eutrophic): Guidance note on information gathering for future designation reviews*. NRA internal guidance note August 1994

Numann W, 1972 The Bodensee: effects of exploitation and eutrophication on the salmonid community. *Journal of the Fisheries Research Board of Canada*, **29**, 833-847

OECD, Organisation for Economic Co-operation and Development, 1982 *Eutrophication of waters: monitoring, assessment and control*. OECD, Paris, 154 pp

Ogden R W, 1996 *The impacts of farming and river regulation on billabongs of the Southeast Murray basin, Australia*. Unpublished PhD thesis. Australian National University, Canberra

Ogilvie B, Nedwell D B, Harrison R M, Robinson A and Sage A, 1997 High nitrate, muddy estuaries as nitrogen sinks: the nitrogen budget of the River Colne estuary (United Kingdom). *Mar Ecol Prog Ser*, **150**, 217-228

Oglesby R T, 1977 Relationship of fish yield to lake phytoplankton standing crop, production and morphoedaphic factors. *Journal of the Fisheries Research Board of Canada,* **34**, 2271-2279

Ohio EPA (Environmental Protection Agency), 1987 *Biological Criteria for the Protection of Aquatic Life Volume III Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities.* Division of Water Quality Monitoring and Assessment, Ohio Environmental Protection Agency, Columbus, OH

Ohio EPA, 1987 *Biological criteria for the protection of aquatic life*. Vols 1-3. Monitoring and Assessment Program, Surface Water Section, Division of Water Quality, Ohio EPA, Columbus, OH

O'Keeffe J, 1997 *Methods of assessing conservation status for natural fresh waters in the Southern hemisphere*. In Freshwater quality: defining the indefinable? (ed. P J Boon and D L Howell), pp. 369-386. Stationery Office, Edinburgh

Oliveira J C and Cabecadas G, 1996 *Portugal.* In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (ed. W Schramm and P Neinhuis), pp. 283-294. Springer-Verlag, Berlin

Omernik J M, 1977 *Non-point source stream nutrient level relationships: a nationwide survey.* Environmental Protection Agency Report, **EPA 600/3-77-105,** In Nutrients - human modification p323

Omernik J M, 1987 Ecoregions of the conterminous United States. *Annals of the Association of American Geographers*, **77,** 118-125

Ormerod S J, 1987 The influences of habitat and seasonal sampling regimes on the ordination and classification of macroinvertebrate assemblages in the catchment of the River Wye, Wales. *Hydrobiologia,* **150,** 143-151

Ormerod S J and Edwards R W, 1987 The ordination and classification of macroinvertebrate assemblages in the catchment of the River Wye in relation to environmental factors. *Freshwater Biology*, **17,** 533-546

Ormerod S J, Wade K R and Gee A S, 1987 Macro-floral assemblages in upland Welsh streams in relation to acidity and their importance to invertebrates. *Freshwater Biology*, **18,** 545-557

Osborne and Wiley, 1988 *Empirical relationships between landuse cover and stream water quality in an agricultural watershed.* Journal Environmental Management, **26,** p9 - 27, In Stream Ecology Structure and function of running waters edited by J D Allen - Chapman and Hall 1995

Osborne PL, 1981 Phosphorus and nitrogen budgets of Barton Broad and the predicted effects of a reduction in nutrient loading on phytoplankton biomass in Barton, Sutton and Stalham Broads, Norfolk, UK. *Internationale Revue der Gesamten Hydrobiologie,* **66**, 171-202

OSPARCOM, 1992 *Nutrients in the convention area*. Oslo and Paris Commissions. ISBN 0946956235.

Overton J, Nicholls K, Dickman M and Foy M, 1994 *Lake Simcoe*. In The book of Canadian Lakes (ed. R J Allan, M Dickman, C B Gray and V Cromie), pp. 125-142. Burlington: Canadian Association on Water Quality

Owens N J P and Stewart W D P, 1983 Enteromorpha and the cycling of nitrogen in a small estuary. *Estuarine Coastal and Shelf Science*, **17**, 287-296

Paerl H W, Pinckney J L, Fear J M and B L Peierls, 1998 Ecosystem responses to internal and watershed organic matter loading: consequences for hypoxia in the eutrophying Neuse River estuary, North Carolina, USA. *Mar Ecol Prog Ser*, 166, 17-25

Palmer C, Palmer A, Okeeffe J and Palmer R, 1994 Macroinvertebrate community structure and altitudinal changes in the upper reaches of a warm temperate southern African river. *Freshwater Biology*, **32,** 337-347

Palmer M A, 1989 *A Botanical Classification of Standing Waters in Great Britain and a Method for the Use of Macrophyte Flora in Assessing Changes in Water Quality*. Research and Survey in Nature Conservation No. 19. Nature Conservancy Council, Peterborough

Palmer M A and Newbold C, 1983 *Wetland and riparian plants in Great Britain*, Focus on Nature Conservation No. 4, Nature Conservancy Council, 100pp

Palmer M A, Bell S L and Butterfield I, 1992 A botanical classification of standing waters in Britain: applications for conservation and monitoring. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **2**, 125-143

Parr W, 1994 *Eutrophication of the Wash: Factors and interactions*. WRc report No CO 3752

Parr W and Norton R L, 1994 *Eutrophication of tidal waters: Classification and screening tools*. WRc report No SR 3707

Parr W and Wheeler M A, 1996 *Trends in nutrient enrichment of sensitive marine areas in England.* WRc/EN report No CO 4055/1

Parr W, Nixon S C, Ashley S and Woodrow D, 1993 *Classification options for trophic status.* WRc report No NR 3402/1/7075. NRA R&D note 248

Parsons M and Norris R H, 1996 The effect of habitat-specific sampling on biological assessment of water quality using a predictive model. *Freshwater Biology*, **36,** 419-434

Patrick R, 1949 A proposed biological measure of stream conditions, based on a survey of the Conestoga Basin, Lancaster County, Pennsylvania. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **101,** 277-341

Patrick R, 1954 Use of algae, especially diatoms, in the assessment of water quality.

Patrick S T, Juggins S, Waters D, Jenkins A, 1991. *The United Kingdom Acid Waters Monitoring Network. Site descriptions and methodology report.* ENSIS, London, 63 pp

Patsch J and Radach G, 1997 Long-term simulation of the eutrophication of the North Sea: temporal development of nutrients, chlorophyll and primary production in comparison to observations. *Journal of Sea Research,* **38**, 275-310

Pauwels H, Lachassagne P, Bordenave P, Foucher J C and Martelat A, 2001 Temporal variability of nitrate concentration in a schist aquifer and transfer to surface waters. *Appl Geochem,* **16**, 583-596

Pearsall W H, 1918 On the classification of aquatic plant communities. *Journal of Ecology*, **6,** 75-84

Pearsall W H, 1920 *The development of vegetation in the English Lakes, considered in relation to the general evolution of glacial lakes and rock-basins'.* In Proceedings of the Royal Society B, **92**, 259-284

Pearsall W H, 1921 The aquatic vegetation of the English lakes. *Journal of Ecology*, **8**, 163-201

Pearsall W H, 1932 Phytoplankton in English Lakes. II. The composition of phytoplankton in relation to dissolved substances. *J. Ecol.*, **20**, 241-262

Pearson T H, Grey J S and Johannesen P J, 1983 Objective selection of sensitive species indicative of pollution-induced change in benthic communities: 2. Data analyses. *Marine Ecology Progress Series*, **12,** 237-255

Pedersen M F, 1995 Nitrogen limitation of photosynthesis and growth: comparison accross aquatic plant communities in a Danish estuary (Roskilde Fjord). *Ophelia*, **41**, 261-272

Peierls B, Caraco N, Pace M and Cole J, 1991 Human influence on river nitrogen. *Nature*, **350**, 386-387

Pen L J and Potter I C, 1992 Seasonal and size related changes in the diet of perch, Perca fluviatilis L, in the shallows of an Australian river and their implications for the conservation of indigenous teleosts. *Aquatic Conservation, Marine and Freshwater Ecosystems*, **2**, 243-253

Penczak T, Mahon R and Balon E K, 1984 The effect of an impoundment on the upstream and downstream fish taxocenes Speed River, Ontario, Canada. *Archiv fur Hydrobiologie*, **99**, 200-207

Pennock J R and Sharp J H, 1994 Temporal Alternation between Light-Limitation and Nutrient- Limitation of Phytoplankton Production in a Coastal-Plain Estuary. *Marine Ecology-Progress Series,* **111**, 275-288

Penny S F, 1985 *The use of macroinvertebrates in the assessment of point source pollution.* In Biological Monitoring in Freshwaters: Proceedings of a Seminar, Hamilton, November 21-23, 1984 Part 2, (ed. R D Pridmore and A B Cooper), Water & Soil Miscellaneous Publication No 83, pp. 205-215, National Water and Soil Conservation Authority, Wellington, NZ

Perez-Llorens J L, 1991 *Estimaciones de biomasa y contenido interno de nutrientes. Ecofisiologia de incorporacion de carbono y fostato en* Zostera noltii *Hornem*. Unpublished PhD thesis, University of Malaga, Spain

Perez-Ruzafa A, Marcos-Diego C and Ros J D, 1991. Environmental and Biological changes related to recent human activities in the Mar Menor (SE of Spain). *Marine Pollution Bulletin*, **23**, 747-751

Perkins E J and Abbott O J, 1972 Nutrient enrichment and sand-flat fauna. *Marine Pollution Bulletin*, **3**, 70-72

Perrone S J and Meade T L, 1977 Protective effects of chloride on nitrite toxicity to coho salmon (*Onchorynchus kisutch*). *J Fish Res Board Can*, **34**, 486-492

Perrow M R, 1989 *Causes and consequences of a two-year cycle in recruitment of roach Rutilis rutilus L. in Alderfern Broad*. PhD Thesis: University of East Anglia

Peters R H, 1991 *A critique for ecology*. Cambridge, MA: Cambridge University Press

Petersen C H and Black R, Density-dependant mortality caused by physical stress interacting with biotic history. *American Naturalist*, **131,** 257-270

Philippart C J M and Cadee G C, 2000 Was total primary production in the western Wadden Sea stimulated by nitrogen loading? *Helgoland Marine Research,* **54**, 55-62

Philippart C J M, Cadee G C, van Raaphorst W and Riegman R, 2000 Long-term phytoplankton-nutrient interactions in a shallow coastal sea: Algal community structure, nutrient budgets, and denitrification potential. *Limnology and Oceanography,* **45**, 131-144

Phillips G L, Eminson D and Moss B, 1978 A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. *Aquatic Botany,* **4**, 103-126

Pichon J J, Labracherie M, Labeyrie L D and Duprat J, 1987 Transfer functions between diatom assemblages and surface hydrology in the southern Ocean. *Palaeogeography*, *Palaeoclimatology & Paleaeoecology*, **61**, 79-95

Pignatti S, 1962 *Associazioni di alghe marine sulla costa veneziana*. Memoriel 1st Veneto Lattere Science Artie, **32**, 1-134

Pihl L, Svenson A, Moksnes P-O and Wennhage H, 1999 Distribution of green algal mats throughout shallow soft bottoms of the Swedish Skagerrak archipelago in relation to *nutrient sources and wave exposure. Journal of Sea Research,* **41**, 281-294

Pinckney J L, Paerl H W and Harrington M B, 1999 Responses of the phytoplankton community growth rate to nutrient pulses in variable estuarine environments. *Journal of Phycology,* **35**, 1455-1463

Pinder L, 1999 *Macro-invertebrates*. In The United Kingdom Environmental Change Network. Protocols for Standard Measurements at Freshwater Sites, pp. 90-96: NERC

Pinder L C V and Farr I S, 1987 Biological surveillance of water quality - 2.: Temporal and spatial variation in the macroinvertebrate fauna of the River Frome - a Dorset chalk stream. *Archiv fur Hydrobiologie*, **109,** 321-331

Pinder L C V and Ladle M, 1987 Biological surveillance of water quality - 1. A comparison of macroinvertebrate surveillance methods in relation to assessment of water quality, in a chalk stream. *Archiv fur Hydrobiologie*, **109,** 207-226

Plafkin J L, Barbour M T, Porter K D, Gross S K and Hughes R M, 1989 *Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish.* EPA/440/4-89-001. USEPA, Office of Water, Washington DC.

