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


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Keywords
Water framework directive; nutrients; nitrogen; phosphorus; rivers; lakes; transitional waters; coastal waters; eutrophication; ecological status; ecology; phytoplankton; phytobenthos, macrophytes; benthic invertebrates; fish; reference conditions.

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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 morphoeaphic 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

<table>
<thead>
<tr>
<th></th>
<th>Rivers</th>
<th>Lakes</th>
<th>Transitional</th>
<th>Coastal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phytoplankton</strong></td>
<td>Carvalho</td>
<td>Carvalho</td>
<td>Johnston</td>
<td>Johnston</td>
</tr>
<tr>
<td><strong>Phytobenthos</strong></td>
<td>Carvalho</td>
<td>Carvalho</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Macrophytes</strong></td>
<td>Dawson</td>
<td>Luckes/Monteith</td>
<td>Taylor</td>
<td>Taylor</td>
</tr>
<tr>
<td>(angiosperms/macroalgae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Benthic Invertebrates</strong></td>
<td>Winder/Furse</td>
<td>Gunn</td>
<td>Hughes/Trimmer</td>
<td>Hughes/Trimmer</td>
</tr>
<tr>
<td><strong>Fish</strong></td>
<td>Maitland</td>
<td>Maitland</td>
<td>Hughes/Trimmer</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Rivers, lakes, transitional and coastal waters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spatial-state reference schemes</strong></td>
<td>Furse/Winder</td>
</tr>
<tr>
<td><strong>Palaeoecological methods</strong></td>
<td>Bennion</td>
</tr>
<tr>
<td><strong>Modelling approaches</strong></td>
<td>May</td>
</tr>
</tbody>
</table>

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 km² (Cardoso et al., 2001)

<table>
<thead>
<tr>
<th></th>
<th>TP (µg/l)</th>
<th>SRP (µg/l)</th>
<th>TN (mg/l)</th>
<th>Nitrate (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>5-50</td>
<td>0-10</td>
<td>0.2-1.0</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>North America</td>
<td>&lt;25</td>
<td>-</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Australia</td>
<td>10</td>
<td>-</td>
<td>0.1-0.2</td>
<td>-</td>
</tr>
<tr>
<td>Large rivers</td>
<td>-</td>
<td>2-25</td>
<td>0.1-1.2</td>
<td>0.05-0.2</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>System B</th>
<th>System A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude</td>
<td>Altitude: High (&gt;800 m), mid (200 – 800 m), or low (&lt;200 m)</td>
</tr>
<tr>
<td>Latitude/longitude</td>
<td>Ecoregion</td>
</tr>
<tr>
<td>Geology</td>
<td>Geology: calcareous, siliceous or organic</td>
</tr>
<tr>
<td>Size</td>
<td>Catchment size (km²): 10-100, 100-1000, 1000-10000, &gt;10000</td>
</tr>
</tbody>
</table>

#### 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)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UK</th>
<th>Ireland</th>
<th>France</th>
<th>JRC Ispra</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (mean) (µg/l)</td>
<td>&gt;100</td>
<td>&gt;50</td>
<td>&lt;100</td>
<td>75-200</td>
</tr>
<tr>
<td>Chl a (mean) (µg/l)</td>
<td>&gt;25</td>
<td>&gt;60</td>
<td>&gt;60</td>
<td>-</td>
</tr>
<tr>
<td>Chl a (max.) (µg/l)</td>
<td>&gt;100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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 (NH₃) 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 community. 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 siltling 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 communities 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 epilithic 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, epipel on 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 fluvial 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>
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<th>Reference</th>
<th>Methodology overview [basis of assessment]</th>
<th>Trophic divisions</th>
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<td>River plants</td>
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<td>Use of plants to reflect general trophic status in national context for open water bodies using P, N, alkalinity</td>
<td>Trophic Ranking number (1-150) given to 150 open water plant species [Expert opinion]</td>
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<td>Assessment of damage using difference from expected for 8 plant communities in habitats derived for stream size, landscape and rock-type [expert opinion]</td>
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<td>Holmes 1983</td>
<td>Typing rivers according to their flora</td>
<td>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]</td>
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<td>Holmes and Newbold 1984</td>
<td>River plant communities – reflectors of water and substrate chemistry</td>
<td>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]</td>
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<td>Standard methods for UK</td>
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<td>British rivers - a working classification</td>
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<td>Reference</td>
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<td>Trophic divisions</td>
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<td>Method for plant distribution, plant habitat with some geomorphology</td>
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<td>Holmes 1995</td>
<td>Water quality assessment through indicator species basis of MTR method [synthesis of expert opinions - no water chemistry]</td>
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<td>Macrophyte indices to assess stream water quality : preliminary proposals</td>
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<td>Holmes, Boon and Rowell 1999</td>
<td>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]</td>
<td>3 ranges oligo - meso- eutrophic</td>
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<td>Users manual for Mean Trophic Rank methodology</td>
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<td>Dawson, Newman, Gravelle, Rouen and Henville 1999</td>
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Table 2.5  Methodologies or studies of plant assessment with lesser degrees of coverage or extent of nutrient classification.

<table>
<thead>
<tr>
<th>Author</th>
<th>Methodology overview [basis of assessment]</th>
<th>Nutrient classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kohler Wonneeberger and Zeltner 1973</td>
<td>Assessment of chemical pollution indicators for a German river (indicated that only N and P had indicator value)</td>
<td>Series of floristic-ecological river zones (c6)</td>
</tr>
<tr>
<td>Wiegleb 1978</td>
<td>Indicator values for plant communities in standing waters differing water chemistry, Lower Saxony [statistical analysis of surveys]</td>
<td>6 clear community based phytosociological indicator groups</td>
</tr>
<tr>
<td>Harding 1981</td>
<td>Plant cover in rivers of NW England</td>
<td>5 ranges of plant species: sensitive to tolerant</td>
</tr>
<tr>
<td>Marieux 1982</td>
<td>Use of phyto-sociological groupings as indicators of water quality</td>
<td>5 zones/levels</td>
</tr>
<tr>
<td>Caffrey, 1987</td>
<td>Comparison of plant species in three Irish catchments with water quality class</td>
<td>Uses 5 water quality classes</td>
</tr>
<tr>
<td>Haury, Peltre, Muller, Tremoliers, Barbes 1991</td>
<td>Comparison of ten French rivers on different geology, conductivity and degree of pollution establishing indices for 200 riparian species [expert opinion plus N, P]</td>
<td></td>
</tr>
<tr>
<td>Kelly, 1996c</td>
<td>Diatoms monitoring eutrophic and organic enrichment</td>
<td>Continuous and broad ranges</td>
</tr>
<tr>
<td>Dawson and Szoszkiewicz 1999</td>
<td>Relationships of some ecological factors with the associations of vegetation in British rivers (N, P etc)</td>
<td>Community based - no bands defined but nutrient data given</td>
</tr>
<tr>
<td>Thiebaut and Muller</td>
<td>Eutrophication with acidification in Vosges, France</td>
<td>4 communities in sequence</td>
</tr>
<tr>
<td>Haslam 2000</td>
<td>Pragmatic method for evaluation of pollution in Malta</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Status</th>
<th>Class definitions</th>
<th>Detailed field studies*</th>
<th>MTR assessment**</th>
<th>Proposed range limits (alternative less rigorous value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Undisturbed or minor anthropogenic alterations</td>
<td>10 µg P l⁻¹</td>
<td>18-40µg P l⁻¹</td>
<td>&lt;10 (&lt;25) µg P l⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.7 mg N l⁻¹</td>
<td>0.2-0.8 mg N l⁻¹</td>
<td>0.2 (0.5) mg N l⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plant types: Mostly bryophytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>Slight deviation in biology from undisturbed. No significant undesirable disturbance</td>
<td>49 µg P l⁻¹</td>
<td>40-80 µg P l⁻¹</td>
<td>30-40 (75) µg P l⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0 mg N l⁻¹</td>
<td>0.8-2.2 mg N l⁻¹</td>
<td>0.8 (1.5) mg N l⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plant types: Mostly bryophytes and submerged species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>Moderate deviation in biology that may produce a significant undesirable disturbance in other biological and physico-chemical elements</td>
<td>112µg P l⁻¹</td>
<td>90-125µg P l⁻¹</td>
<td>60 (100) µg P l⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.8 mg N l⁻¹ (increased to 8 mg N l⁻¹ in 2000)</td>
<td>2.2-5.5 mg N l⁻¹</td>
<td>2 (4) mg N l⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plant types: Submerged and emergent species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>Major alterations in biology. Substantial deviation from undisturbed</td>
<td>125-c450 µg P l⁻¹</td>
<td>5.5-8.0 mg N l⁻¹</td>
<td>100 (200) µg P l⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plant types: Includes Cladophora agg.</td>
<td></td>
<td>6 (8) mg N l⁻¹</td>
</tr>
<tr>
<td>Bad</td>
<td>Severe alterations in biology. Undisturbed communities largely absent</td>
<td>&gt;450(-c6000) µg P l⁻¹</td>
<td>&gt;500 µg P l⁻¹</td>
<td>&gt;500 µg P l⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;8.0 (- c12) mg N l⁻¹</td>
<td>&gt; 8 mg N l⁻¹</td>
<td></td>
</tr>
</tbody>
</table>

