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Use of diatoms for evaluating ecological status in UK freshwaters

Science Report: SC030103/SR4

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This report is the result of research commissioned and funded by the Environment Agency's Science Programme.

Published by:
Environment Agency, Rio House, Waterside Drive, Aztec West, Almondsbury, Bristol, BS32 4UD
Tel: 01454 624400 Fax: 01454 624409
www.environment-agency.gov.uk

ISBN: 978-1-84432-816-1

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Dissemination Status:
Publicly available / released to all regions

Keywords:
Diatoms, fresh water, ecological status, uncertainty, Water Framework Directive, phytobenthos

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Science Project Number:
SC030103

Product Code:
SCHO0907BNFW-E-P

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Executive summary

This report describes the development and testing of a diatom-based tool to fulfil UK obligations to include phytobenthos in the assessment of ecological status of freshwaters. Separate tools have been developed for lakes and rivers, although they share many features, including a conceptual underpinning, in common. The new tool is based on the Trophic Diatom Index (TDI), a metric already in use with UK statutory agencies to monitor eutrophication in rivers.

The conceptual framework for the model is based around a quantitative and qualitative 'visualisation' of the state of biofilms in UK rivers and lakes in the absence of anthropogenic pressures. This recognises that such biofilms are dynamic, with both composition and abundance of the taxa present changing over relatively short periods of time. The hydrological regime and grazing pressure, in particular, will influence the phytobenthos and, as a result, there is a considerable amount of within-site variability, particularly in flowing waters.

Reference sites for rivers were defined by an iterative series of screening stages that included nutrient concentrations (N and P) and the presence of a healthy invertebrate fauna. In addition, samples with TDI values that suggested anthropogenic enrichment were purged, even if other screening factors did not indicate this.

Two systems for predicting 'expected' TDI values in rivers were tested, one based on type-specific predictions and the other on site-specific predictions. The former used multivariate regression trees (MRT) to define four 'types' of diatom assemblage found in UK running waters, separated on the basis of alkalinity and altitude. The latter predicted the 'expected' TDI value for individual sites, using a multiple regression equation that incorporated alkalinity and seasonality. The latter explained 33% of the variance in the reference samples, compared to 10% using type-specific predictions. Site-specific predictions also had a lower prediction error and this system is recommended for future use.

Using site-specific predictions of expected TDI values enabled EQRs (Ecological Quality Ratios) to be calculated for all samples in the database. The high/good status boundary was defined as the 25th percentile of the EQRs of all reference sites; good/moderate as the point at which the proportions of valves belonging to nutrient sensitive and nutrient tolerant taxa were approximately equal (the 'crossover') and lower status class boundaries were determined as equal divisions of the remaining EQR scale.

Lakes were divided into three types, based on their alkalinity, and reference sites were established using a combination of palaeoecological techniques and expert judgement. A separate index – the Lake Trophic Diatom Index (LTDI) – was established via re-calibration of the TDI and EQR values were calculated in the same manner as for rivers except that a separate reference LTDI value was defined for each lake type. Again, the high/good status class boundary was defined as the 25th percentile of reference samples and good/moderate as the 'crossover'. This approach worked well for High Alkalinity (HA) lakes and, to a lesser extent, for those at Medium Alkalinity (MA). However, for Low Alkalinity (LA) lakes, there were very few samples whose EQR values fell below the 'crossover' and, even though there is evidence from other sources of change within the lake biota at higher EQRs, the littoral phytobenthos seems to be relatively resistant to change.

Aspects of spatial and temporal variability associated with these values were explored and from these estimates of the uncertainty associated with EQR predictions for rivers were developed (there were too few data for a similar exercise to be performed for lakes). Spatial variability in diatom assemblages in rivers is higher than that in lakes

and temporal variability is also substantial. Converting these measurements of variability into estimates of uncertainty suggests that six temporal replicates are required to get estimates of status with > 95% confidence at mid-class.

Whereas the lake tool can be validated using a combination of spatial and palaeoecological studies, the river tool was developed using only contemporary spatial data. In order to establish that there have, in fact, been changes in diatom assemblages over time and that these are driven by nutrients, we removed diatoms from herbarium specimens of common aquatic macrophytes collected before 1930. In some cases, specimens were > 100 years old. Compared with the contemporary diatom flora, almost all the herbarium samples had assemblages that suggested much lower nutrient concentrations.

Both tools have been tested extensively, and results of tests of the river tool on the Rivers Wye and Axe, and the lakes tool on the Lake District lakes are also reported.

Acknowledgements

We owe an enormous debt of gratitude to the ecology staff of the Environment Agency, SEPA and EHS who collected all the samples and associated environmental data and sent them to us for processing.