Pollard P and Huxham M, 1998 The European Water Framework Directive: a new era in the management of aquatic ecosystem health? *Aquatic Conservation: Marine and Freshwater Ecosystems*, **8**, 773-792

Portt C B, Balon E K and Noakes D L G, 1986 Biomass and production of fishes in natural and channelized streams. *Canadian Journal of Fisheries and Aquatic Science*, **43**, 1926-1934

Powell H T, 1963 New records of *Fucus distichus* subspecies for the Shetland and Orkney Islands. *British Phycological Bulletin*, **2**, 247-254

Prakash A, 1987 Coastal organic pollution as a contributing factor to red-tide development. *Rapports et Proces verbaux des reunions du conseil permanent Internation pour l'exploration de la mer.,* **187**, 61-65

Premazzi G and Chiaudani G, 1992 *Ecological Quality of Surface Waters*, pp. 124: Commission of the European Communities

Preston C D and Croft J M, 1970 Aquatic plants in Britain and Ireland. Harley Books, Colchester, UK, 365pp

Price N M and Morel F M, 1991 Colimitation of phytoplankton growth by nickel and nitrogen. *Limnology and Oceanography,* **36**, 1071-1077

Pridmore R D and Cooper A B (eds), 1985 *Biological Monitoring in Freshwaters:* Proceedings of a Seminar, Hamilton, November 21-23, 1984 Part 1 & 2, Water & Soil Miscellaneous Pub No 82 & 83, National Water and Soil Conservation Authority, Wellington, NZ

Pringle *et al*, 1993 *Environmental problems of the Danube Delta*. American Scientist, **81,** 350-361, In Stream Ecology Structure and function of running waters J D Allen, Chapman and Hall 1995

Prosser C L, 1973 *Comparative physiology 3rd*. Saunders W B, Philadelphia, PA, USA

Prygiel J and Coste M, 1996 *Progress in the use of diatoms for monitoring rivers in France*. In Use of Algae for Monitoring Rivers III (ed. J Prygiel, B A Whitton and J Bukowska), pp. 165-179. Douai, France: Agence de l'Eau Artois-Picardie

Prygiel J, Whitton B A and Bukowska J, 1998 *Use of Algae for monitoring rivers III.* Proceedings of 3rd European Workshop, Douai Agence de l'eau Artois-Picardie.

Puskaric S, Berger G W and Jorissen F J, 1990 Successive appearance of subfossil phytoplanktonic species in Holocene sediments of the northern Adriatic and its relation to the increased eutrophication pressure. *Estuarine, Coastal and Shelf Science*, **31**, 117-187

Pye K E, 2000 *The effects of eutrophication on the marine benthic flora of Langstone Harbour, South Coast of England.* Unpublished PhD thesis, University of Portsmouth, UK

Quignard J P and Douchement C, 1991 *Alosa alosa Linnaeus, 1758*. In The Freshwater fishes of Europe. Volume 2. Clupeidae, Anguillidae (ed. H Hoestlandt), pp. 86-126. Wiesbaden: AULA-Verlag

Quinlan R, Smol J P and Hall R I, 1998 Quantitative inferences of past hypolimnetic anoxia in south-central Ontario lakes using fossil midges (Diptera: Chironomidae). *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 587-596

Quinn J M and Hickey C W, 1993 Effects of sewage waste stabilization lagoon effluent on stream invertebrates. *Journal of Aquatic Ecosystem Health*, **2**, 205–219

Rabalais N N, Turner R E, Justic D, Dortch Q, Wiseman W J and Sen Gupta B K, 1996 Nutrient changes in the Mississippi River and system responses on the adjacent continental shelf. *Estuaries*, **19**, 386-407

Radach G, Berg J and Hagmeier E, 1990 Long term changes of the annual cycles of meterological, hydrographic, nutrient and phytoplankton time series at Helgoland and at LV ELBE 1 in the German Bight. *Continental Shelf Research,* **10**, 305-328

Rader R B and Richardson C J, 1994 Response of macroinvertebrates and small fish to nutrient enrichment in the northern Everglades. *Wetlands - Wilmington*, **14,** 134pp

Raffaelli D G, 1999 Nutrient enrichment and trophic organisation in an estuarine food web. *Acta Oecologica-International Journal of Ecology,* **20**, 449-461

Raffaelli D G, Hull S and Milne H, 1989 Long-term changes in nutrients, weed mats and shorebirds in an estuarine system. *Cahiers de Biologie Marine,* **30**, 259-270

Raffaelli D G, Raven J A and Poole L J, 1998 Ecological impact of green macroalgal blooms. *Oceanography and Marine Biology: an Annual review*, **36**, 97-125

Raffaelli D G, Limia J, Hull S and Pont S, 1991 Interactions between invertebrates and macroalgal mats on estuarine mudflats. *Journal of the Marine Biological Association of the United Kingdom*, **71**, 899-908

Raffaelli D G, Balls P, Way S, Patterson I J, Hohmann S and Corp N, 1999 Major long term changes in the ecology of the Ythan estuary, Aberdeenshire, Scotland: how important are physical factors? *Aquatic Conservation: Marine and Freshwater Ecosystems*, **9**, 219-236

Randall R G, Kelso J R M and Minns C K, 1995 Fish production in fresh waters: are rivers more productive than lakes? *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 631-643

Rankin E T, 1995 *Habitat indices in water resource quality assessments.* In Biological assessment and criteria (ed. W S Davis and T P Simon), pp. 181-208. Tools for water resource planning and decision making

Ranta E and Lindstrom K, 1993 Water-quality and prediction of lake-specific fish yield - a northern European perspective. *Annales Zoolici Fennici*, **30**, 89-100

Ratcliffe D A, (Ed) 1977 *A Nature Conservation Review*, Cambridge University Press, Cambridge

Rauch W and Vanrolleghem P A, 1997 Modelling benthic activity in shallow eutrophic rivers. *Water Science and Technology. Conference: Eutrophication research, Wageningen, The Netherlands Aug 1997*, **37**, 129-138

Raven J A, 1984 *Energetics and Transport in Aquatic Plants*. New York, AR Liss

Raven J A, 1986 Physiological consequences of extremely small size for autotrophic organisms in the sea. *Canadain Bulletin of Fisheries and Aquatic Sciences.,* **214**, 1-70

Raven J A, 1996 The Bigger the Fewer - Size, Taxonomic Diversity and the Range Of Chlorophyll(Ide)Pigments In Oxygen-Evolving Marine Photolithotrophs. *Journal of the Marine Biological Association of the United Kingdom,* **76**, 211-217

Raven J A and Richardson K, 1986 *Marine environments*. In Photosynthesis in contrasting environments (ed. N R Baker and S P Long), pp. 337-398. Amsterdam: Elsevier

Rawson D S, 1951 The total mineral content of lake waters. *Ecology,* **32**, 669-672

Rawson D S, 1952 Mean depth and fish production of large lakes. *Ecology,* **33**, 513-521

Rawson D S, 1955 Morphometry as a dominant factor in the productivity of large lakes. *Verh. int. Verein. Limnol.,* **12**, 164-175

Reavie E, Hall R I and Smol J P, 1995 An expanded weighted-averaging model for inferring past total phosphorus concentrations from diatom assemblages in eutrophic British Colombia (Canada) lakes. *Journal of Paleolimnology*, **14**, 49-67

Reavie E D and Smol J P, 1997 Diatom-based model to infer past littoral habitat characteristics in the St. Lawrence River. *Journal of Great Lakes Research*, **23**, 339-348

Reckermann M, 2000 Flow sorting in aquatic ecology. *Scientia Marina,* **64**, 235-246

Regier H A and Henderson H F, 1973 Towards a broader ecological model of fish communities. *Transactions of the American Fisheries Society*, **102**, 56-72

Reice S R and Wohlenberg M, 1993 *Monitoring Freshwater Benthic Macroinvertebrates and Benthic Processes: Measures for Assessment of Ecosystem Health*. In Freshwater Biomonitoring and Benthic Macroinvertebrates (ed. D M Rosenberg and V Resh), pp. 287-305. London: Chapman and Hall

Reid C R, Edwards M, Hunt H G and Warner A J, 1998 Phytoplankton Change in the North

Reid M A, 1997 *A diatom-based palaeoecological study of the dynamics of the Goulbourn River billabongs, southeastern Australia*. Unpublished PhD thesis. Monash University, Melbourne

Reid M A, Fluin J, Ogden R W, Tibby J and Kershaw A P (in press). Long-term perspectives on human impacts on floodplain-river ecosystems, Murray-Darling Basin, Australia. *Proceedings of the International Association of Theoretical and Applied Limnology*, **28**

Reid P C, Holliday N P and Smyth T J, 2001 Pulses in the eastern margin current and warmer water off the north west European shelf linked to North Sea ecosystem changes. *Marine Ecology-Progress Series,* **215**, 283-287

Reise K, 1983 Sewage, green algal mats anchored by lugworms and the effects on Turbellaria and small polychaeta. *Helgolander wissenschaftliche Meeresuntersuchungen*, **36**, 151-162

Reise K, 1994 Changing life under the tides of the Wadden Sea during the 20th Century. *Ophelia*, **6**, 117-125

Resh V H, 1995 *Freshwater benthic macroinvertebrates and rapid assessment procedures for water quality monitoring in developing and newly industrialised countries*. In Biological assessment and criteria. Tools for water resource planning and decision making. (ed. W S Davis and T P Simon), 167-180

Resh V H and Jackson J K, 1993 *Rapid assessment approaches to biomonitoring using benthic macroinvertebrates.* In Freshwater biomonitoring and benthic macroinvertebrates (ed. Rosenberg and Resh)**,** 195-233

Resh V H and McElravy E P, 1993 *Contemporary quantitative approaches to biomonitoring using benthic macroinvertebrates.* In Freshwater biomonitoring and benthic macroinvertebrates (ed. Rosenberg and Resh), 159-194

Resh V H and Rosenberg D M, 1989 Spatial-temporal variability and the study of aquatic insects. *Canadian Entomologist*, **121,** 941-963

Resh V H and Unzicker J D, 1975 Water quality monitoring and aquatic organisms. *Journa*l *of Water Pollution Control Federation*, **47,** 9-19

Resh V H, Norris R H and Barbour M T, 1995 Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. *Australian Journal of Ecology*, **20,** 108-121

Resh V H, Rosenberg D M and Reynoldson T B, 2000 *Selection of benthic macroinvertebrate metrics for monitoring water quality of the Fraser River, British Colombia: implications for both multimetric approaches and multivariate models.* In Assessing the Biological quality of fresh waters: RIVPACS and other techniques (ed. J F Wright, D W Sutcliffe and M T Furse), 195-206, Freshwater Biological Association, Ambleside

Revsbech N P and Jørgensen B B, 1986 *Microelectrodes: their use in microbial ecology*. In Advances in microbial ecology 9 (ed. K C Marshall), pp. 293-356. Plenum. Press. New York

Revsbech N P, Jørgensen B B and Blackburn T H, 1980 Oxygen in the sea bottom measured with a microelectrode. *Science*, **207**,1355-1356

Reynolds C S, 1984 *The ecology of freshwater phytoplankton*: Cambridge University Press

Reynolds C S, 1998 The control and management of cyanobacterial blooms. *Special Publications of the Australian Society for Limnology,* **12**, 6-22

Reynolds C S, 1998 What factors influence the species composition of phytoplankton in lakes of different trophic status? *Hydrobiologia,* **370**, 11-26

Reynolds C S, 1999 Modelling phytoplankton dynamics and its application to lake management. *Hydrobiologia,* **396**, 123-131

Reynolds C S and Bellinger E G, 1992 Patterns of Abundance and Dominance of the Phytoplankton of Rostherne Mere, England - Evidence from an 18-Year Data Set. *Aquatic Sciences,* **54**, 10-36

Reynolds C S and Petersen A C, 2000 The distribution of planktonic Cyanobacteria in Irish lakes in relation to their trophic states. *Hydrobiologia,* **424**, 91-99

Reynolds C S, Jaworski G H M, Roscoe J V, Hewitt D P and George D G, 1998 Responses of the phytoplankton to a deliberate attempt to raise the trophic status of an acidic, oligotrophic mountain lake. *Hydrobiologia,* **370**, 127-131