*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 fluvial 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 µg l$^{-1}$ proposed by English Nature is not in agreement with experimental data which has shown no inhibition of growth at 10 µg 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 µg 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 Ephemeroptera, 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, NH₄-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 < TP µg 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 ≤15 µg TP l⁻¹, n = 246) and sites affected by eutrophication (≥50 µg 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) (Kirkegaard et al. 1992). The DFI is similar to the Trent Biotic Index (Woodiwillis1964).

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.
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),
O₂ 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](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](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:

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

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 salmonid 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  Size  Altitude  Fish  Diversity
(rock and soil)  (area/depth)  (and latitude)  (community)  (species)

Poorest          Smallest     Highest         Perhaps     None
Dystrophic and  shallow   cooler       Stenothermic  Very low
Oligotrophic    pools       waters       Species (e.g.  low
systems        and         often        Salmonidae)      
TRICKLES       frozen       Frozen       May dominate

Mesotrophic systems

Eutrophic and  Deeper   Warmer       Eurythermic  High
Hypertrophic    lakes     waters       Species (e.g.  Medium
systems        and       rarely       Cyprinidae)    May dominate
RIVERS         Frozen       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 \textit{Abramis brama} and Roach \textit{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 \textit{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:
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 rheophytic 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.

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>REGRESSION COEFFICIENT</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>River size</td>
<td>$r = 0.73$</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>Thermal regime</td>
<td>$r = 0.05$</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>Flow velocity</td>
<td>$r = 0.53$</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>BOD$_5$</td>
<td>$r = 0.84$</td>
<td>$p &lt; 0.0006$</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>$r = 0.72$</td>
<td>$p &lt; 0.008$</td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>$r = 0.89$</td>
<td>$p &lt; 0.0005$</td>
</tr>
<tr>
<td>Zooplankton</td>
<td>$r = 0.43$</td>
<td>$p &lt; 0.029$</td>
</tr>
</tbody>
</table>

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.
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

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

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 comparative 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 confidence 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)

<table>
<thead>
<tr>
<th>System B</th>
<th>System A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude</td>
<td>Altitude: High (&gt;800 m), mid (200 – 800 m), or low (&lt;200 m)</td>
</tr>
<tr>
<td>Latitude/longitude</td>
<td>Ecoregion</td>
</tr>
<tr>
<td>Depth</td>
<td>Mean depth: shallow (&lt;3 m), mid (3-15 m), or deep (&gt;15 m)</td>
</tr>
<tr>
<td>Geology</td>
<td>Geology: calcareous, siliceous or organic</td>
</tr>
<tr>
<td>Size</td>
<td>Size: 0.5-1 km², 1-10 km², 10-100 km², or &gt;100 km²</td>
</tr>
</tbody>
</table>

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 µg l⁻¹ TP), both clear water plant-dominated
and turbid algal-dominated lake states can exist, each stabilised by buffering mechanisms. Below 30 µg l\(^{-1}\) TP submerged macrophytes tend to be abundant, while above 1000 µg l\(^{-1}\) 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 \textit{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 \textit{Ceratium hirundinella}, \textit{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).

\textbf{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 \textit{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 \textit{Microcystis}, \textit{Aphanizomenon} and \textit{Anabaena} arefavoured 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 \textit{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 (µg l⁻¹) for selected diatom species

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

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 µg/l⁻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, Chlorophyll\text{mean} and Chlorophyll\text{max.} are the annual mean and annual maximum in-lake chlorophyll *a* concentrations respectively and P\text{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**

<table>
<thead>
<tr>
<th>Class</th>
<th>Annual mean TP (µg/l)</th>
<th>Annual mean chlorophyll <em>a</em> (µg/l)</th>
<th>Annual maximum chlorophyll <em>a</em> (µg/l)</th>
<th>Annual mean secchi depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultra-oligotrophic</td>
<td>&lt;4</td>
<td>&lt;1</td>
<td>&lt;2.5</td>
<td>&gt;12</td>
</tr>
<tr>
<td>Oligotrophic</td>
<td>&lt;10</td>
<td>&lt;2.5</td>
<td>&lt;8</td>
<td>&gt;6</td>
</tr>
<tr>
<td>Mesotrophic</td>
<td>10-35</td>
<td>2.5-8</td>
<td>8-25</td>
<td>6-3</td>
</tr>
<tr>
<td>Eutrophic</td>
<td>35-100</td>
<td>8-25</td>
<td>25-75</td>
<td>3-1.5</td>
</tr>
<tr>
<td>Hyper-eutrophic</td>
<td>&gt;100</td>
<td>&gt;25</td>
<td>&gt;75</td>
<td>&lt;1.5</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Excellent</th>
<th>Good</th>
<th>Fair</th>
<th>Poor</th>
<th>Bad</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (mean) (µg l(^{-1}))</td>
<td>Natural levels</td>
<td>&lt;125% of excellent</td>
<td>125-150% of excellent</td>
<td>150-200% of excellent</td>
<td>&gt;200% of excellent</td>
</tr>
<tr>
<td>Chl. (a) (mean) (µg l(^{-1}))</td>
<td>&lt;2</td>
<td>&lt;5</td>
<td>&lt;10</td>
<td>&lt;25</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Chl. (a) (max.) (µg l(^{-1}))</td>
<td>&lt;5</td>
<td>&lt;10</td>
<td>&lt;20</td>
<td>&lt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Secchi depth (mean) (m)</td>
<td>&gt;5</td>
<td>2-5</td>
<td>1.5-2</td>
<td>1-1.5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Secchi depth (min.) (m)</td>
<td>&gt;3</td>
<td>1-3</td>
<td>0.7-1</td>
<td>&lt;0.7</td>
<td>&lt;0.7</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UK</th>
<th>Ireland</th>
<th>France</th>
<th>Spain</th>
<th>JRC Ispra</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (µg l(^{-1}))</td>
<td>&gt;50</td>
<td>&gt;25</td>
<td>&gt;30</td>
<td>reference conditions</td>
<td></td>
</tr>
<tr>
<td>Chl. (a) (mean) (µg l(^{-1}))</td>
<td>-</td>
<td>&gt;60</td>
<td>&gt;8</td>
<td>&gt;10</td>
<td></td>
</tr>
<tr>
<td>Chl. (a) (max.) (µg l(^{-1}))</td>
<td>&gt;30</td>
<td>&gt;35</td>
<td>-</td>
<td>&gt;25</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Secchi depth (m)</td>
<td>&lt;3</td>
<td>-</td>
<td>-</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