Chris Adams (Environment Agency), Gina Clarke (UCL), Sarah Pritchard (Beacon Biological) and Gayle Reddihough (University of Bristol) all helped to count samples. Ellie Liptrot, Liam Macrae, Sophie Theophile and Simon Turner all helped with sample preparation at UCL and Maggie Gamble helped with sample preparation at the University of Bristol.

Lesley Batty (University of Newcastle) helped with the early stages of compiling the DARES database.

Tim Colborn (University of Bristol) helped with video photography for the training materials that were developed early in the project to help field staff collect samples.

Paul Craze (University of Bristol) helped with statistical analyses in section 6.1 while Veronique Adriaenssens (Environment Agency) and Julian Ellis (WRc) developed the framework for uncertainty analyses used in section 6.2

Several curators supported this work by granting supervised access to historical collections: Sam Trebilcock (Bristol City Museum), Tim Rich (National Museum and Gallery of Wales, Cardiff), Leander Wolstenholme (Liverpool Museum) and Stephen Harris (Oxford University Herbaria).

Jeremy Tanner and Andrew Haigh (both Environment Agency) lent us their expertise on the Wye and Axe catchments respectively.

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1 Introduction

1.1 Phytobenthos and the Water Framework Directive

1.1.1 Objectives of the Water Framework Directive

The Water Framework Directive (WFD: European Union, 2000; Foster *et al*., 2001) has created a statutory obligation for EU Member States to monitor the ecological status of water bodies with the aim of achieving 'good ecological status' (i.e. the biota is the same as or only slightly different from that expected in the absence of human activity) for all water bodies by 2015. Annex V of the WFD provides definitions of ecological status in rivers and lakes that are based on four biological quality elements: 'phytoplankton', 'macrophytes and phytobenthos', 'benthic invertebrate fauna' and 'fish fauna'. However, the element 'macrophytes and phytobenthos' comprises two groups of organisms that have traditionally been treated more-or-less separately by researchers for a number of reasons, not least because of the difference in size, with six orders of magnitude between the largest rooted macrophytes and the smallest unicellular algae.

In recent years, with the increased interest in aquatic eutrophication, assessment methods for rivers based on both macrophytes and algae have been developed in several European countries, leading to the development of European standards (EN 14184, EN 13946; EN 14407, CEN, 2003a, b, 2004). In the UK, two methods, the Trophic Diatom Index (TDI: Kelly and Whitton, 1995; Kelly *et al.*, 2001) and the macrophyte-based Mean Trophic Rank (MTR: Holmes *et al*., 1999) have both been in use for a number of years. However, the mode of assessment required for the WFD (i.e. focusing on a holistic concept of 'ecological status' rather than on the impact of individual pressures such as eutrophication) means that almost all existing approaches need to be refined or, possibly, replaced entirely in order to provide guidance appropriate to the WFD. Methods for monitoring lakes using benthic organisms are generally less well developed than those for rivers although, in the case of phytobenthos, similar methods can often be used (King *et al*., 2006).

1.1.2 Normative definitions for 'macrophytes and phytobenthos'

The normative definitions in Annex V of the WFD include reference to four different aspects of the 'macrophytes and phytobenthos' in lakes and rivers: taxonomic composition, abundance, undesirable disturbances and the presence of bacterial tufts (Table 1.1). Taxonomic composition is examined in both the TDI and MTR, but the TDI looks at relative, rather than absolute, abundance. The MTR measures 'abundance' as the percentage of the streambed covered, which may be a useful proxy for abundance *per se*. 'Undesirable disturbances' are not defined any further in the WFD itself, but ECOSTAT (2005; Table 1.2) defines an undesirable disturbance as: 'a direct or indirect anthropogenic impact on an aquatic ecosystem that appreciably degrades the health or threatens the sustainable human use of that ecosystem'. Recording of 'bacterial tufts' has not been part of any previous UK method for examining either 'phytobenthos' or 'macrophytes' and there is some confusion about exactly what this part of the normative definitions means, and how best it should be assessed.

A further feature of the normative definitions is the loose use of adjectives: the difference in taxonomic composition between 'high', 'good' and 'moderate' status, for example, requires

distinguishing the taxonomic composition corresponding 'totally or nearly totally' to undisturbed conditions with that which is subject to 'slight' and 'moderate' changes. Interpreting these normative definitions is as much about how words such as 'slight' and 'moderate' are understood as it is about how biological parameters are assessed.

1.2 Diatoms as proxies for 'phytobenthos'

The term 'phytobenthos' is not defined in the WFD. 'Phytobenthos' is, however, one part of a biological quality element termed 'macrophytes and phytobenthos' which suggests a definition that comprises all non-macrophytic components of the benthic flora. Yet this is still problematical, largely because the definition of 'macrophyte' is itself rather vague. It is perhaps easiest to accept that there is no scientific basis for a clear separation of 'macrophytes' and 'phytobenthos' with the implication that there may be some overlap between taxa included within each definition. In this report, the definition of 'phytobenthos' proposed by the Comité European de Normalisation (CEN) is followed ('All phototrophic algae and cyanobacteria that live on or attached to substrata or other organisms, rather than suspended in the water column': CEN, in preparation).