Reynolds J D and Guillaume H P, 1998 Effects of phosphate on the reproductive symbiosis between bitterling and freshwater mussels: implications for conservation. *J Appl Ecol*, **35**, 575-581

Reynoldson T B and Metcalfe-Smith J L, 1992 *An overview of the assessment of aquatic ecosystem health using benthic macroinvertebrates*. Journal of Aquatic Ecosystem Health

Reynoldson T B and Wright J F, 2000 *The Reference Condition: problems and solutions.* In Assessing the biological quality of freshwaters: RIVPACS and similar techniques. (ed. J F Wright, D W Sutcliffe and M T Furse)**,** pp. 293-303, Freshwater Biological Association, Ambleside

Reynoldson T B and Zarull M A, 1993 *An approach to the development of biological sediment guidelines*. In Ecological Integrity and the Management of Ecosystems (ed. S Woodley, J Kay and G Grancis), pp.177-200. St Lucie Press

Reynoldson T B, Bailey R C, Day K E and Norris R H, 1995 Biological guidelines for freshwater sediment based on BEnthic Assessment of SedimenT (the BEAST) using a multivariate approach for predicting biological state. *Australian Journal of Ecology*, **20,** 198-219

Reynoldson T B, Norris R H, Resh V H, Day K E and Rosenberg D M, 1997 The reference condition: a comparison of multimetric and multivariate approaches to assess water quality impairment using benthic macroinvertebrates. *Journal of the North American Benthological Society*, **16**, 833-852

Rhoads D C and Young D K, 1970 The influences of deposit-feeding organisms on sediment stability and community trophic structure. *J Mar Res*, **28**, 150-178

Richardson D H S (ed), 1987 *Biological indicators of pollution.* Royal Irish Academy, Dublin, Ireland

Richardson K, 1991 Comparison of C-14 Primary Production Determinations Made By Different Laboratories. *Marine Ecology-Progress Series,* **72**, 189-201

Richardson K, 1996 In Eutrophication in Coastal Marine Ecosystems (ed. B Jorgensen and K Richardson), pp. 95-114. Washington: American Geophysical Union

Richardson K, 1997 *Harmful or exceptional phytoplankton blooms in the marine ecosystem*. In Advances in Marine Biology, Vol 31, vol. 31, pp. 301-385

Richardson K and Heilmann J P, 1995 Primary Production in the Kattegat - Past and Present. *Ophelia,* **41**, 317-328

Richardson K and Pedersen F B, 1998 Estimation of new production in the North Sea: consequences for temporal and spatial variability of phytoplankton. *Ices Journal of Marine Science,* **55**, 574-580

Richardson R E, 1929 The bottom fauna of the Middle Illinois River 1913-1925. Its distribution, abundance, valuation and index value in the study of stream pollution. *Bulletin of the Illinois Natural History Survey*, **17,** 387-475

Ricker W E, 1934 An ecological classification of Ontario streams. *Publications of the Ontario Fisheries Research Laboratory*, **49**, 1-114

Rippey B, Anderson N J and Foy R H, 1997 Accuracy of diatom-inferred total phosphorus concentrations and the accelerated eutrophication of a lake due to reduced flushing and increased internal loading. *Canadian Journal of Fisheries and Aquatic Sciences,* **54**, 2637-2646

Risberg J, 1990 Siliceous microfossil stratigraphy in a superficial sediment core from the Northwestern part of the Baltic proper. *Ambio*, **19**, 161-172

Riseng C M, Wiley M J, Stevenson R J, Seelback P W and Holomuzki J R, 2000 *Effects of fish on the trophic structure of Michigan streams: evidence from a nutrient gradient study.* North American Benthological Society Annual Meeting, Keystone. Abstract.

Roback S S, 1974 *Insects (Arthropoda: Insecta).* In Pollution Ecology of Freshwater Invertebrates (ed. C W Hart Jr and S L H Fuller), pp. 313-376. Academic Press, New York

Roberts J, Chick A, Oswald L and Thompson P, 1995 Effects of carp, *Cyprinus carpio*, an exotic benthivorous fish, on aquatic plants and water quality in experimental ponds. *Australian Journal of Marine and Freshwater Research,* **46**, 1171-1180

Rodwell J S, 1995 *British Plant Communities: Volume 4. Swamps, Tall Herb Fen and Aquatic Communities.* Cambridge University Press, Cambridge

Roelke D L, Eldridge P M and Cifuentes L A, 1999 A model of phytoplankton competition for limiting and nonlimiting nutrients: Implications for development of estuarine and nearshore management schemes. *Estuaries,* **22**, 92-104

Rogers K M, 1999 Effects of sewage contamination on macro-algae and shellfish at Moa Point, New Zealand using stable carbon and nitrogen isotopes. *New Zealand Journal of Marine and Freshwater Research*, **33**, 181-188

Romero J, Niell F X, Martinez-Arroyo A, Perez M and Camp J, 1996. *The Spanish Mediterranean coasts.* In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (ed. W Schramm and P Neinhuis), pp. 295-306. Springer-Verlag, Berlin

Rönnberg O, 1984 Recent changes in the distribution of Fucus vesiculosus (L.) around the Aland Islands (N Baltic). *Ophelia*, **3**, 189-193

Roper D S, 1985 *The role of biological surveys and surveillance.* In Biological Monitoring in Freshwaters: Proceedings of a seminar, Hamilton, November 21-23, 1984 Part I, (eds R D Pridmore and A B Cooper) Water & Soil Miscellaneous Publication No 82, 17-20, National Water and Soil Conservation Authority, Wellington, NZ

Rosen G., 1981 Phytoplankton indicators and their relations to certain chemical and physical factors. *Limnologica*, **13**, 262-290

Rosenberg R, 1985 Eutrophication. The future marine coastal nuisance? *Marine Pollution Bulletin*, **6**, 227-231

Rosenberg D M, 1992 Freshwater biomonitoring and Chironmidae. *Netherlands Journal of Aquatic Ecology,* **26**, 101-122

Rosenberg D M and Resh V H (eds), 1993 *Freshwater biomonitoring and benthic macroinvertebrates.* Chapman and Hall, New York and London

Rosenberg D M and Resh V H, 1993 *Introduction to freshwater biomonitoring and benthic macroinvertebrates.* In Freshwater biomonitoring and benthic macroinvertebrates (ed. Rosenberg and Resh)**,** pp. 1-9. Chapman and Hall, New York and London

Rosenberg D M, Danks H V and Lehmkuhl D M, 1986 Importance of insects in environmental impact assessment. *Environmental Management*, **10,** 773-783

Rosenberg D M, Reynoldson T B and Resh V H, 2000 *Establishing reference conditions in the Fraser River catchment, British Colombia, Canada, using the BEAST predictive model.* In Assessing the Biological quality of fresh waters: RIVPACS and other techniques. (ed. J F Wright, D W Sutcliffe and M T Furse), 181-194, 0-900386-62-2, Freshwater Biological Association, Ambleside

Rosenberg R, Elmgren R, Fleischer S, Jonsson P, Persson G and Dahlin H, 1990 Marine eutrophication case studies in Sweden. *Ambio*, **19**, 102-110

Rosenberg R, Hellman B and Johansson B, 1991 Hypoxic tolerance of marine benthic fauna. *Mar Ecol Prog Ser*, **79**, 127-31

Round F E, 1981 *The ecology of algae*. Cambridge: University Press

Round F E, 1993 *A Review and Methods for the use of Epilithic Diatoms for Detecting and Monitoring Changes in River Water Quality 1993.* (Blue Book) Methods for the Examination of Waters and Associated Materials. London. Her Majesty's Stationery Office, 68pp

Royal Commission on Environmental Pollution, Sixteenth report. Freshwater Quality. June 1992

Rueness J, 1973 Pollution effects on littoral algal communities in the inner Oslofjord, with special reference to Ascophyllum nodosum. *Helgolander wissenschaftliche Meeresuntersuchungen*, **24**, 446-454

Ruhle C, 1989 *The management of Arctic Charr Salvelinus alpinus L. in eutrophied Lake Zug*. In Biology of the Arctic Charr (ed. L Johnson and B L Burns), pp. 487-492. University of Manitoba Press: Winnipeg.

Ruiz Z, Rippey B, Carter C and Bennion H, 2001. *A transfer function between lake anoxic factor and sedimentary chironomid subfossil species abundance*. Final Report to NERC on Research Grant GR9/04033 by University of Ulster, Coleraine

Rumeau A and Coste M, 1988 Initiation a la systematique des Diatomees d'eau douce pour l'utilisation pratique d'un indice diatomique generique. *Bulletin Francais de la Peche et de la Pisciculture*, **309**, 1-69

Ruse L, 2000 Thienemann's technique - the use of chironomid pupal exuviae for classifying and monitiring lakes. *Freshwater Biology*

Ruse L and Love A, 1997 Predicting phytoplankton composition in the River Thames, England. *Regulated Rivers: Research and Management*, **13**, 171-183.

Russell G, 1974 Fucus distichus communities in Shetland. *Journal of Applied Ecology*, **11**, 679-684

Russo R C, 1985 *Ammonia, nitrite and nitrate*. In Fundamentals of Aquatic Toxicology (ed. G Rand, S R Petrocelli), pp. 455-471. Hemisphere Publishing Corporation, Washington, DC

Russo R C and Thurston R V, 1977 *The acute toxicity of nitrite to fishes*. In Recent advances in fish toxicology (ed. R A Tubb) pp. 118-131. Environmental Protection Agency, Ecological Research Series EPA-600/3-77-085, Corvalis, Oregon

Ryder R A, 1965 A method for estimating the potential fish production of north-temperate lakes. *Transactions of the American Fisheries Society*, **94**, 214-218

Ryder R A, 1972 The limnology and fishes of oligotrophic glacial lakes in North America about 1800 A.D. *Journal of the Fisheries Research Board of Canada*, **29**, 617-628

Ryder R A, 1982 The morphoedaphic index - use, abuse, and fundamental concepts. *Transactions of the American Fisheries Society*, **111**, 154-164

Ryder R A, Kerr S R, Taylor W W and Larkin P A, 1981 Community consequences of fish stock diversity. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 1867-1876

Ryder R A, Kerr S R, Loftus K H and Regier H A, 1974 The morphoedaphic index as a fish yield estimator: review and evaluation. *J. Fish. Res. Bd. Can.,* **31**, 663-688

Ryder R D, 1961 Fisheries management in northern Ontario. *Ont. Fish. Wildl. Rev,* **1**, 13-19

Ryther J H, 1981 *Impact of nutrient enrichment on water uses*. In Estuaries and nutrients. (ed. B J Neilson and L E Cronin), pp. 247-261, Humana Press, New Jersey

Ryther J H and Dunstan W M, 1971 Nitrogen, phosphorus and eutrophication in the coastal marine environment. *Science*, **171**, 1008-1112

Saether O A, 1975 Nearctic chironomids as indicators of lake typology. *Verh. internat. Verein. Limnol.,* **19**, 3127-3133

Saether O A, 1979 Chironomid communities as water quality indicators. *Holarctic Ecology,* **2**, 65-74

Sampou P and Oviatt C A, 1991 Seasonal patterns of sedimentary carbon and anaerobic respiration along a simulated eutrophication gradient. *Mar Ecol Prog Ser*, **72**, 271-282

Sanders R, Klein C and Jickells T, 1997 Biogeochemical nutrient cycling in the upper Great Ouse estuary, Norfolk, UK. *Estuarine Coastal and Shelf Science,* **44**, 543-555

Sandin L and Johnson R K, 2000 The statistical power of selected indicator metrics using macroinvertebrates for assessing acidification and eutrophication of running waters. *Hydrobiologia*, **422/423,** 233-243

Sand-Jensen K, 1997 *Macrophytes as biological engineers in the ecology of Danish streams*. In Freshwater Biology in Denmark. Historical Perspectives and Current Research (ed. K Sand-Jensen and O Pedersen)

Sand-Jensen K and Borum J, 1991 Interactions among phytoplankton, periphyton and macrophytes in temperate freshwaters and estuaries. *Aquatic Botany*, **41**, 137-176

Sand-Jensen K and Søndergaard M, 1981 Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. *Internationale Revue Der Gesamten Hydrobiologie,* **66**, 529-552

Say P J, Harding J P C and Whitton B A, 1981 Aquatic mosses as monitors of heavy metal pollution in the River Etherow, Great Britain. *Environmental Pollution,* **B2**, 295-307

Sayer C D, 2001 Problems with the application of diatom-total phosphorus transfer functions: examples from a shallow English lake. *Freshwater Biology,* **46**, 743-757

Sayer C D and Roberts N, 2001 Establishing realistic restoration targets for nutrient-enriched shallow lakes: linking diatom ecology and palaeoecology at the Attenborough Ponds, U.K. *Hydrobiologia*, **448**, 117-142

Sayer C, Roberts N, Sadler J, David C and Wade P M, 1999 Biodiversity changes in a shallow lake ecosystem: a multi-proxy palaeolimnological analysis. *Journal of Biogeography*, **26**, 97-114

Schaeffer D J, Herricks E E and Kerster H W, 1988 Ecosystem Health: I. Measuring ecosystem health. *Environmental Management,* **12,** 445-455

Scheffer M, Hosper S H, Meijer M L, Moss B and Jeppesen E, 1993 Alternative Equilibria in Shallow Lakes. *Trends in Ecology & Evolution,* **8**, 275-279

Schelske C L, 1991 Historical nutrient enrichment of Lake Ontario: paleolimnological evidence. *Canadian Journal of Fisheries and Aquatic Sciences,* **48**, 1529-1538

Schiefele S and Kohmann F, 1993 Bioindikation der Trophie in Fliessgewassern.