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 cyanobacteria 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 cyanobacteria 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 % \textit{Anabaena}, \textit{Aphanizomenon} and \textit{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 community. 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 epipelagic 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 \textit{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

\textbf{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 epipelagic habitats, or on other loose substrates (Wetzel 2001). Epipelagic communities on fine, organic sediments largely comprise motile algae, as mobility is necessary to move to the surface following disturbance of the sediments. Episammic communities tend to be dominated by algae attached firmly to crevices in sand-grains.

\textbf{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.

\textbf{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).

\textbf{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 $\mu$S/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, Potamogeton 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
Spirodea 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 \(\mu\)gl\(^{-1}\)). 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 attenuation 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 mg l\(^{-1}\); 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**

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

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 TWINSPLAN 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.

TWINSPLAN 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)

<table>
<thead>
<tr>
<th>Type</th>
<th>Site Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dystrophic</td>
</tr>
<tr>
<td>2 and 3</td>
<td>Oligotrophic/base-poor</td>
</tr>
<tr>
<td>4</td>
<td>Mixture of influences</td>
</tr>
<tr>
<td>5</td>
<td>Mesotrophic</td>
</tr>
<tr>
<td>6</td>
<td>Brackish</td>
</tr>
<tr>
<td>7,8,9 and 10</td>
<td>Eutrophic/base rich</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Lake Type</th>
<th>Botanical lake type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertrophic</td>
<td><em>Lemna-Nuphar</em></td>
</tr>
<tr>
<td>Eutrophic</td>
<td><em>Typha-Alisma</em></td>
</tr>
<tr>
<td>Clear-water-meso-eutrophic</td>
<td><em>Mesotrophic elodeid</em></td>
</tr>
<tr>
<td>Mesotrophic</td>
<td><em>Equisetum-Phragmites</em></td>
</tr>
<tr>
<td>Clear-water oligotrophic</td>
<td><em>Myriophyllum</em></td>
</tr>
<tr>
<td>Brown-water oligotrophic</td>
<td><em>Equisetum-Nuphar</em></td>
</tr>
<tr>
<td></td>
<td><em>Nuphar</em></td>
</tr>
<tr>
<td></td>
<td><em>Nuphar</em> (clear water variant)</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>TP Concentration May-October</th>
<th>TN Concentration May-October</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low concentrations (oligotrophic)</td>
<td>≤ 12.5</td>
<td>≤ 300</td>
</tr>
<tr>
<td>2</td>
<td>Moderately high concentrations (mesotrophic)</td>
<td>12.5 – 25</td>
<td>300-625</td>
</tr>
<tr>
<td>3</td>
<td>High concentrations (eutrophic)</td>
<td>25 – 50</td>
<td>625-1.250</td>
</tr>
<tr>
<td>4</td>
<td>Very high concentrations (eutrophic)</td>
<td>50 – 100</td>
<td>1.250-5.000</td>
</tr>
<tr>
<td>5</td>
<td>Extremely high concentrations (hyper-eutrophic)</td>
<td>&gt;100</td>
<td>&gt;5.000</td>
</tr>
</tbody>
</table>
Table 3.10  Current condition assessment for ratio of total nitrogen/total phosphorus in lakes

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Ratio June - September</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nitrogen surplus</td>
<td>≥ 30</td>
</tr>
<tr>
<td>2</td>
<td>Nitrogen – phosphorus balance</td>
<td>15 – 30</td>
</tr>
<tr>
<td>3</td>
<td>Moderate nitrogen deficit</td>
<td>10 – 15</td>
</tr>
<tr>
<td>4</td>
<td>Large nitrogen deficit</td>
<td>5 - 10</td>
</tr>
<tr>
<td>5</td>
<td>Extreme nitrogen deficit</td>
<td>&lt; 5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Total number of species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Very high numbers of species</td>
<td>&gt; 18</td>
</tr>
<tr>
<td>2</td>
<td>High numbers of species</td>
<td>14 – 18</td>
</tr>
<tr>
<td>3</td>
<td>Moderate number of species</td>
<td>9 – 14</td>
</tr>
<tr>
<td>4</td>
<td>Relatively few species</td>
<td>4 – 9</td>
</tr>
<tr>
<td>5</td>
<td>Very few species</td>
<td>≤ 4</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Recorded concentration/reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No or insignificant deviation</td>
<td>≤ 1.5</td>
</tr>
<tr>
<td>2</td>
<td>Significant deviation</td>
<td>1.5 – 2.0</td>
</tr>
<tr>
<td>3</td>
<td>Large deviation</td>
<td>2.0 – 3.0</td>
</tr>
<tr>
<td>4</td>
<td>Very large deviation</td>
<td>3.0 – 6.0</td>
</tr>
<tr>
<td>5</td>
<td>Extreme deviation</td>
<td>&gt; 6.0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Lake area km²</th>
<th>Northern Sweden</th>
<th>Southern Sweden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of species</td>
<td>Indicator ratio</td>
</tr>
<tr>
<td>&gt; 60 m above sea level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 0.1</td>
<td>3 – 5</td>
<td>5.5</td>
</tr>
<tr>
<td>0.1 – 1</td>
<td>9 – 14</td>
<td>6.5</td>
</tr>
<tr>
<td>1 – 10</td>
<td>10 – 18</td>
<td>6.3</td>
</tr>
<tr>
<td>≥10</td>
<td>17 – 21</td>
<td>6.5</td>
</tr>
<tr>
<td>60 – 199 m above sea level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.1 – 1</td>
<td>5 – 13</td>
<td>6.3</td>
</tr>
<tr>
<td>1 – 10</td>
<td>10 – 16</td>
<td>5.8</td>
</tr>
<tr>
<td>≥10</td>
<td>13 – 20</td>
<td>6.3</td>
</tr>
<tr>
<td>&gt;200 m above sea level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 – 1</td>
<td>4 – 11</td>
<td>6.3</td>
</tr>
<tr>
<td>1 – 10</td>
<td>8 – 15</td>
<td>6.2</td>
</tr>
<tr>
<td>≥10</td>
<td>13 – 17</td>
<td>5.9</td>
</tr>
</tbody>
</table>