Table 1.1. Normative definitions for ecological status classes in lakes and rivers (Annex V, 1.2.1 and 1.2.2).

Class	Definition
High	The taxonomic composition corresponds totally or nearly totally to undisturbed conditions
	There are no detectable changes in the average macrophytic and the average phytobenthic abundance.
Good	There are slight changes in the composition and abundance of macrophytic and phytobenthic taxa compared to the type-specific communities. Such changes do not indicate any accelerated growth of phytobenthos or higher forms of plant life resulting in undesirable disturbances to the balance of organisms present in the water body or in any physicochemical quality of the water or sediment.
	The phytobenthic community is not adversely affected by bacterial tufts and coats present due to anthropogenic activity.
Moderate	The composition of macrophytic and phytobenthic taxa differs moderately from the type-specific community and is significantly more distorted than at good status.
	Moderate changes in the average macrophytic and the average phytobenthic abundance are evident.
	The phytobenthic community may be interfered with and, in some areas, displaced by bacterial tufts and coats present as a result of anthropogenic activities.
Poor/bad	Waters achieving a status below moderate shall be classified as poor or bad.

Table 1.2. Significant undesirable disturbances that may result from accelerated growth of phytoplankton, macroalgae, phytobenthos, macrophytes or angiosperms. From ECOSTAT (2005).

1. Causes the condition of other elements of aquatic flora in the ecosystem to be moderate or worse (e.g. as a result of decreased light availability due to increased turbidity and shading)
2. Causes the condition of benthic invertebrate fauna to be moderate or worse (e.g. as a result of increased sedimentation of organic matter; oxygen deficiency; release of hydrogen sulphide; changes in habitat availability)
3. Causes the condition of fish fauna to be moderate or worse (e.g. as a result of oxygen deficiency; release of hydrogen sulphide; changes in habitat availability)
4. Compromises the achievement of the objectives of a Protected Area for economically significant species (e.g. as a result of accumulation of toxins in shellfish)
5. Compromises the achievement of objectives for a Natura 2000 Protected Area
6. Compromises the achievement of objectives for a Drinking Water Protected Area (e.g. as a result of disturbances to the quality of water)
7. Compromises the achievement of objectives for other protected areas (e.g. bathing water, sensitive areas or polluted waters)
8. Causes a change that is harmful to human health (e.g. shellfish poisoning; wind borne toxins from algal blooms)
9. Causes a significant impairment of, or interference with, amenities and other legitimate uses of the environment (e.g. impairment of fisheries)
10. Causes significant damage to material property

Note: If eutrophication were to lead to an environmental quality standard for a specific pollutant being exceeded (e.g. through changes to the conditions in sediments), this would also constitute a significant undesirable disturbance.

Current approaches for using phytobenthos for monitoring in Europe generally focus on a representative taxonomic group (usually diatoms) sampled from a representative habitat (usually cobbles or boulders) (Kelly *et al.*, 1998; Prygiel *et al.*, 2002; Rott *et al.*, 2003; King *et al.*, 2006). Fewer studies adopt a holistic approach to the flora (e.g. Jarlman *et al*., 1996; Pipp and Rott, 1996; Lindstrøm *et al*., 2004; Schaumburg *et al.*, 2004a, b), in contrast to North America (Stevenson and Bahls, 1999) and New Zealand (Biggs and Kilroy, 2000) where the entire phototrophic assemblage is analysed routinely. The justification for using diatoms is that they offer a similar insight into the pressures shaping the benthic flora but in a more cost-effective manner than when the entire flora is examined. This is particularly true where the primary purpose of the analysis is to assess the impact of a pressure such as pH or nutrients but the validity of this approach needs to be re-examined where the purpose of the analysis is to assess ecological status.

Kelly (2006) looked at the relationship between diatoms and the entire phototrophic flora (i.e. including macroalgae and macrophytes) in UK rivers and suggested that diatoms were acting as cost-effective proxies. Kelly *et al*. (2006), on the other hand, found no relationship between non-diatoms and pressures from rivers in Ireland and a canonical correspondence analysis based on all algae was actually weaker than one based on diatoms alone. It appeared that non-diatoms were introducing 'noise' to the algae–environment relationship in rivers, rather than strengthening the 'signal'. The situation was slightly different in lakes, where the signal based on 'all algae' was slightly stronger than that based on diatoms alone, possibly because diatoms were less dominant than in rivers (Kelly *et al*., 2006).