Schiffner V and Vatova A, 1938 *Le alghe della Laguna: Chlorophyceae, Phaeophyceae, Rhodophyceae, Myxophyceae.* In La Laguna di Venezia (ed. M Mino), pp. 250.Venezia

Schindler D W, 1971 A hypothesis to explain differences and similarities among lakes in the Experimental Lakes Area, northwestern Ontario. *Journal of the Fisheries Research Board of Canada,* **28**, 295-301

Schindler D W, 1977 The evolution of phosphorus limitation in lakes. *Science,* **195**, 260-262

Schindler D W, 1987, Detecting ecosystem responses to anthropogenic stress. *Canadian Journal of Fisheries and Aquatic Sciences,* **44**, 6-25

Schlesinger D A and Regier H A, 1982 Climatic and morphoedaphic indices of fish yields from natural lakes. *Transactions of the American Fisheries Society,* **111**, 141-150

Schlosser I J, 1982a Fish community structure and function along two habitat gradients in a headwater stream. *Ecological Monographs*, **52**, 395-414

Schlosser I J, 1982b Trophic structure, reproductive success, and growth rate of fishes in a natural and modified headwater stream. *Canadian Journal of Fisheries and Aquatic Sciences*, **39**, 968-978

Schmitz W, 1955 Physiographische Aspekte der LimnologischenFliesgewasserntypen. *Archiv fur Hydrobiologie,* **22**, 510-523

Schmutz S, Kaufmann M *et al.*, 2000 A multi-level concept for fish-based river-type-specific assessment of ecological integrity. *Hydrobiologia*, **422**, 279-289

Schramm W, 1988 *Untersuchungen zur Rolle benthischer Primärproduzenten im organischen und anorganischen Stoffhaushalt der Keiler Bucht*. UBA Ber Wasser 10204215/127, 34pp

Schramm W, 1996 *The Baltic sea and its transition zones*. In Ecological Studies, Vol. 123. Marine benthic vegetation: recent changes and the effects of eutrophication (ed. W Schramm and P Neinhuis), pp. 131-164. Springer-Verlag, Berlin

Schubauer-Berigan M K, Monson P D, West C W and Ankley G T, 1995 Influence of pH on the toxicity of ammonia to *Chironomus tentans* and *Lumbriculus variegatus*. *Environ Toxicol Chem*, **14**, 713-717

Scott G and Crunkilton R L, 2000 Acute and chronic toxicity of nitrate to fathead minnows (*Pimephales promelas*), *Ceriodaphnia dubia* and *Daphnia magna. Environment Toxicol Chem*, **19**, 2918-2922

Scottish Executive 2001 *Rivers, Lochs, Coasts: The Future for Scotland's Waters*. www.scotland.gov.uk/consultations/environment/ffsw-16.asp

Scrimgeour G J and Chambers P A, 1996 *Identification of spatial and temporal patterns in nutrient limitation with herbivory effects, Wapiti, Smoky and Athabasca Rivers.* Northern River Basins Study Technical Report No 96

Scrimgeour G J, Chambers P A, Culp J M and Podemski C, 1995 *Identification of spatial and temporal patterns in nutrient limitation, Athabasca River, October to December, 1993.* Northern River Basins Study Technical Report No 49

Scrimgeour G J, Chambers P A, Culp J M, Cash K J and Ouellette M, 1995 *Long term trends in ecosystem health: quantitative analysis of river benthic invertebrate communities, Peace and Athabasca Rivers.* Northern River Basins Study Technical Report No 56

Seddon B, 1972 Aquatic macrophytes as limnological indicators. *Freshwater Biology*, **2**, 107-130

SEPA, 2000 Water quality in the Forth Estuary 1980-1999, pp. 1-21: SEPA

Sfriso A, 1987 Flora and vertical distribution of macroalgae in the lagoon of Venice: a comparison with previous studies. *Giornale Botanico Italiano,* **121**, 69-85

Sfriso A, Marcomini A and Pavoni B, 1987 Relationships between macroalgal biomass and nutrient concentrations in a hypertrophic area of the Venice Lagoon. *Marine Environmental Research*, **22**, 297-312

Sfriso A, Pavoni B and Marcomini A, 1989 Macroalgae and phytoplankton standing crops in the central Venice Lagoon: primary production and nutrient balance. *Science of the Total Environment*, **13**, 139-159

Sfriso A, Pavoni B, Marcomini A and Orio A A, 1992 Macroalgae, nutrient cycles and pollutants in the Lagoon of Venice. *Estuaries*, **14**, 517-528

Sfriso A, Raccanelli S, Pavoni B and Marcomini A, 1991 Sampling strategies for measuring macroalgal biomass in the shallow waters of the Venice Lagoon. *Environmental Technology Letters,* **6**, 24-29

Shaltout K H and El-Sheikh M A, 1993 Vegetation-environment relationships along water courses in the Nile Delta region. *Journal of Vegetation Science*, **4**, 567-570

Shapiro J, 1990 Current beliefs regarding dominance by blue-greens: the case for the importance of CO_2 and pH. *Verhandlungen Internationale Vereinigung Limnologie,* **24**, 38-54

Shapiro J, Edmonson W T and Allinson D E, 1971 Changes in the chemical composition of sediments of Lake Washington, 1958-1970. *Limnology and Oceanography*, **16**, 437-452

Shepherd S A, McComb A J, Bulthius D A, Neverauskas V, Steffensen D A and West R, 1989 *Decline of seagrasses.* In Biology of seagrasses: a treatise on the biology of seagrasses with special reference to the Australian region (ed. A W D Larkum, A J McComb and S A Shepherd), pp. 346-387. Elsevier, Amsterdam

Shields F D, Knight S S and Cooper C M, 1995 Use of the index of biotic integrity to assess physical habitat degradation in warmwater streams. *Hydrobiologia,* **312,** 191-208

Short F T and Burdick D M, 1996 Quantifying eelgrass habitat loss in relation to housing development and nitrogen loading in Waquoit Bay, Massachusetts. *Estuaries*, **19**, 730-739

Short F T, Burdick D M and Kaldy J E, 1995 Mesocosm experiments quantify the effects of eutrophication on eelgrass Zostera marina. *Limnology and Oceanography*, **40**, 740-749

Shuval H I and Gruener N, 1972 Epidemiological and toxicological aspects of nitrates and nitrites in the environment. *Am J Pub Health*, 62, 1945-1051

Simon T P and Lyons J, 1995 *Application of the Index of Biotic Integrity to evaluate water resource integrity in freshwater ecosystems*, In Biological assessment and criteria. Tools for water resource planning and decision making. (ed. W S Davis and T P Simon), pp. 167-180

Simonsen R, 1969 Diatoms as indicators in estuarine environments. *Veröffentl. Inst. Meeresforsch. Bremerhaven*, **11**, 287-291

Simpson G, 2001 *Biological targets for recovery from lake acidification: developing the analogue matching procedure*. In Critical Loads of Acidity and Metals (CLAM): Work Package 2 - Recovery. Final Project Report to the Department of the Environment, Transport and the Regions, Contract No. EPG 1/3/117 (ed. C J Curtis). Environmental Change Research Centre, University College London

Simpson J C and Norris R H, 2000 *Biological assessment of river quality: development of AUSRIVAS models and outputs.* In Assessing the Biological quality of fresh waters: RIVPACS and other techniques (ed. J F Wright, D W Sutcliffe and M T Furse)**,** pp.125-142

Simpson J, Norris R, Barmuta L and Blackman P, 1996 *Australian River assessment system: National river health program predictive model manual,* http.//ausrivas.canberra.au

Simpson J C and Norris R H, 2000 *Biological assessment of river quality: development of AUSRIVAS models and outputs.* In Assessing the biological quality of fresh waters: RIVPACS and other techniques (ed. Wright J F, Sutcliffe D W and Furse M T)**,** pp.125-142, Proceedings of an International Workshop, Oxford, September 1998. Freshwater Biological Association, Ambleside.

Sin Y, Wetzel R L and Anderson I C, 2000 Seasonal variations of size-fractionated phytoplankton along the salinity gradient in the York River estuary, Virginia (USA). *Journal of Plankton Research,* **22**, 1945-1960

SIS, 1994 *Water quality – methods for biological sampling – guidance on handnet sampling of aquatic benthic macroinvertebrates*. SS-EN 27 828

Slack K V, Averett R C, Greeson P E and Lipscomb R G, 1973 *Methods for collection and analysis of aquatic biological and microbiological samples.* In Techniques of Water-Resources Investigations of the United States Geological Survey, Ch 4A, Bk 5, 1-165, US Department of the Interior, Geological Survey, Washington DC

Sladecek V, 1965 The future of the saprobity system. *Hydrobiologia,* **25,** 518-537

Sladecek V, 1973 System of water quality from the biological point of view. *Archiv fur Hydrobiologie Ergebnisse der Limnologie*, **7,** 1-218

Sladecek V, 1985 Scale of saprobity. *Verhandlungen der Internationale Vereinigung f*ur *theoretische und angewandte Limnologie*, **22**, 2337-2341

Smayda T J, 1990 *Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic*. In Toxic marine phytoplankton (ed. E Graneli, B Sundstrom, L Edler and D M Anderson), pp. 29-40. New York: Elsevier

Smayda T J, 1997 Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnology and Oceanography,* **42**, 1137-1153

Smayda T J and Reynolds C S, 2001 Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. *Journal of Plankton Research,* **23**, 447-461

Smayda T J and Villareal T A, 1986 *The 1985 "brown tide" and the open phytoplankton niche in Narragansett Bay during summer*. In Novel phytoplankton blooms (ed. E M Cosper, V M Bricelj and E J Carpenter), pp. 159-187. Berlin: Springer

Smit G L and Hattingh J, 1978 The effect of respiratory stress on carp haemoglobin. *Comp Biochem Physiol,* **59**, 369-374

Smith P, 2001 Can fish determine the conservation value of shallow lakes in the UK? *British Wildlife,* **13**, 10-15

Smith V H, Tilman G D and Nekola J C, 1999 Eutrophication: impacts of excess nutrient inputs on freshwater, marine and terrestrial ecosystems. *Environmental Pollution*, **100**, 179-196

Smol J P, 1985 The ratio of diatom frustules to chrysophycean statospores: a useful paleolimnological index. *Hydrobiologia*, **123**, 199-208

Smol J P, 1992 Paleolimnology: an important tool for effective ecosystem management. *Journal of Aquatic Ecosystem Health,* **1**, 49-58

Smoot J L, Liebermann T D, Evaldi R D and White K D and United States Geological Survey, 1995 *Water quality assessment of the Kentucky River Basin, Kentucky - analysis of available surface water quality data through 1986. With section on biological indicators of water quality.* USGS Water Supply Paper 2351-B, Washington DC, US Government Printing Service

Solic M, Krstulovic N, Marasovic I, Baranovic A, PucherPetkovic T and Vucetic T, 1997 Analysis of time series of planktonic communities in the Adriatic Sea: Distinguishing between natural and man-induced changes. *Oceanologica Acta,* **20**, 131-143