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

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

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
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 1 g 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)

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

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

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

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 it 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 nutrients

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 sphaerid 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)**

<table>
<thead>
<tr>
<th>Lake type/trophic level</th>
<th>Indicator chironomids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultraoligotrophic</td>
<td><em>Heterotrisocladius subpilosus</em></td>
</tr>
<tr>
<td>Oligotrophic</td>
<td><em>Tanytarsus lugens</em> or <em>Heterotrisocladius grismhawi</em> or <em>H. scutellatus</em></td>
</tr>
<tr>
<td>Mesotrophic</td>
<td><em>Stictochironomus rosenchoeldi</em> or <em>Sergentina coracina</em></td>
</tr>
<tr>
<td>Moderately eutrophic</td>
<td><em>Chironomus anthracinus</em></td>
</tr>
<tr>
<td>Strongly eutrophic</td>
<td><em>Chironomus plumosus</em></td>
</tr>
<tr>
<td>Dystrophic</td>
<td><em>Chironomus tenuistylus</em> or <em>Zalutschia zalutschicola</em></td>
</tr>
</tbody>
</table>

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 plumosus* to 5 in oligotrophic lakes where *Heterotriussocladius 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 scarce 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 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 communities, 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 communities 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 salmon 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/m²/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 physicochemical 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

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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:

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dystrophic</td>
<td>Sometimes no species, always very few</td>
</tr>
<tr>
<td>Oligotrophic</td>
<td>Few species, dominated by salmonids and sometimes coregonids</td>
</tr>
<tr>
<td>Mesotrophic</td>
<td>Fewer species, sometimes dominated by percids and cyprinids</td>
</tr>
<tr>
<td>Eutrophic</td>
<td>Usually a wide range of species, often dominated by cyprinids</td>
</tr>
</tbody>
</table>

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$^3$) (Klifffmuller 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 produce strong 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 a consequence (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 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>
<thead>
<tr>
<th>Lake Schemes</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoplankton: Chlorophyll $a$ (OECD)</td>
<td>Highly sensitive to nutrients</td>
<td>Highly dynamic</td>
</tr>
<tr>
<td></td>
<td>Simple, rapid response</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Integrates limiting factors</td>
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</tr>
<tr>
<td></td>
<td>Present in all lakes</td>
<td></td>
</tr>
<tr>
<td>Planktonic and benthic diatoms: DI-TP (Bennion et al.)</td>
<td>Highly sensitive to nutrients</td>
<td>Specialist EA training requirement</td>
</tr>
<tr>
<td></td>
<td>Integrates over space/time</td>
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<tr>
<td></td>
<td>Sediment records for defining reference conditions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Present in all lakes</td>
<td></td>
</tr>
<tr>
<td>Macrophytes (CCW, NILS)</td>
<td>Sensitive to nutrients</td>
<td>Not consistently present</td>
</tr>
<tr>
<td></td>
<td>Practical relevance</td>
<td>Species poor</td>
</tr>
<tr>
<td></td>
<td>Data rich</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Historical records for defining reference conditions</td>
<td></td>
</tr>
<tr>
<td>Chironomid pupal exuviae (Ruse)</td>
<td>Integrates over space/time</td>
<td>Nutrient response difficult to interpret</td>
</tr>
<tr>
<td></td>
<td>Sediment records for defining reference conditions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Present in all lakes</td>
<td>Specialist EA training requirement</td>
</tr>
<tr>
<td>Fish</td>
<td>None established</td>
<td>Nutrient response difficult to interpret</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Species poor</td>
</tr>
</tbody>
</table>

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 km² 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 maximum) 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 SNIFTER, 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\textsubscript{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\textsubscript{mol} y\textsuperscript{-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\textsubscript{mol} N y\textsuperscript{-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\textsubscript{3}{-} + NO\textsubscript{2}{-} or TO\textsubscript{x}{N}) was by far the greatest fluvial input of N, with the greatest loads in both TO\textsubscript{x}{N} 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\textsubscript{3}{-} have doubled from 4 µM in the 1960s to 8 µM in the 1990s. In addition, winter NO\textsubscript{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 cryptomonad 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 stria*ta 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 estuary, 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 al., 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*, *Navicula 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 limitation
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\(^{-3}\)) \(y = 0.53x + 39.4, r^2 = 0.84\) (ii) the chlorophyll \(a\) concentration (mg m\(^{-3}\)) \(y = 0.7x + 16.9, r^2 = 0.96\). 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 \(\mu\)m) and pico (<3 \(\mu\)m) plankton dominated in the mesohaline region of the estuary in the summer bloom where as the microplankton (>20 \(\mu\)m) 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](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>
<thead>
<tr>
<th>Month</th>
<th>Deben Melton</th>
<th>Deben Felixstowferry</th>
<th>Conwy Deganwy</th>
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<tbody>
<tr>
<td>Feb</td>
<td>3.2</td>
<td>3.2</td>
<td>0</td>
</tr>
<tr>
<td>Mar</td>
<td>8.2</td>
<td>1.9</td>
<td>0</td>
</tr>
<tr>
<td>Apr</td>
<td>13.9</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>17.1</td>
<td>8.2</td>
<td>13.4</td>
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<tr>
<td>Jun</td>
<td>14.5</td>
<td>2.5</td>
<td>6.4</td>
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<tr>
<td>Jul</td>
<td>17.7</td>
<td>5.1</td>
<td>1.1</td>
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<tr>
<td>Aug</td>
<td>48.6</td>
<td>3.8</td>
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<tr>
<td>Sept</td>
<td>10.7</td>
<td>9.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Oct</td>
<td>17.1</td>
<td>4.4</td>
<td>5.7</td>
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<tr>
<td>Nov</td>
<td>5.7</td>
<td>2.5</td>
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<td>Dec</td>
<td>2.5</td>
<td>1.3</td>
<td>1.1</td>
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<tr>
<td>Mean</td>
<td>14.5</td>
<td>3.9</td>
<td>3.2</td>
</tr>
<tr>
<td>Min</td>
<td>2.5</td>
<td>0.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Max</td>
<td>48.6</td>
<td>9.5</td>
<td>13.4</td>
</tr>
</tbody>
</table>

Table 4.1 Monthly chlorophyll $a$ concentrations from the Deben and Conway estuaries (Dong, 2000)
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 $a : \text{Carbon}$. 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 NIFTS 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 seagrasses and slow-growing macroalgae (including brackish charophytes) by blooming, fast-growing macroalgae. This is shown schematically in Figure 1.
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

<table>
<thead>
<tr>
<th>Location</th>
<th>Available N and P data</th>
<th>Changes in local flora</th>
<th>Reference</th>
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<tbody>
<tr>
<td>British Isles</td>
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<tr>
<td>Ythan Estuary, Scotland</td>
<td>2 to 3-fold increase in N loading between mid 1960’s and mid 1980’s. N in river Ythan increased from 3 mg l⁻¹ in 1960’s to 6 mg l⁻¹ in 1980’s to &gt; 9 mg l⁻¹ in 1990’s</td>
<td>Increased growth of green macroalgae forming blooms – Predominantly <em>Enteromorpha</em>, but also <em>Chaetomorpha</em> and <em>Ulva</em> spp.</td>
<td>Hull 1987, Raffaelli <em>et al.</em> 1989, 1998, 1999</td>
</tr>
<tr>
<td>Firth of Forth, Scotland</td>
<td>Increased sewage discharge since 1800’s</td>
<td>41.6% reduction in macroalgal species. Restricted flora of often-stunted growths of <em>Ulva lactuca</em>, <em>Chondrus crispus</em>, <em>Gigartina stellata</em>, <em>Ceramium rubrum</em>, <em>Polysiphonia nigrescens</em>, <em>Chaetomorpha</em> sp., <em>Hildenbrandia rubra</em>, <em>Dumontia incrassata</em> and <em>Corallina officinalis</em></td>
<td>Traill 1886, Johnston 1971/1972, Wilkinson and Titley 1979</td>
</tr>
<tr>
<td>Firth of Clyde, Scotland</td>
<td>-</td>
<td>Reduced flora comprising species of the genera <em>Dumontia</em>, <em>Gigartina</em>, <em>Hildenbrandia</em> and <em>Ralfsia</em></td>
<td>Clokie and Boney 1979</td>
</tr>
<tr>
<td>Humber-Wash estuarine system, England</td>
<td>Nutrients in water column: 3-10 mg l⁻¹ TON 2-10 mg l⁻¹ PO₄ (1990 data)</td>
<td>Mass occurrence of <em>Ulva</em>, <em>Enteromorpha</em> or <em>Chaetomorpha</em> since 1989</td>
<td>MacGarvin 1995</td>
</tr>
<tr>
<td>Helford Estuary, England</td>
<td>Nutrients in water column: 2 mg l⁻¹ TON 20 mg l⁻¹ PO₄ (1990 data)</td>
<td>Total loss of <em>Zostera marina</em> beds between 1986 and 1988. Luxuriant growth of <em>Enteromorpha</em> and <em>Ulva</em> in certain years</td>
<td>Tompsett 1994</td>
</tr>
<tr>
<td>Deben, Orwell and Stour Estuaries, England</td>
<td>Nutrients in water column: 0.9-2.5 mg l⁻¹ TON (annual mean)</td>
<td>Increasing <em>Enteromorpha</em> weed mats</td>
<td>MacGarvin 1995</td>
</tr>
<tr>
<td>Location</td>
<td>Available N and P data</td>
<td>Changes in local flora</td>
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<tr>
<td>Plymouth Sound, Tamar and Yealm Estuaries, England</td>
<td>Nutrients in water column: 0.3-2.8 mg l⁻¹ TIN (1990-93 data)</td>
<td>Decline in <em>Zostera</em> beds</td>
<td>Davies 1991, Parr and Wheeler 1996</td>
</tr>
<tr>
<td>Europe</td>
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<tr>
<td>Venice Lagoon, Italy</td>
<td>Nutrients in water column: 0.09-0.9 mg l⁻¹ NH₄ 3.1-4.3 mg l⁻¹ NO₃ (1985-90 data)</td>
<td>Increase in the green macroalga <em>Ulva rigida</em> and corresponding decline of the seagrasses <em>Cymodocea nodosa</em> and <em>Zostera noltii</em>. Replacement by <em>Dictyota dichotoma</em>, <em>Punctaria latifolia</em>, <em>Codium fragile</em>, <em>Ulva fasciata</em>, <em>Petalonia zosterifolia</em> and <em>Corallina officinalis</em> 95 species of macrophyte recorded in 1987 compared to 104 recorded in 1960’s and 141 recorded in 1930’s</td>
<td>Sfriso 1987, Sfriso et al. 1989, 1992</td>
</tr>
<tr>
<td>Orbetello Lagoon, Italy</td>
<td>Nutrients in water column: Up to 0.01 mg l⁻¹ NH₄ Up to 1.4 mg l⁻¹ NO₃ Up to 0.01 mg l⁻¹ PO₄ but large variations in nutrient concentrations reported over recent years</td>
<td>Replacement of seagrasses <em>Posidonia oceanica</em> and <em>Cymodocea nodosa</em> by <em>Chaetomorpha linum</em>, <em>Cladophora vagabunda</em> and <em>Gracilaria verrucosa</em> from 1976 onwards</td>
<td>Bombelli and Lenzi 1996</td>
</tr>
<tr>
<td>Location</td>
<td>Available N and P data</td>
<td>Changes in local flora</td>
<td>Reference</td>
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<tr>
<td>Sacca di Goro, Italy</td>
<td>Nutrients in water column: 2 mgl⁻¹ N 0.2 mgl⁻¹ P</td>
<td><em>Ulva</em> blooms and proliferation of <em>Gracilaria verrucosa</em></td>
<td>Viaroli <em>et al.</em> 1994</td>
</tr>
<tr>
<td>Palmones Estuary, Spain</td>
<td>‘high’ nutrient concentrations in water column</td>
<td>Proliferation of <em>Ulva</em> and corresponding disappearance of <em>Zostera noltii</em> and <em>Gracilaria</em> mats</td>
<td>Perez-Llorens 1991 Niell <em>et al.</em> 1996</td>
</tr>
<tr>
<td>Tancada Lagoon, NE Spain</td>
<td>-</td>
<td>Decrease in the abundance of submerged rooted macrophyte cover, and increase in floating macroalgae – <em>Chaetomorpha linum</em> and <em>Cladophora</em> sp.</td>
<td>Menendez and Comin 2000</td>
</tr>
<tr>
<td>Miro and Modego Estuaries, Portugal</td>
<td>-</td>
<td>Progressive increase in <em>Enteromorpha</em> and corresponding decline in seagrasses <em>Zostera marina</em> and <em>Z. noltii</em></td>
<td>Oliveira and Cabecadas 1996</td>
</tr>
<tr>
<td>Diana and Urbino Lagoons, Corsica</td>
<td>Nutrients in water column: Diana: 0.026 mgl⁻¹ NH₄ 0.092 mgl⁻¹ NO₃ 0.019 mgl⁻¹ PO₄ Urbino: 0.019 mgl⁻¹ NH₄ 0.066 mgl⁻¹ NO₃ 0.011 mgl⁻¹ PO₄</td>
<td>Shift from a high diversity of communities and species to a single community with a low species diversity. Invasion of <em>Cladophora vagabunda</em>, <em>Ulva lactuca</em>, <em>Chaetomorpha linum</em> and <em>Enteromorpha intestinalis</em></td>
<td>De Cassabianca 1996</td>
</tr>
<tr>
<td>Rinkøbing Fjord, Denmark</td>
<td>-</td>
<td>Significant reduction of eelgrass coverage since 1972</td>
<td>North Sea Task Force 1994</td>
</tr>
<tr>
<td>Nissum Fjord, Denmark</td>
<td>-</td>
<td>Significant restriction of eelgrass coverage from 1966 to 1988</td>
<td>North Sea Task Force 1994</td>
</tr>
<tr>
<td>Isefjord-Roskilde Fjord, Denmark</td>
<td>Increasing nitrate concentrations since 1941</td>
<td>Significant increase in relative abundance of <em>Cladophora, Chaetomorpha, Enteromorpha</em> and <em>Ulva</em> spp. and significant decrease in relative abundance of large perennial brown algae e.g. <em>Fucus serratus, Fucus vesiculosus</em></td>
<td>Middelboe and Sand-Jensen 2000</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Algal taxa</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phaeophyceae</strong></td>
<td></td>
</tr>
<tr>
<td><em>Pilayella</em></td>
<td>Wilce <em>et al.</em> 1982, Lotze <em>et al.</em> 1999</td>
</tr>
<tr>
<td><em>Myriotrichia</em></td>
<td>Wear <em>et al.</em> 1999</td>
</tr>
<tr>
<td><strong>Rhodophyceae</strong></td>
<td></td>
</tr>
<tr>
<td><em>Furcellaria</em></td>
<td>Baden <em>et al.</em> 1990</td>
</tr>
<tr>
<td><em>Phycodrys</em></td>
<td>Baden <em>et al.</em> 1990</td>
</tr>
<tr>
<td><em>Acrochaetium</em></td>
<td>Wear <em>et al.</em> 1999</td>
</tr>
</tbody>
</table>

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 respectively (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 worldwide. Table 4.4 lists localities relevant to this review and indicates the algal genera associated with floral changes at such sites.