Solidoro C, Pastres R, Franco D, Pecenik G and Dejak C, 1996 *Sensitivity analysis of a eutrophication model for shallow water environments colonized by Ulva rigida Annali di Chimica*, **86**, 677-684

Sommer U, 1989 *The role of competition for resources in phytoplankton succession*. In Plankton ecology: succession in plankton communities. (ed. U Sommer), pp. 57-106. Berlin: Springer

Sørensen J, Jørgensen B B and Revsbech N P, 1979 A comparison of oxygen, nitrate and sulphate respiration in coastal marine sediments. *Microbial Ecol*, **5**, 105 - 115

Soulsby P G, Lowthion D and Houston M C M, 1978 Observations on the effects of sewage discharged into a tidal harbour. *Marine Pollution Bulletin*, **9**, 242-245

Soulsby P G, Lowthion D, Houston, M C M and Montgomery H A L, 1985 The role of sewage effluent in the accumulation of macroalgal mats on intertidal mud flats in two basins in Southern England. *Netherlands Journal of Sea Research*, **16**, 257-263

Sournia A, 1995 *Red tide and toxic marine phytoplankton of the world ocean: an enquiry into biodiversity*. In Harmful marine algal blooms (ed. P Lassus, G Arzul, P Erard, P Gentien and C Marcaillou), pp. 103-112. Paris: Lavoisier/Intercept

Southerland M T and Stribling J B, 1995 *Status of biological criteria development and implementation*. In Biological assessment and criteria. Tools for water resource planning and decision making. (ed. W S Davis and T P Simon), pp. 81-96, Lewis Publishers, Boca Raton, Florida

Southgate B A, 1972 *Langstone Harbour study*. Report to Hampshire River Authority and the Hampshire County Council, 20pp

Southy J (19..) Flora of some Welsh Rivers, MSc Thesis

Sparks R E and Sandusky M J, 1981 Identification of factors responsible for decreased production of fish food organisms in the Illinois and Mississippi Rivers. Final report Project 3-291-R. Illinois Natural History Survey River Research Laboratory, Havana, IL, USA

Spence D H N, 1964 '*The macrophytic vegetation of lochs, swamps and associated fens''* In The Vegetation of Scotland (ed. J H Burnett), pp. 306-425. Oliver and Boyd Ltd, Edinburgh

Spence D H N, 1967 Factors controlling the distribution of freshwater macrophytes with particular reference to the lochs of Scotland. *Journal of Ecology*, **55**, 147-170

Spindler P, 1996 *Using ecoregions for explaining macroinvertebrate community distribution among reference sites in Arizona, 1992.* Arizona Department of Environmental Quality, Hydrologic Support and Assessment Section, Flagstaff, Arizona

Srivastava D S, Staicer A and Freedman B, 1995 Aquatic vegetation of Nova Scotian lakes differing in acidity and trophic status. *Aquatic Botany*, **51**, 181-196

Standing Committee of Analysts, 1986 Methods for the Use of Aquatic Macrophytes for Assessing Water Quality 1985 - 86. HMSO 1987

Staver K W and Brinsfield R B, 1996 Seepage of groundwater nitrate from a riparian agroecosystem into the Wye River estuary. *Estuaries*, **19**, 359-370

Steedman R J, 1988 Modification and assessment of an index of biotic integrity to quantify stream quality in southern Ontario. *Canadian Journal of Fisheries and Aquatic Science,* **45,** 492-501

Steemann Nielsen E, 1952 The use of radio-active carbon (C^{14}) for measuring organic production in the sea. *Journal du Conseil Permanent International pour l'Exploration de la Mer.,* **18**, 117-140

Stevenson R J, 1996 *An introduction to algal ecology in freshwater benthic habitats*. In Algal ecology: freshwater benthic ecosystems (ed. R J Stevenson, M L Bothwell and R L Lowe), pp. 3-30. San Diego: Academic Press

Stockner J G and MacIsaac E A, 1996 British Columbia lake enrichment programme: two decades of habitat enhancement for Sockeye Salmon. *Regulated Rivers Research and Management*, **12**, 547-561

Stockner J G, Rydin E and Hyenstrand, 2000 Cultural oligotrophication: causes and consequences for fisheries resources. *Fisheries*, **25**, 7-14

Stribling J B, Snyder B D and Davis W S, 1996 *Biological assessment methods, biocriteria, and biological indicators. Bibliography of selected technical, policy* *and regulatory literature*. EPA 230-B-96-001, US Environmental Protection Agency, Office of Policy, Planning, and Evaluation, Washington, DC

Sullivan B K and Banzon P V, 1990 Food limitation and benthic regulation of populations of the copepod *Acartia hudsonica* Pinhey in nutrient-limited and nutrient-enriched systems. *Limnol Oceanogr*, **35**, 1618-1631

Sullivan B K and Ritacco P J, 1985 Ammonia toxicity to larval copepods in eutrophic marine ecosystems: a comparison of results from bioassays and enclosed experimental ecosystems. *Aqua Toxic*, **7**, 205-217

Sullivan M J, 1999 *Applied diatom studies in estuaries and shallow coastal environments*. In The Diatoms: Applications for the Environmental and Earth Sciences (ed. E F Stoermer and J P Smol), pp. 334-351. Cambridge University Press, Cambridge

Summers J K and Engle V D 1993 Evaluation of sampling strategies to characterise dissolved oxygen conditions in northern Gulf of Mexico estuaries. *Environmental Monitoring and Assessment,* **24**, 219-229

Sunda W G, 1989 Trace metal interactions with marine phytoplankton. *Biological Oceanography,* **6**, 411-442

Sündback K, Carlson L, Nilsson C, Jonsson B, Wulff A and Odmark S, 1996 Response of benthic microbial mats to drifting green algal mats. *Aquatic Microbial Ecology*, **10**, 195-208

Sündback K, Jonsson B, Nilsson P and Lindstrom I, 1990 Impact of accumulating drift macroalgae on a shallow-water sediment system: an experimental study. *Marine Ecology Progress Series*, **58**, 261-274

Swedish Environmental Protection Agency, 2000 *Lakes and Watercourses: Environmental Quality Criteria*. Report 5050

Sweeting R A, Lowson D, Hale P and Wright J F, 1993 *1990 Biological assessment of rivers in the UK.* In River water quality - ecological assessment and control (ed. P J Newman, M A Piavaux and R A Sweeting), pp. 319-326

Sykes J M, Lane A M J and George D G, 1999 *The United Kingdom Environmental Change Network Protocols for Standard Measurements at Freshwater Sites*: ITE Monks Wood

Tang E P Y, 1996 Why do dinoflagellates have lower growth rates? *Journal of Phycology,* **32**, 80-84

Tansley A G, 1939 *The British islands and their vegetation*. Cambridge: Cambridge University Press

Taylor D I, Nixon S W, Granger S L, Buckley B A, McMahon J P and Lin H J, 1995 Responses of coastal lagoon plant communities to different forms of nutrient enrichment – a mesocosm experiment. *Aquatic Botany*, **52**, 19-34

Taylor P, 1993 The state of the marine environment: A critique of the work and role of the Joint Group of Experts on Scientific Aspects of Marine Pollution (GESAMP). *Marine Pollution Bulletin*, **26**, 120-127

Taylor R, 1999 The ‘green tide’ threat in the UK – a brief overview with particular reference to Langstone Harbour, south coast of England and the Ythan Estuary, east coast of Scotland. *Botanical Journal of Scotland*, **51**, 195-203

Taylor R and Raven J A, 2001 *Survival strategies of* Enteromorpha *from eutrophic sites*. Coastal Zone Topics Series, In press

Taylor R, Fletcher R L and Pye K, 1998 *The ‘green tide’ phenomenon in the North Sea and English Channel: case studies of the Ythan estuary and Langstone Harbour*. In Changes in the marine flora of the North Sea (ed. G W Scott and I Tittley), pp. 166 CERCI Publications

Taylor R, Fletcher R L and Raven J A, 2001 Preliminary studies on the growth of selected 'green tide' algae in laboratory culture – effects of iradiance, temperature, salinity and nutrients on growth rate. *Botanica Marina*, **44**, In press

ter Braak C J F and Juggins S, 1993 Weighted averaging partial least squares (WA-PLS): an improved method for reconstructing environmental variables from species assemblages. *Hydrobiologia,* **269/270**, 485-502

ter Braak C J F and van Dam H, 1989 Inferring pH from diatoms: a comparison of old and new calibration methods. *Hydrobiologia*, **178**, 209-223

Tett P, 1992 The Ecology of Plankton in Scottish Coastal Waters. *Proceedings of the Royal Society of Edinburgh Section B- Biological Sciences,* **100**, 27-54

Tett P, Kennaway G M, Boon D, Mills D K, O'Connor G T, Walne A W and Wilton R, 2001 Optical monitoring of phytoplankton blooms in Loch Striven, a eutrophic fjord. *International Journal of Remote Sensing,* **22**, 339-358

Thienemann A, 1910 Das Sammeln von Puppenhauten der Chironomiden. *Archiv Fur Hydrobiologie,* **6**, 213-214

Thienemann A, 1921 Biologische Seetypen und die Grundung einer Hydrobiologischen Ansalt am Bodensee. *Archiv Fur Hydrobiologie,* **13**, 347-370

Thienemann A, 1922 Die beiden Chironomus-arten der Tiefenfauna der norddeutschen Seen. Ein Hydrobiolgisches Problem. *Archiv Fur Hydrobiologie,* **13**, 609-646

Thienemann A, 1925 *Die Binnengewasser Mitteleuropas*. Stuttgart: Schweizerbart'sche Verlagbuchhandl.

Thoms M C, Ogden R W and Reid M A, 1999 Establishing the condition of lowland floodplain rivers: a palaeo-ecological approach. *Freshwater Biology*, **41**, 407-423

Thorne R S J and Williams W P, 1997 The response of benthic macroinvertebrates to pollution in developing countries: a multimetric system of bioassessment. *Freshwater Biology*, **37**, 671-686

Thurston R V, 1983 Acute toxicity of ammonia to rainbow trout. *Trans Am Fish Soc*, **112**, 696-704

Thurston R V, Russo R C and Phillips G R, 1983 Acute toxicity of ammonia to fathead minnows. *Trans Am Fish Soc,* **112**, 705-711

Thurston R V, Russo R C and Vinogradov G A, 1981 Ammonia toxicity to fishes. Effect of pH on the toxicity of the un-ionised ammonia species. *Environ Sci Technol*, **15**, 837-840

Toivonen H and Huttunen P, 1995 Aquatic macrophytes and ecological gradients in 57 small lakes in southern Finland. *Aquatic Botany,* **51,** 197-221

Toivonen J, 1972 The fish fauna and limnology of large oligotrophic glacial lakes in Europe about 1800 A.D. *Journal of the Fisheries Research Board of Canada*, **29**, 629-637

Tomasso J R and Carmichael G J, 1986 Acute toxicity of ammonia, nitrite and nitrate to the Guadalupe bass (*Micropterus treculi*). *Bull Environ Contam Toxicol*, 36, 866-870

Tomasso J R, Simco B A and Davis K B, 1979 Chloride inhibition of nitrite-induced methaemoglobinemia in channel catfish (*Ictalurus punctatus*). *J Fish Res Board Can*, 36, 1141-1144

Tompsett P E, 1994 *Helford river survey. Monitoring report No 4 for 1993*. Report to World Wide Fund for Nature UK

Townsend C R and Perrow M R, 1989 Eutrophication may produce population cycles in roach *Rutilus rutilus* L., by two contrasting mechanisms. *Journal of Fish Biology*, **34**, 161-164

Townsend D W, Cammen L M, Holligan P M, Campbell D E and Pettigrew N R, 1994 Causes and consequences of variability in the timing of spring phytoplankton blooms. *Deep-Sea Research Part a-Oceanographic Research Papers,* **41**, 747-766

Traill G W, 1886 *The marine algae of Joppa in the county of Mid-Lothian*. Transcripts of the Proceedings of the Botanical Society of Edinburgh, **16**, 395-402

Trama F B, 1954 The acute toxicity of some common salts of sodium, potassium and calcium to the bluegill (*Lepomis macrochirus* Rafinesque). *Proc Acad Nat Sci. Phili*, **106**, 185-205