<table>
<thead>
<tr>
<th>Location</th>
<th>Algal taxa</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medway Estuary, England</td>
<td>Enteromorpha</td>
<td>Wharfe 1977</td>
</tr>
<tr>
<td>Forfar Loch, Scotland</td>
<td>Enteromorpha</td>
<td>Coleman and Stewart 1979, Ho 1979</td>
</tr>
<tr>
<td>Clyde Estuary, Scotland</td>
<td>Enteromorpha</td>
<td>Perkins and Abbot 1972</td>
</tr>
<tr>
<td>Ythan Estuary, Scotland</td>
<td>Enteromorpha</td>
<td>Raffaelli et al. 1989, 1991</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Location</th>
<th>Algal taxa</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rogerstown Estuary, Ireland</td>
<td><em>Enteromorpha</em></td>
<td>Fahy et al. 1975</td>
</tr>
<tr>
<td>Belfast Lough, Ireland</td>
<td><em>Ulva</em></td>
<td>Cotton 1910, 1911, Letts and Richards 1911</td>
</tr>
<tr>
<td>Europe Lagune du Provost, France</td>
<td><em>Ulva</em></td>
<td>Casabianca-Chassany 1983, 1989</td>
</tr>
<tr>
<td>Mar Menor Lagoon, Spain</td>
<td><em>Chaetomorpha</em></td>
<td>Perez-Ruzafa et al. 1991</td>
</tr>
<tr>
<td>Odense Fjord, Norway</td>
<td><em>Ulva</em></td>
<td>Frederiksen 1987</td>
</tr>
<tr>
<td>Roskilde Fjord, Denmark</td>
<td><em>Ulva</em></td>
<td>Geertz-Hansen and Sand-Jensen 1990</td>
</tr>
</tbody>
</table>

**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 mg l\(^{-1}\) TON and are mindful of evidence of effects on growth or species composition at concentrations lower than between 0.15 and 0.25 mg l\(^{-1}\) 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 been 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). Ecotoxicology 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 (NH₃) and ionised (ammonium NH₄⁺) 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 (NH₃) = 100 (1 + antilog (pKₐs (T) (P) (I) – pH)-1

Where pKₐs 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(1000-1.005109 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 NH₃ 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 NH₃ fraction (Erickson 1985, Miller et al. 1990). Studies have been carried out to assess the relative toxicity of NH₃ to NH₄⁺ 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 NH₃ predominates) it was concluded that NH₃ 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 NH₄⁺ 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 NH₃, with the proportionate amount of each fraction reported (NH₄⁺ : NH₃) 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 NH₃ concentration. Hence, ammonia toxicity studies that quote NH₃ concentrations to 3
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 may be 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 macroepibenthic 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 offshore 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 NH₄⁺ and NH₃, although Frazier et al. (1996) demonstrated significant temporal and vertical variation in sediment porewater distributions of total ammonia and unionised ammonia (NH₃) 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 × 10⁶ m³ 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 NH₃ 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). NH₃ was more toxic to mysids at pH 7 than at pH 8 or 9, but, in contrast, NH₃ 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 NH₃ 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.
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$_4$Cl 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 molting 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 NH₄Cl 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 NH₄Cl 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 NO₃⁻ and NO₂⁻ are naturally generated in water via the chemoautotrophic oxidation of ammonia. In turn NO₃⁻ and NO₂⁻ are returned to the atmospheric pool of dinitrogen gas (N₂) via the anaerobic process of denitrification (see below), completing the nitrogen cycle. However, NO₃⁻ is now regarded as the most abundant form of ground water pollution (Pauwels *et al.* 2001) and, as discussed above, vast quantities of NO₃⁻ flow into estuaries from both fluvial and sewage sources (Nedwell *et al.* 1999). As a result of this it is not uncommon for winter NO₃⁻ concentrations to exceed 1 mM in the east coast estuaries of the UK (Trimmer *et al.* 2000b). The total annual load of NO₃⁻ flowing down UK east coast estuaries has been estimated at 5 Gmol N or 70000 t N (Trimmer *et al.* 2000b). Obviously NO₃⁻ concentrations are much higher within estuaries than the receiving coastal waters (Vannekom and Salomons 1980, Allen *et al.* 1998), as NO₃⁻ 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$_4$Cl, 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 NaNO3 with bluegills (Lepomis macrochirus) of 12 g l−1 and that they had also reported 96 h LC 50 for NaCl and Na2SO4 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 NO3− 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 NO3− 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 NO3− i.e. 6.2 g l−1 NaNO3 or a total salinity of 21.2. Although Camargo and Ward (1992) did not use as high NaNO3 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 petitti, or 13 and 15 mM NO3− respectively, which are still high with regard to NO3− for any receiving water.

Few data exist for either NO2− or NO3− (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 NO2− 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 coastal waters, with both NO2− and NO3− 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 NO2− 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 NO2− and NO3− in estuarine waters. Lewis and Morris (1986) argued that the effect of Cl− on the toxicity of NO2− 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 NO2− 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 NO2− ions for transport across the gills, hence uptake of NO2− is inhibited at higher Cl− salinities. Lewis and Morris (1986) regressed the NO2− toxicity of Russo and Thurston (1977) for rainbow trout and used
this to model the effect of Cl⁻ on NO₂⁻ toxicity, concluding that an increase of 1 mM in Cl⁻ raises the LC 50 for NO₂⁻ 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 NO₂⁻ (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 NO₂⁻ toxicity than fathead minnows, although interestingly, the attenuating effect of Cl⁻ on the LC 50 for NO₂⁻ is greatest for the least sensitive species (Lewis and Morris 1986).

A similar consistent trend of attenuating NO₂⁻ toxicity by Cl⁻ ions has been demonstrated for commercial prawn species. Chen and Lee (1997) reported 96 h LC 50 NO₂⁻ concentrations for the giant prawn Macrobrachium rosenbergii of 8.48, 11.21 and 12.87 mg l⁻¹ NO₂⁻-N at 15, 24 and 34 mg l⁻¹ Cl⁻, respectively. Similar results were reported for juvenile stages of Penaeus penicillatus, where NO₂⁻ 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 NO₃⁻ being predominantly higher in estuarine waters relative to sediment porewaters. For example, the Great Ouse estuary in Norfolk has high seasonal NO₃⁻ 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 NO₃⁻ via the anaerobic respiration process of denitrification. Stimulation of organic mineralisation leads to a rapid consumption of dissolved O₂ 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 NO₂⁻, may be utilised as alternative electron acceptors to O₂ and reduced by anaerobic bacteria. This in turn sets up a vertical concentration gradient and maintains the flux of NO₃⁻ 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 NO₃⁻ and NO₂⁻ will be generated through nitrification of ammonia arising from organic ammonification. Indeed concentrations of NO₂⁻ (1-5 µM) in this surface layer may exceed those in the overlying water despite anthropogenic sources of NO₂⁻ to the overlying water. As with ammonia, when assessing or assaying for the toxicity of either NO₃⁻ or NO₂⁻ 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 (*Gnathomenus petersi* and *G. tamandua*, Mormyridae) 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 O₂ l⁻¹ (∼ 45 µM) (Rosenberg et al. 1991). Due to its neutral charge H₂S 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 H₂S 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 Rosenberg (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 H₂S in the sediment, and to a lesser extent in the overlying water. H₂S is toxic to most invertebrates, fish and plants but the toxicity varies between species. In general the resistance to anoxia and to H₂S 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 NaNO₃ 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, NO₂⁻, NO₃⁻ 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 stations, indicated clearly that the former was inadequate in terms of understanding fish communities 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 Prymnesiophyte 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 V\(\text{max}\), “storage strategy species” with high internal stores and “affinity strategy species” with low K\(s\). Smayda possibly confused uptake (V\(\text{max}\)) with growth (\(\mu\)), because a high V\(\text{max}\) 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 V\(\text{max}\) 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 hysteresis 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 mesocosms 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, intensive 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 increasingly 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 sequestered 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 straightforward 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 microalgae (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 epibenthic 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 status 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)

<table>
<thead>
<tr>
<th>Location</th>
<th>Algal taxa</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>British Isles</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portsmouth Harbour, England</td>
<td><em>Ulva</em></td>
<td>Soulsby <em>et al.</em> 1985</td>
</tr>
<tr>
<td>Thanet Bay, England</td>
<td><em>Ulva</em></td>
<td>Fletcher 1974</td>
</tr>
<tr>
<td>Holes Bay, England</td>
<td><em>Ulva</em></td>
<td>Southgate 1972</td>
</tr>
<tr>
<td><strong>Europe</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saint-Brieuc Bay, France</td>
<td><em>Ulva</em></td>
<td>Briand and Morand 1987, Le Bozec 1993</td>
</tr>
<tr>
<td>Alfaques Bay, Spain</td>
<td><em>Ulva</em></td>
<td>Martinez-Arroyo and Romero 1990</td>
</tr>
<tr>
<td>Corsica</td>
<td><em>Chaetomorpha</em></td>
<td>Grimes and Hubbard 1971</td>
</tr>
<tr>
<td>Naples Bay, Italy</td>
<td><em>Ulva</em></td>
<td>Golubic 1970</td>
</tr>
<tr>
<td>Island of Sylt, Germany</td>
<td><em>Cladophora</em></td>
<td>Grenager 1957, Bokn and Lein 1978,</td>
</tr>
</tbody>
</table>

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 in Section 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 (Clendennen 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**

<table>
<thead>
<tr>
<th>Location</th>
<th>Available N and P data</th>
<th>Changes in local flora</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>British Isles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shetland Isles, Scotland</td>
<td>Nutrient-enriched waters due to sewage input</td>
<td>Abundant occurrence of <em>Fucus disticus</em> subsp. <em>edentatus</em></td>
<td>Powell 1963</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decline in <em>Zostera marina</em> beds due to prolific growth of green filamentous algae</td>
<td>Russell 1974</td>
</tr>
<tr>
<td>Belfast Bay, Ireland</td>
<td>-</td>
<td>Blooms of <em>Ulva lactuca</em></td>
<td>Wilkinson 1963</td>
</tr>
<tr>
<td>Location</td>
<td>Available N and P data</td>
<td>Changes in local flora</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------------</td>
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</tr>
<tr>
<td>Portsmouth Harbour, England</td>
<td>Input of sewage effluent</td>
<td>Appearance of green algal mats</td>
<td>Soulsby et al. 1978</td>
</tr>
<tr>
<td></td>
<td>Nutrients in water column:</td>
<td></td>
<td>Parr and Wheeler 1996</td>
</tr>
<tr>
<td></td>
<td>0.085-0.794 mg l⁻¹ NH₄+ NO₂</td>
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<td></td>
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<tr>
<td></td>
<td>(1988-93 data)</td>
<td></td>
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<tr>
<td></td>
<td>0.004-0.1 mg l⁻¹ NO₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.04-0.3 mg l⁻¹ PO₄</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>(1985-90 data)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Langstone Harbour, England</td>
<td>Input of sewage effluent – 5-fold increase in amount of effluent entering the harbour between 1959-1981</td>
<td>Excessive growth of Enteromorpha and Ulva</td>
<td>Montgomery and Soulsby 1981</td>
</tr>
<tr>
<td></td>
<td>Nutrients in water column:</td>
<td></td>
<td>Tubbs and Tubbs 1983</td>
</tr>
<tr>
<td></td>
<td>0.121-0.788 mg l⁻¹ NH₄+ NO₂</td>
<td></td>
<td>Montgomery et al. 1985</td>
</tr>
<tr>
<td></td>
<td>(1988-93 data)</td>
<td></td>
<td>Lowthion et al. 1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increase in Zostera marina and Zostera noltii during 1960’s, 70’s and 80’s</td>
<td>Fletcher 1996a, b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suffocation of Zostera by Enteromorpha radiata during 1990’s</td>
<td>Parr and Wheeler 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tubbs 1977</td>
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<td></td>
<td></td>
<td></td>
<td>Tubbs and Tubbs 1993</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>den Hartog 1994</td>
</tr>
<tr>
<td>Poole Harbour, England</td>
<td>-</td>
<td>Proliferations of green algae</td>
<td>Farnham 1986</td>
</tr>
<tr>
<td>Thanet, Kent, England</td>
<td>-</td>
<td>Abundance of Ulva lactuca and Griffithsia floculosa</td>
<td>Fletcher 1974</td>
</tr>
<tr>
<td>Location</td>
<td>Available N and P data</td>
<td>Changes in local flora</td>
<td>Reference</td>
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</tr>
<tr>
<td><strong>Europe</strong></td>
<td></td>
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</tr>
<tr>
<td>German Wadden Sea</td>
<td>-</td>
<td>Explosive growth of <em>Enteromorpha</em> forming thick algal mats since late 1970’s. Loss of red algae from deeper waters and disappearance of <em>Zostera marina</em> coinciding with dramatic increase in green algal mats</td>
<td>Reise 1983, 1994 Nienhuis 1996</td>
</tr>
<tr>
<td>Island of Sylt, Germany</td>
<td>-</td>
<td>Blooms of <em>Enteromorpha</em>, <em>Ulva</em>, <em>Chaetomorpha</em>, <em>Cladophora</em> and the red alga <em>Porphyra</em></td>
<td>Reise 1983</td>
</tr>
<tr>
<td>Baltic Sea general</td>
<td>-</td>
<td>Reduction in <em>Fucus</em>, <em>Zostera</em> and <em>Chara</em>. Increased growth of filamentous algae <em>Pilayella</em>, <em>Ceramium</em>, <em>Cladophora</em>, <em>Enteromorpha</em> and <em>Monostroma</em></td>
<td>Schramm 1996</td>
</tr>
<tr>
<td>Coast of Denmark</td>
<td>Increased nutrient loading</td>
<td>50% reduction in areas covered by <em>Zostera marina</em> since 1960’s</td>
<td>Funen Island Council 1991 Nienhuis 1996</td>
</tr>
<tr>
<td>Location</td>
<td>Available N and P data</td>
<td>Changes in local flora</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Dutch coast, including Dutch Wadden Sea</td>
<td>Increasing nutrient concentrations Nutrients in water column: 0.5-4.6 mg l⁻¹ N</td>
<td>Decline in <em>Zostera marina</em> and <em>Zostera noltii</em> and increasing floating algae, <em>Ulva</em>, <em>Enteromorpha</em> and <em>Chaetomorpha</em></td>
<td>den Hartog and Polderman 1975, Nienhuis 1996</td>
</tr>
<tr>
<td>Brittany coast, France</td>
<td>Intensive agriculture contributing to high N and P loading</td>
<td>Blooms of <em>Ulva</em> and some blooms of <em>Pilayella littoralis</em> since late 1970’s</td>
<td>Briand 1989, Dion and Le Bozec 1996</td>
</tr>
<tr>
<td>Bay of Saint-Brieuc, Brittany, France</td>
<td>-</td>
<td>Proliferation of free-floating <em>Ulva</em></td>
<td>Menesguen and Piriou 1995</td>
</tr>
<tr>
<td>Bay of Arcachon, France</td>
<td>Increased N loading due to mariculture Nutrients in water column: 0.2 mg l⁻¹ TON</td>
<td>Mass production of <em>Enteromorpha</em> since 1982. Increase in <em>Monostroma obscurum</em> since 1989</td>
<td>Auby <em>et al.</em> 1994, Dion and Le Bozec 1996</td>
</tr>
<tr>
<td>Spanish Atlantic coasts</td>
<td>Increase of nitrogen</td>
<td>Proliferation of <em>Ulva</em> blooms</td>
<td>Fuentes 1986, Niell <em>et al.</em> 1996</td>
</tr>
<tr>
<td>Alfas Bay, Spain</td>
<td>Nutrients in water column: 0.2-0.9 mg l⁻¹ NH₄, 0.6-3.1 mg l⁻¹ NO₃, 0.1-0.2 mg l⁻¹ PO₄</td>
<td>Domination of green algal communities, predominantly <em>Ulva sp.</em></td>
<td>Romero <em>et al.</em> 1996</td>
</tr>
<tr>
<td>Gulf of Trieste, Northern Adriatic</td>
<td>Increased eutrophication due to sewage input Nutrients in water column: 0.21-4.39 mg l⁻¹ NO₃, 0.01-0.28 mg l⁻¹ PO₄</td>
<td>Profound changes in benthic algal vegetation since late 1970’s. Reduction or absence of Fucoids linked with increasing <em>Enteromorpha</em>, <em>Ulva</em>, <em>Blidingia</em> and <em>Cladophora</em>. Appearance of turf-like mats of <em>Gelidium</em>. Reduction or absence of <em>Cystoseira</em> stands, replaced by populations of <em>Halopteris scoparta</em>, <em>Dictyota dicotoma</em>, <em>Halopithys incurvus</em>, <em>Padina pavonica</em> and under extreme conditions by <em>Ulva rigida</em>, <em>Scytosiphon lomentaria</em> and <em>Enteromorpha spp.</em></td>
<td>Munda 1993, 1996</td>
</tr>
</tbody>
</table>
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, Jeffery 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 in Section 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 oligotrophic 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 liaison 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. Macralsal 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 than 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”.