Traunspurger W and Drews C, 1996 Toxicity analysis of freshwater and marine sediments with meio- and macrobenthic organisms: a review. *Hydrobiol*, **328**, 215-261

Trimmer M, Nedwell D B, Sivyer D B and Malcolm S J, 1998 Nitrogen fluxes through the lower estuary of the river Great Ouse, England: the role of the bottom sediments. *Marine Ecology-Progress Series,* **163**, 109-124

Trimmer M, Nedwell D B, Sivyer D and Malcolm S J, 2000a Seasonal organic mineralisation and denitrification in intertidal sediments and their relationship to the abundance of *Enteromorpha* sp. and *Ulva* sp. *Mar Ecol Prog Ser*, **203**, 67-80

Trimmer M, Nedwell D B, Sivyer D B and Malcolm S J, 2000b Seasonal benthic organic matter mineralisation measured by oxygen uptake and denitrification along a transect of the inner and outer River Thames estuary, UK. *Mar Ecol Prog Ser*, **197**, 103-119

Tsai C F, Wiley M and Chai A L, 1991 Rise and fall of the Potomac River striped bass stock- a hypothesis of the role of sewage. *Trans Am Fish Soc*, **120**, 1-22

Tubbs C R, 1977 Wildfowl and waders in Langstone Harbour. *British Birds*, **70**, 177-199

Tubbs C R and Tubbs J M, 1983 Macroalgal mats in Langstone Harbour, Hampshire, England*. Marine Pollution Bulletin*, **4**, 148-149

Turner R E and Rabelais N N, 1991 *Changes in Mississippi water quality this century.* Bioscience*,* **41,** 140-7, In Stream Ecology Structure and Function of Running waters J D Allen - Chapman and Hall 1995

Turner R E and Rabalais N N, 1994 Coastal eutrophication near the Mississippi river delta. *Nature*, **368**, 619-621

Twilley R R, Kemp W M, Staver K W, Stevenson J C and Boynton W R, 1985 Nutrient enrichment of estuarine submersed vascular plant communities.1. Algal growth and *effects on production of plants and associated communities. Marine Ecology Progress Series*, **23**, 179-191

Twomey L and Thompson P, 2001 Nutrient limitation of phytoplankton in a seasonally open bar- built estuary: Wilson Inlet, Western Australia. *Journal of Phycology,* **37**, 16-29

UK Biodiversity Action Plan http://www.ukbap.org.uk/

Umweltforschungsplan des Bundesministers fur Umwelt, Naturschutz und Reaktorsicherheit. Wasserwirtschaft. Forschngsbericht 102 01 504. Munchen: Bayerisches Landesamt fur Wasserwirtschaft, Germany.

Underwood G J C, 1994 Seasonal and spatial variation in epipelic diatom assemblages in the Severn estuary. *Diatom Research*, **9,** 451-472

Underwood G J C, Phillips J and Saunders K, 1998 *Distribution of estuarine benthic diatom species along salinity and nutrient gradients*. European Journal of Phycology, **33**, 173-183

US Department of Agriculture (USDA) Soil Conservation Service, 1981 *Land Resource regions and major land resource areas of the United States*. Agricultural Handbook 296, US Government Printing Service, Washington DC

US Environmental Protection Agency (USEPA), 1973 *Appendix II-B Community structure and diversity indices*. In 'Water Quality Criteria' 1972, 408-409, EPA-R3-73-033 EPA Washington USA

US Environmental Protection Agency, 1985 Ambient water quality criteria for ammonia-1984. US EPA Office of Water, Washington DC, USA.

US Environmental Protection Agency (USEPA), 1987 *Surface water monitoring: A framework for change*. USEPA, Office of Water, Office of Policy, Planning and Evaluation, Washington DC

US Environmental Protection Agency, 1989 *Ambient water quality criteria for ammonia (saltwater) –1989*. EPA 440/5-88-004. Office of water regulations and standards, criteria and standards division, Washington DC, USA

US Environmental Protection Agency (US EPA), 1991 *Biological criteria: State development and implementation efforts*. EPA-440/5-91-003, US Environmental Protection Agency, Office of Water, Washington DC

US Environmental Protection Agency (US EPA), 1991 *Biological criteria: Guide to technical literature*. EPA-440-5-91-004, US Environmental Protection Agency, Office of Water, Washington DC

US Environmental Protection Agency, 1994 *Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods*. EPA/600/R-94/025. Final Report. Washington, DC

US Environmental Protection Agency (US EPA), 1996 *Biological Criteria, Technical Guidance for streams*. Rivers Revised Edition EPA 822-B-96-001

US Environmental Protection Agency 1998 *Lake and Reservoir Bioassessment and Biocriteria. Technical Guidance Document.* EPA 841-B-98-007. Office of Water, Washington DC (4504F)

US Environmental Protection Agency (US EPA), 1998 *Technical Guidance Document: About Indices employing macroinvertebrates as indicators of nutrient pollution*

US Environmental Protection Agency (US EPA), 1999 *Rapid Bioassessment Protocols for use in wadeable streams and rivers: periphyton, benthic macroinvertebrates, and fish*. EPA 841-B-99-002

US Environmental Protection Agency (US EPA), 2000 *National Nutrient Assessment Strategy: An overview of Available Endpoints and Assessment Tools*. 2000, http://www.epa.gov/OWOW/NPS/proceedings/overview.html, USEPA: Office of Water: Washington DC

US Environmental Protection Agency, 2000a *Nutrient criteria technical guidance manual. Lakes and reservoirs*. 1st edition. EPA-822-B00-001. Office of Water and Technology, Washington DC. 20460

US Environmental Protection Agency, Office of Water 2000b *Estuarine and coastal marine waters: Bioassessment and Biocriteria Technical Guidance.* EPA 822-B-00-024. www.epa.gov/ost/biocriteria/States/estuaries/chpt_04.htm

Usseglio-Polatera P and Bournaud M, 1989 Trichoptera and Ephemeroptera as indicators of environmental changes of the Rhone River at Lyons over the last twenty-five years. *Regulated Rivers: Research and Management,* **4,** 249-262

Valiela I, Foreman K, LaMontagne M, Hersh D, Costa J, Peckol P, DeMeo-Anderson B, d'Avanzo C, Babione M, Sham C H, Brawley J and Lajtha K, 1992 Coupling of watersheds and coastal waters: sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. *Estuaries*, **15**, 443-457

Valiela I, McClelland J, Hauxwell J, Behr P J, Hersh D and Foreman K, 1997 Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography*, **42**, 1105-1118

Valiela I, Tomasky G, Hauxwell J, Cole M L, Cebrian J and Kroeger K D, 2000 Operationalizing sustainability: Management and risk assessment of land-derived nitrogen loads to estuaries. *Ecological Applications*, **10**, 1006-1023

Van Sprang P A and Janssen C R 1997 Identification and confirmation of ammonia toxicity in contaminated sediments using a modified toxicity identification evaluation approach. *Environ Toxicol Chem*, **16**, 2501-2507

Vannekom P M and Salomons W, 1980 *Pathways of nutrients and organic matter from land to ocean through rivers*. In River inputs to ocean systems (ed. J M Martin, J D Burton and D Eisma), pp 33-51. UNESCO, Paris

Vannote R L, Minshall G W, Cummins K W, Sedell J R and Cushing C E, 1980 The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences,* **37**, 130-137

Vermont Agency of Natural Resources, Department of Environmental Conservation – Information and Resources, 1998 *Biocriteria Development for Vermont Lakes. Pilot and Field Phases.* Project Summary, April, 1998.

Vernaux J and Tuffery T, 1976 Une methode zoologique pratique de determination de la qualite biologique des eaux courantes. *Annales Scientifiques de l'Universite de Besancon, Zoologie*, **3,** 79-90

Vernaux J, Galmiche P, Janier F and Monnot A, 1982 Une nouvelle methode pratique d'evaluation de la qualite des eaux courantes. Un indice biologique de qualite generale (BIG). *Annales Scientifiques de l'Universite de Besancon, Biologie Animale*, **3,** 11-21

Vernberg F J, 1972 Dissolved gases. Animals. In Marine Ecology (ed. O Kinne),

pp. 1491-1526. London. Wiley-Interscience

Vestergaard O and Sand-Jensen K, 2000a Aquatic macrophyte richness in Danish lakes in relation to alkalinity, transparency, and lake area. *Can. J. Aquat. Sci.*, **57**, 2022-2031

Viaroli P, Bartoli M, Bondavalli C and Christian R R, 1996 Macrophyte communities and their impact on benthic fluxes of oxygen, sulphide and nutrients in shallow eutrophic environments. *Hydrobiol*, **329**, 105-119

Viaroli P, Bartoli M, Bondavalli C, Naldi M and Ferrari I, 1994 *Macroalgae growth and decomposition and nutrient recycling in the Sacca di Gora (Po River Delta).* In Proceedings of the CLEAN Meeting - Coastal Lagoons Eutrophication and Anaerobic Processes (Alicante 1993) (ed. P Caumette), pp. 281-307. Commission of the European Communities, Environmental Research Programme, Brussels – Laboratoire d'Oceanographie biologique, Universite Bordeaux I, France

Vieira J M P, Pinho J L S and Duarte A A L S, 1997 Eutrophication vulnerability analysis: a case study, Eutrophication Research. State of the art: inputs, processes, effects modelling, management, 1998 *Water Science & Technology*, **37**, 121-128

Vighi M and Chiaudani G, 1985 A Simple Method to Estimate Lake Phosphorus Concentrations Resulting from Natural, Background, Loadings. *Water Research,* **19**, 987-991

Virbickas T, 1998 Regularities of changes in the production of fish populations and communities in Lithuanian rivers of different types. *Acta Zoologica Lituanica Hydrobiologia*, **8**, 1-67

Vismann B, 1990 Sulphide detoxification and tolerance in *Nereis* (*Hediste*) *diversicolor* and *Nereis* (*Neanthes*) *virens* (Annelida: Polychaeta). *Mar ecol Prog Ser*, **59**, 229-38

Vogt H and Schramm W, 1991 Conspicuous decline of Fucus in Kiel Bay (Western Baltic): what are the causes? *Marine Ecology Progress Series*, **69**,189-194

Vollenweider R A, 1968 *Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication*. Technical Report DAS/CSI 68.27, Organisation for Economic Cooperation and Development, Paris

Volstad J H, Heimbuch D H, Southerland M T, Jacobson P T, Chaillou J A, Weisberg S B and Wilson H T, 1995 Maryland Biological Stream Survey: The 1993 pilot study, Maryland Department of Natural Resources, Chesapeake Bay Research and Monitoring Division, Annapolis Maryland

Vos P C and de Wolf H, 1993 Diatoms as a tool for reconstructing sedimentary environments in coastal wetlands; methodological aspects. *Hydrobiologia*, **269/270**, 285-296

Wade A J, Whitehead P G, Hornberger G M and Snook D L, 2001a (in press) On modelling the flow controls on macrophyte and epiphyte dynamics in a lowland UK catchment: River Kennet, southern England. *Science of the Total Environment*

Wade A J, Whitehead P G, Hornberger G M, Jarvie H P and Flynn N, 2001b (in press) On modelling the impacts of phosphorus stripping at sewage works on in-stream phosphorus and macrophyte/epiphyte dynamics: a case study for the River Kennet. *Science of the Total Environment*

Wajsbrot N, Gasith A, Krom M D and Samocha T M, 1990 Effect of dissolved oxygen and the molt stage on the acute toxicity of ammonia to the juvenile tiger prawn *Penaeus semisulcatus*. *Environ Toxicol Chem*, **9**, 497-504

Walker I R, 1993 *Paleolimnological biomonitoring using freshwater benthic macroinvertebrates*. In Freshwater Biomonitoring and Benthic Macroinvertebrates (ed. D M Rosenberg and V Resh), pp. 306-343. London: Chapman and Hall

Wallentinus I, 1981 *Chemical constituents of some Baltic macroalgae in relation to environmental conditions*. In Proceedings of the 10th International Seaweed Symposium (ed. T Levring), pp. 363-370. Walker de Gruyter & Co., New York

Walley W J and Fontama V N, 2000 *New approaches to river quality classification based on RIVPACS*. In Assessing the biological quality of fresh waters. RIVPACS and other techniques. Proceedings of an International Workshop, Oxford, September 1998 (ed. J F Wright, D W Sutcliffe and M T Furse)**,** pp.263-280. Freshwater Biological Association, Ambleside

Walton S P, Welch E B and Horner R R, 1995 Stream periphyton response to grazing and changes in phosphorus concentration. *Hydrobiologica,* **302,** 31-46

Ward R C, Loftis J C and McBride G B, 1986 The 'data-rich but information poor' syndrome in water quality monitoring. *Environmental Management,* **10,** 291-297

Warwick R M, Davey J T, Gee J M and George C L, 1982 Faunistic control of Enteromorpha blooms: a field experiment. *Journal of Experimental Marine Biology and Ecology*, **56**, 23-31

Washington H G, 1984 Diversity, biotic and similarity indices. A review with special relevance to aquatic ecosystems. *Water Research,* **18,** 653-694

Watson P G, Frickers P E and Howland R J M, 1993 Benthic fluxes of nutrients and some trace metals in the Tamar estuary, S. W. England. *Neth J Aqua Ecol*, **24**, 135-146

Watson V J (ed), 1990 *Proceedings, 1990 Clark Fork River Symposium.* Montana Academic Sciences

Wear D J, Sullivan M J, Moore A D and Millie D F, 1999 Effects of water-column enrichment on the production dynamics of three seagrass species and their epiphytic algae. *Marine Ecology Progress Series*, **179**, 201-213

Weber C I (ed), 1973 *Biological Field and Laboratory Methods for Measuring Quality of Surface Waters and Effluents.* EPA-670/4-73-001, US Environmental Protection Agency, Cincinnati, OH

Weckstrom K, Clark A, Juggins S, Korhola A and Anderson N J, 2000 *Inferring long-term trends in eutrophication and nutrient concentrations in the coastal zone of the Baltic Sea using diatom transfer functions.* 8th International Palaeolimnology Symposium Abstracts Volume, p 106-107

Weisberg S B, Frithsen J B, Holland A F, Paul J F, Scott K J, Summers J K, Wilson H T, Heimbuch D G, Gerritsen J, Schimmel S C and Latimer R W, 1993 *EMAP-Estuaries Virginian Province 1990 Demonstration Project Report.* EPA/620/R-93/006. Environment Research Laboratory, USEPA, Marragansett, RI

Welch E B, Horner R R and Patmont C R, 1989 Prediction of nuisance periphytic biomass: a management approach. *Water Research,* **23**, 401-405

Wendker S, 1990 *Untersuchungen zur subfossilen und rezenten Diatomeenflora des Schlei-Ästuars (Ostsee).* In Bibliotheca Diatomologica 20 (ed. H Lange-Bertalot), pp. 1-268. J. Cramer, Berlin

Wennberg T, 1992 Colonization and succession of macroalgae on a breakwater in Laholm Bay, a eutrophicated brackish water area (SW Sweden). *Acta Phytogeographica Suecica*, **78**, 65-77

West G, 1910 *A further contribution to a comparative study of the dominant phanerogamic and higher cryptogamic flora of aquatic habit in Scottish Lakes* In Proceedings of the Royal Society of Edinburgh, **30**, 65-81

Westin D T, 1974 Nitrate and nitrite toxicity to salmonid fishes. *Prog Fish Culturist,* **36**, 86-89

Wetzel R G, 1983 *Limnology*, Saunders, New York

Wetzel R G, 2001 *Limnology. Lake and River Ecosystems*. San Diego: Academic Press

Wetzel R G and Likens G E, 2000 *Limnological Analyses*. New York: Springer-Verlag

Wharfe J R, 1977 The intertidal sediment habitats of the lower Medway Estuary, Kent. *Environmental Pollution*, **13**, 79-91

Whiles M R, Brock B l, Franzen A C and Dinsmore S C, 2000 Stream invertebrate communities, water quality, and landuse patterns in an agricultural drainage basin of northeastern Nebraska, USA. *Environmental Management,* **26**, 563-576

Whipple G C, 1898 Classification of lakes according to temperature. *American Naturalist*, **32**, 25-53

Whitehurst I T and Lindsey B I, 1990 The impact of organic enrichment on the benthic macroinvertebrate communities of a lowland river. *Water Research,* **24,** 625-630

Whiteman F W, Ankley G W, Kahl M D, Rau D M and Balcer M D 1996 Evaluation of interstitial water as a route of exposure for ammonia in sediment tests with benthic macroinvertebrates. *Environ Toxicol Chem*, **15**, 794-801

Whitfield M, 1974 The hydrolysis of ammonium ions in seawater -a theoretical study. *J Mar Biol Assoc UK,* **54**, 565-580

Whittier T, Hughes R M and Larsen D P, 1988 Correspondence between ecoregions and spatial patterns in stream ecosystems in Oregon. *Canadian Journal of Fisheries and Aquatic Sciences*, **45,** 1264-1278

Whitton B A (ed), 1975 *River Ecology*. University of California Press, Berkley CA

Whitton B A and Kelly M G, 1995 Use of algae and other plants for monitoring rivers. *Australian Journal of Ecology*, **20**, 45-56

Whitton B A and Rott E, 1991 *Use of Algae for Monitoring Rivers II.* Institut fur Botanik, Innsbruck. 200pp

Whitton B A and Rott E (Eds), 1996 *Use of Algae for Monitoring Rivers III.* Proceedings of an international conference, Innsbruck, Austria Sept 1995, 196pp

Whitton B A, Holmes N T H and Sinclair C, 1978 *A coded list of 1000 Freshwater Algae of the British Isles*. Water Archive Manual Series No 2, Department of the Environment Water Data Unit, 335 pp

Whitton B A, Kelly M G, Harding J P C and Say P J, 1991 *Use of Plants to Monitor Heavy Metals in Freshwaters*. Methods for the Examination of water and associated materials. HMSO, London

Whitton B A, Rott E and Friedrich G (Eds), 1991 *Use of Algae for Monitoring Rivers I.* Institute fur Botanik, Univ. Innsbruck, Austria 1991, 200pp

Wiederholm I, 1984 *Responses of aquatic insects to environmental pollution*. In Ecology of aquatic insects (ed. V H Resh and D M Rosenberg), pp. 508-557, Praeger Publications New York

Wiederholm T, 1976 Chironomids as indicators of water quality in Swedish lakes. *Naturvardsverkets Limnologiska Undersokningar,* **10**, 1-17

Wiederholm T, 1980 Use of benthos in lake monitoring. *Journal of the Water Pollution Control Federation,* **52**, 537-547

Wiederholm T, 1984 *Responses of aquatic insects to environmental pollution*. In The ecology of aquatic insects (ed. D M Rosenberg & V H Resh). New York: Praeger

Wiegleb G, 1978 Investigation of the relationship between hydrochemical environmental factors and the macrophyte vegetation in standing waters. *Archive fur Hyrobiologia*, **83**, 443-484

Wiener J G, 1983 Comparative analyses of fish populations in naturally acidic and circumneutral lakes in northern Wisconsin. *U.S. Fish and Wildlife Service Air Pollution and Acid Rain Report*, **16**, 1-108

Wilander A R K, Johnson W, Goedkoop and Lundin L, 1998 *Riksinventering 1995. En synoptisk studie av vattenkemi och bottenfauna I svenska sjoar och vattendrag.* SNV 4813, Statens Naturvardsverk, Stockholm, Sweden

Wilce R T, Schneider C W, Quinlan A V and van den Bosch K, 1982 The life history and morphology of free living Pilayella littoralis (L.) Kjellm (Ectocarpaceae, Ectocarpales) in Natiant Bay, Massachusetts. *Phycologia***,** **21**, 336-354

Wilhm J L, 1970 Range of diversity index in benthic macroinvertebrate populations. *Journal of the Water Pollution Control Federation,* **42,** 221-224

Wilhm J L, 1975 *Biological indicators of pollution*. In River Ecology (ed, BA Whitton)**,** pp. 375-402, University of California Press, Berkley, CA

Wilhm J L and Doris T C, 1968 Biological parameters for water quality criteria. *Bioscience,* **18,** 477-480

Wilkinson L, 1963 Nitrogen transformations in a polluted estuary. *International Journal of A*ir *and Water Pollution*, **7**, 737-752

Wilkinson M and Titley I, 1979 The marine algae of Elie, Scotland: a reassessment. *Botanica Marina*, **22**, 249-256

Williams P, Biggs J, Whitfield M, Corfield A, Fox G and Adare K, 1998 *Biological techniques of still water quality assessment: Phase II Method development*, R&D Technical Report E56, Bristol: Environment Agency

Williams S J, Dodd D and Gohn K K, 1991 *Coasts in crisis*. U S Geol Surv Circ 1075

Wilson D M, Teresa J N, Wiener J G, Anderson R V, Sandheinrich M B and Sparks R E, 1995 Declining populations of the fingernail clam *Musculium transversum* in the upper Mississippi River. *Hydrobiol*, **304**, 209-220

Wilson J G, Jeffrey D W, Madden B and Rafferty C, 1990 *Algal mats and eutrophication in Dublin Bay, Ireland.* Presentation made at the 25th European Marine Biology Symposium, Sept. 10-15, 1990, Ferrara, Italy

Wilson R S and McGill J D, 1977 A new method of monitoring water quality in a stream receiving sewage effluent, using chironmid pupal exuviae. *Water Research,* **11**, 959-962

Wilson W H, 1991 Competition and predation in marine soft-sediment communities. *Ann Rev Ecol Syst*, **21**, 221-241

Windust A J, Wright J L C and McLachlan J L, 1996 The effects of the diarrhetic shellfish poisoning toxins, okadaic acid and dinophysistoxin-1, on the growth of microalgae. *Marine Biology,* **126**, 19-25

Winget R N and Magnum F A, 1979 *Biotic condition index: Integrated biological, physical, and chemical stream parameters for management*, Intermountain Region, US Department of Agriculture, Forest Service, Ogden, Utah

Winter J G and Duthie H C, 2000 Stream epilithic, epipelic and epiphytic diatoms: habitat fidelity and use in biomonitoring. *Aquatic Ecology,* **34**, 345-353

Wollast R, 1983 Interactions in estuaries and coastal waters. In The major biogeochemical cycles and their interactions (ed. B Bolin and R B Cook), pp. 385-407. SCOPE Report 21. Wiley, New York

Woo N Y S and Chiu S F, 1997 Metabolic and osmoregulatory responses of the sea bass Lates calcarifer to nitrite exposure. *Environ Toxicol Chem*, **12**, 257-264

Wood S C, Johansen K and Weber R E, 1975 Effects of ambient PO_2 on haemoglobin oxygen affinity and red cell ATP concentrations in a benthic fish, *Pleuronectes platessa*. *Resp Physiol*, 25, 259-267

Woodiwiss F S, 1964 The biological system of stream classification used by the Trent River Board. *Chemistry and Industry,* **83,** 443-447

Worf D L (ed), 1980 *Biological Monitoring for Environmental Effects,* D C Heath, Lexington, MA

World Health Organisation, 1998. *Guidelines for safe recreational-water environments. Vol 1: Coastal and freshwaters*. Draft for consultation, October 1998, WHO, Geneva. http://www.who.int/water_sanitation_health/Recreational_water/eosdraft9814.htm

Worm B and Sommer U, 2000 Rapid direct and indirect effects of a single nutrient pulse in a seaweed-epiphyte-grazer system. *Marine Ecology Progress Series*, **202**, 283-288

Wright J F, 1985 Predicting biological change. *Water Bulletin,* **188,** 8-9

Wright J F, 1994 Development of RIVPACS in the UK and the value of the underlying database. *Limnetica,* **10**, 15-31

Wright J F, 1995a Development and use of a system for predicting macroinvertebrates in flowing waters. *Australian Journal of Ecology,* **20,** 181-197

Wright J F, 1995b *Development and use of RIVPACS in the UK*. Abstract of an invited paper given at the first Nordic Benthological Meeting, Uppsala, Sweden May 1995

Wright J F, 1995c *Biological assessment of river quality using RIVPACS.* Abstract of a paper presented at the SNH conference 'Freshwater Quality: Defining the Undefinable?' in Sept 1995