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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 log₁₀-transformed annual mean TP data, is strong (apparent r²= 0.85 and r²= 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 r²= 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, AUSTRIVAS 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 BEThic 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 BEnectic 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 optimal 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 multimetric 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 macroinvertebrate 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](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 CIRES. 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, Reynolds 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 SPerie 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:

\[
\log (TP) = 1.48 + 0.33 (±0.09) \log (\text{MEI}_{\text{alk}}) \quad (r = 0.83)
\]
\[
\log (TP) = 0.75 + 0.27 (±0.11) \log (\text{MEI}_{\text{cond}}) \quad (r = 0.71)
\]

where:
- TP is the mean in-lake phosphorus concentration (µg 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 (µs 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
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.

<table>
<thead>
<tr>
<th>Approach</th>
<th>Lakes</th>
<th>Rivers</th>
<th>Transitional</th>
<th>Coastal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site-specific</td>
<td>Palaeolimnology/Analogue-matching</td>
<td>Export-coefficient modelling</td>
<td>MEI</td>
<td>Palaeoecology (MOLTEN)</td>
</tr>
<tr>
<td>Spatial-state</td>
<td>LAKEPACS? PSYM</td>
<td>RIVPACS</td>
<td>COASTPACS</td>
<td>COASTPACS</td>
</tr>
</tbody>
</table>

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 R&D 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 R&D programme which is probably beyond the scope of Phase II.
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Appendix 1  List of lakes where reference nutrient conditions have been established using the diatom-TP transfer function approach

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<th>Lake</th>
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<td><strong>England</strong></td>
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<td>1. Barton Broad</td>
<td>Bennion et al. 2001a</td>
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<td>2. Bassenthwaite Lake</td>
<td>Bennion et al. 2000b</td>
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<td>4. Clarepool Moss</td>
<td>Bennion et al. 1997b</td>
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<td>5. Crag Lough</td>
<td>Lewis 1996</td>
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<td>7. Eleven Acre lake</td>
<td>Bennion 1993</td>
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<td>8. Esthwaite Water</td>
<td>Bennion et al. 2000b</td>
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<td>10. Greenlee Lough</td>
<td>Bennion et al. 1997b</td>
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<td>14. Loweswater</td>
<td>Bennion et al. 2000a</td>
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<td>15. Malham Tarn</td>
<td>Bennion et al. 1997b</td>
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<td>16. Marsworth Reservoir</td>
<td>Bennion 1994</td>
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<td>17. Martham South Broad</td>
<td>Bennion et al. 1997b</td>
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<td>18. Oak Mere</td>
<td>Bennion et al. 1997b</td>
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<td>19. Rollesby Broad</td>
<td>Bennion et al. 2001a</td>
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<tr>
<td>21. Sunbiggin Tarn</td>
<td>In progress</td>
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<td>22. Upton Broad</td>
<td>Bennion et al. 2001a</td>
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<td>23. Wastwater</td>
<td>Bennion et al. 2000b</td>
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<td>25. Wroxham Broad</td>
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<td><strong>Wales</strong></td>
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<td>1. Llyn Alwen</td>
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<tr>
<td>2. Bosherston Lake</td>
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<td>3. Llyn Cau</td>
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<td>8. Llyn Fach</td>
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<td>10. Llyn Glanmerin</td>
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<td>11. Llyn Glasfryn</td>
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<td>15. Llyn Hir</td>
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<td>17. Llyn Lech Owain</td>
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<td>20. Llyn Mymbyr</td>
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<td>22. Lower Talley Lake</td>
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