Wright J F, 1996 *Application of a prediction technique for determining the biological quality of high mountain rivers using macroinvertebrates*. Proceedings of the 1st International Conference on the Sierra Nevada, March 1996

Wright J F, 2000 *An Introduction to RIVPACS*. In Assessing the biological quality of freshwaters: RIVPACS and similar techniques. (ed. J F Wright, D W Sutcliffe and M T Furse.)**,** pp. 1-24, Freshwater Biological Association, Ambleside

Wright J F, Armitage P D and Furse M T, 1981 *Analysis of natural river communities in Great Britain, Phase 1 (October 1977-September 1981)*. Report to the Department of the Environment

Wright J F, Furse M T and Armitage P D, 1993 *RIVPACS* - a technique for evaluating the biological quality of rivers in the UK. *European Water Pollution Control,* **3**, 15-25

Wright J F, Furse M T and Armitage P D, 1994 *Use of macroinvertebrate communities to detect environmental stress in running waters*. In Water quality and stress indicators in marine and freshwater systems: linking levels of organisation (ed. D W Sutcliffe), pp. 15-34, Freshwater Biological Association

Wright J F, Furse M T and Moss D, 1998 River classification using Invertebrates: RIVPACS applications, Aquatic Conservation: *Marine and Freshwater Ecosystems*, **8,** 617-631

Wright J F, Moss D and Furse M T, 1998 Macroinvertebrate richness at running-water sites in Great Britain: A comparison of species and family level data. *Verhandlungen der Internationalen Vereinigung Theoretische und Angewandte Limnologie*, **26,** 1174-1178

Wright J F, Armitage P D, Furse M T and Moss D, 1984 The classification of sites on British rivers using macroinvertebrates. *Verhandlungen der Internationalen Vereinigung Theoretische und Angewandte Limnologie,* **22,** 1939-1943

Wright J F, Armitage P D, Furse M T and Moss D, 1985 The classification and prediction of macroinvertebrate communities in British rivers. *Annual Report of the Freshwater Biological Association,* **53,** 80-93

Wright J F, Armitage P D, Furse M T and Moss D, 1988 A new approach to the biological surveillance of river quality using macroinvertebrates. *Verhandlungen der Internationalen Vereinigung Theoretische und Angewandte Limnologie*, **23,** 1548-1552

Wright J F, Armitage P D, Furse M T and Moss D, 1989 Prediction of invertebrate communities using stream measurement. Regulated Rivers: *Research and Management,* **4,** 147-155

Wright J F, Blackburn J H et al, 1996 Macroinvertebrate frequency data for the RIVPACS III sites in Great Britain and their use in conservation evaluation. Aquatic Conservation: *Marine and Freshwater Ecosystems*, **6**, 141-167

Wright J F, Furse M T, Armitage P D and Moss D, 1993 New procedures for identifying running-water sites subject to environmental stress and for evaluating sites for conservation, based on the macroinvertebrate fauna. *Archiv fur Hydrobiologie Ergebnisse der Limnologie,* **127**, 319-326

Wright J F, Gunn R J M, Winder J M, Wiggers R, Vowles K, Clarke R T and Harris I, 2001/in press *A comparison of the macrophyte cover and macroinvertebrate fauna at three sites on the River Kennet in the mid 1970s and late 1990s.* Science of the Total Environment

Wright J F, Moss D, Armitage P D and Furse M T, 1984 A preliminary classification of running-water sites in Great Britain based on macroinvertebrate species and the prediction of community type using environmental data. *Freshwater Biology,* **14,** 221-256

Wright J F, Moss D, Clarke R T and Furse M T, 1997 *Biological assessment of river quality using the new version of RIVPACS (RIVPACS III).* In Proceedings of a Conference organised by Scottish Natural Heritage 'Freshwater Quality: Defining the Indefinable?', London: HMSO

Wright J F, Sutcliffe D W and Furse M T (eds), 2000 *Assessing the biological quality of freshwaters: RIVPACS and similar techniques,* Freshwater Biological Association, Ambleside

Wright J F, Moss D, Armitage P D and Furse M T, 1984 A preliminary classification of running-water sites in Great Britain based on macroinvertebrate species and the prediction of community type using environmental data. *Freshwater Biology*, **14**, 221-256

Wright J F, Sutcliffe D W and Furse M T (ed.) 2000 *Assessing the biological quality of fresh waters. RIVPACS and other techniques*. Proceedings of an International Workshop, Oxford, September 1998. Ambleside: Freshwater Biological Association, 373pp

Wright J F, Armitage P D, Dawson F H, Westlake D F, Furse M T, Gunn R J M, Hopgood H A and Moss D, 1988 *An investigation of the relationship between the aquatic plant and animal communities of rivers*. Unpublished report to Nature Conservancy Council.

Wright R M and Phillips V E, 1992 Changes in the aquatic vegetation of two gravel pits after reducing the fish population density. *Aquatic Botany*, **43**, 43-49

Wunsam S and Schmidt R, 1995 A diatom-phosphorus transfer function for Alpine and pre-alpine lakes. *Memorie Ist. ital. Idrobiol.,* **53**, 85-99

Wunsam S and Schmidt R, 1995 A diatom-phosphorus transfer function for Alpine and pre-alpine lakes. *Memorie dell'Istituto Italiano di Idrobiologia,* **53**, 85-99

Yang M D and Sykes R M, 1998 Trophic-dynamic modelling in a shallow eutrophic river ecosystem. *Ecological Modelling*, **105**, 129-139

Young-Lai W W, Charmantier-Daures M and Charmantier G, 1991 Effect of ammonia on survival and osmoregulation in different life stages of the lobster *Homarus americanus*. *Mar Biol*, **110**, 293-300

Youngs W D and Heimbuch D G, 1982 Another consideration of the morphoedaphic index. *Transactions of the American Fisheries Society*, **111**, 151-153

Zamora-Munoz C and Alba-Tercedor J, 1996 Bioassessment of organically polluted Spanish rivers, using a biotic index and multivariate methods, *Journal of the North American Benthological Society*, **15**, 332-352

Zeeb B A, Christie C E, Smol J P, Findlay D L, Kling H J and Birks H J B, 1994 Response of diatom amd chrysophyte assemblages in Lake 227 sediments to experimental eutrophication. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 2300-2311

Zelinka and Marvan, 1961 Zur Prazisierung der biologischen Klassifikation des Reinheit fliessender Gewasser. *Archiv fur Hydrobiologie*, **57**, 389-407

Appendix 1 List of lakes where reference nutrient conditions have been established using the diatom-TP transfer function approach

Lake	Literature Reference
England	
1. Barton Broad	Bennion *et al*. 2001a
2. Bassenthwaite Lake	Bennion *et al*. 2000b
3. Betton Pool	Brooks *et al*. 2001
4. Clarepool Moss	Bennion *et al*. 1997b
5. Crag Lough	Lewis 1996
6. Crose Mere	Bennion *et al*. 1997b, Johnes *et al*., 2000
7. Eleven Acre lake	Bennion 1993
8. Esthwaite Water	Bennion *et al*. 2000b
9. Felbrigg Lake	Burgess 1998
10. Greenlee Lough	Bennion *et al*. 1997b
11. Groby Pool	Sayer 2001
12. Hatchet Pond	Bennion *et al*. 1997b
13. Hornsea Mere	Johnes *et al*. 2000
14. Loweswater	Bennion *et al*. 2000a
15. Malham Tarn	Bennion *et al*. 1997b
16. Marsworth Reservoir	Bennion 1994
17. Martham South Broad	Bennion *et al*. 1997b
18. Oak Mere	Bennion *et al*. 1997b
19. Rollesby Broad	Bennion *et al*. 2001a
20. Slapton Ley	Johnes *et al*. 2000
21. Sunbiggin Tarn	In progress
22. Upton Broad	Bennion *et al*. 2001a
23. Wastwater	Bennion *et al*. 2000b
24. Windermere South Basin	Johnes *et al*. 2000
25. Wroxham Broad	Bennion *et al*. 2001a
Wales	
1. Llyn Alwen	Allott *et al*. 2001
2. Bosherston Lake	In progress
3. Llyn Cau	Allott *et al*. 2001
4. Llyn Coron	Bennion *et al*. 1996b, Allott *et al*. 2001
5. Llyn Cwellyn	Bennion *et al*. 1997a, Allott *et al* 2001
6. Llyn Dinam	Bennion *et al*. 1996b, Allott *et al*. 2001
7. Llyn Eiddwen	Bennion *et al*. 1998, Allott *et al*. 2001
8. Llyn Fach	Allott *et al*. 2001
9. Llyn Fanod	Bennion *et al*. 1998, Allott *et al*. 2001
10. Llyn Glanmerin	Allott *et al*. 2001
11. Llyn Glasfryn	Allott *et al*. 2001
12. Gloyw Lyn	Allott *et al*. 2001
13. Llyn Gynon	Allott *et al*. 2001
14. Hanmer Mere	Allott *et al*. 2001
15. Llyn Hir	Allott *et al*. 2001
16. Llyn Idwal	Bennion *et al*. 1997a, Allott *et al*. 2001
17. Llyn Lech Owain	Allott *et al*. 2001
18. Llyn Llagi	Allott *et al*. 2001
19. Llangorse Lake	Bennion and Appleby 1999, Allott *et al*. 2001
20. Llyn Mymbyr	Allott *et al*. 2001
21. Llyn Penrhyn	Bennion *et al*. 1996b, Allott *et al*. 2001
22. Lower Talley Lake	Allott *et al*. 2001
23. Upper Talley lake	Allott *et al*. 2001
24. Llyn Tegid	Allott *et al*. 2001, Bennion *et al*. in press

Scotland	
1. Loch Awe North	Bennion *et al*. 2001b
2. Loch Awe South	Bennion *et al*. 2001b
3. Loch of Butterstone	Bennion *et al*. 2001b
4. Carlingwark Loch	Bennion *et al*. 2001b
5. Castle Loch	Bennion *et al*. 2001b
6. Castle Semple Loch	Bennion *et al*. 2001b
7. Loch of Clunie	Finney,1998
8. Loch of Craiglush	Finney 1998
9. Loch Davan	Bennion *et al*. 2001b
10. Loch Doon	Bennion *et al*. 2001b
11. Loch Earn	Bennion *et al*. 2001b
12. Loch Eck	Bennion *et al*. 2001b
13. Loch Eye	Bennion *et al*. 2001b
14. Loch of Harray	Bennion *et al*. 2001b
15. Kilbirnie Loch	Bennion *et al*. 2001b
16. Loch Kinord	Bennion *et al*. 2001b
17. Loch Leven	Bennion *et al*. 2001b
18. Loch Lomond North	Bennion *et al*. 2001b
19. Loch Lomond South	Bennion *et al*. 2001b
20. Loch of Lowes	Bennion *et al*. 2001b
21. Loch Lubnaig	Bennion *et al*. 2001b
22. Loch Maree	Bennion *et al*. 2001b
23. Loch of Marlee	Finney 1998
24. Lake of Menteith	Bennion *et al*. 2001b
25. Mill Loch	Bennion *et al*. 2001b
26. Loch Ness	Jones *et al*. 1997
27. Loch Rannoch	Bennion *et al*. 2001b
28. Loch Shiel	Bennion *et al*. 2001b
29. Loch of Skene	Bennion *et al*. 2001b
30. Loch Ussie	Bennion *et al*. 2001b
Northern Ireland	
1. Lough Allen	Anderson *et al*. 1999
2. Augher Lough	Anderson and Rippey 1994
3. Back Lough	Anderson *et al*. 1999
4. Lough Ballywillin	Anderson 1997b
5. Lough Brantry	Anderson 1997b
6. Lough Bresk	Anderson *et al*. 1999
7. Lough Corbet	Anderson 1997b
8. Lough Creeve	Anderson 1997b
9. Lower Lough Erne	Anderson *et al*. 1999
10. Upper Lough Erne	Anderson *et al*. 1999
11. Friary Lough	Jordan *et al*. 2001
12. Lough Heron	Anderson 1997b
13. Lower Lough MacNean	Anderson *et al*. 1999
14. Upper Lough MacNean	Anderson *et al*. 1999
15. Lough Mann	Anderson *et al*. 1993
16. Lough Oughter	Anderson *et al*. 1999
17. Lough Patrick	Anderson 1997b
18. White Lough	Anderson *et al*. 1993