The conclusion that Kelly *et al*. (2006) reached was that there probably was a strong relationship between non-diatoms and their environment but that this was lost due to the practical difficulties encountered when examining 'live' (or preserved) epilithon samples, compared to cleaned diatom samples (Table 1.3). Even with the availability of Cox (1996), the *Freshwater Algae Flora of the British Isles* (John *et al*., 2002) and CD-ROMs (Whitton *et al*., 2000, 2002), identification beyond genus is difficult and experience from diatoms (where species–environment relationships are relatively well understood) show that genus-level generalisations about environmental preferences are often unreliable (Chessman *et al*., 1999). If diatoms give similar results to diatoms plus other algae, while the effort for diatoms plus other algae is invariably greater than that required to analyse diatoms alone, then there is a strong case for focusing on diatoms, in order to make efficient use of a limited budget.

Table 1.3. Advantages and disadvantages of microscopic analysis of algae (after Kelly *et al*., 2006).

	All microalgae	**Diatoms**
Density	Low. Organisms often occluded by organic and inorganic sample matrix	High. Organic components of sample matrix removed through use of oxidising agents
Contagion	High. Filaments and coenobia remain intact	Low. Most diatom filaments destroyed (a few Fragilariophyceae remain partially intact) and frustules are separated into valves
Identification	Often only genus-level identification is possible from vegetative material alone. Sometimes only family or order-level identification is possible (e.g. thin cyanobacterial filaments, some small unicellular Chlorophyta, or if only some cells of colonial forms are available)	Species-level identification is routine. A few girdle-views can only be identified to genus
Durability	Samples have only limited lifetime and require a lot more attention. Samples need to be prepared every time analysis is to be carried out	Once prepared, slides can be kept and referred to for many years. Exchange and quality assurance therefore easily possible
Size range	Wide range of size requires counts at various magnifications in order to optimise identification.	Smaller range of size: magnification of 1000× suitable for most diatoms.

1.3 Defining and estimating ecological status using diatoms

The WFD defines ecological status as 'an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters …' (Article 2). Although Annex V goes on to define ecological status classes for macrophytes and phytobenthos in

rivers and lakes in terms of species composition and abundance, and it is possible to develop WFD-compatible methods that are based on composition alone (Rimet *et al*., 2004; Schaumburg *et al.*, 2004a, b), such approaches offer little guidance on how status class boundaries should be placed and, in particular, on how to differentiate between 'good' and 'moderate' ecological status.

It is important, however, to draw a distinction between **defining** ecological status, for which all components of the normative definition need to be considered, and of **estimating** ecological status. Kelly *et al*. (2006) argue that if there is a strong ecological rationale for placing class boundaries then it would be possible to use a metric with a linear response to ecological status to predict the status of individual sampling sites even if the metric did not itself encompass all criteria in the normative definition. The argument that will be developed here is that an understanding of the structure and function of phytobenthic communities can provide a basis for defining the properties of ecological status classes and that this, in turn, can be translated into a list of taxa expected to be associated with each status class. The diatom assemblage becomes, therefore, a proxy for structural and functional properties of the biofilm, fulfilling the basic requirement of the normative definitions for ecological status while still permitting a cost-effective implementation.

The established approach to using diatoms in palaeoecological investigations is to use them as proxies for abiotic 'pressure' variables such as pH (Battarbee *et al*., 1999) nutrients (Bennion *et al.,* 1996) and salinity (Fritz *et al.,* 1999), in which metrics based on (usually) weighted-averaging (Birks *et al*., 1990) are used to integrate the autecological responses of all the diatom taxa present in a sample. Similar approaches are used for monitoring contemporary environments (Zelinka and Marvan, 1961; Coste in CEMAGREF, 1982; Kelly and Whitton, 1995), leading to numerical expressions of the intensity of pressures. If this value is then divided by the value expected in the absence of anthropogenic pressures, then the outcome is an Ecological Quality Ratio (EQR) that is compatible with the reporting requirements of the WFD (Annex V, 1.4.1). If the metric is calibrated against the most likely pressure, then there will be a high likelihood that impacts due to this pressure will be detected. There will, however, be a low probability that unexpected pressures will also be detected and, therefore, a risk of 'false negatives' (a site is classified as 'good ecological status' when it is, in fact, 'moderate ecological status' or lower).

One alternative is to combine metrics in order to provide simultaneous coverage of a number of pressures with distinct responses. The lowest EQR (assuming a scale 0–1 where 1 = high status) then indicates the true ecological status. This approach has been adopted for phytobenthos in Austria and Germany where separate metrics for 'saprobicity' (i.e. organic pollution) and nutrients are combined with a metric based on the proportion of taxa expected at reference conditions for a given type (Schaumburg *et al.,* 2004a, b; Pfister and Pipp, 2005). Such methods reduce (but do not eliminate) the risk of 'false negatives' but this apparent benefit may not be realised if there is strong collinearity between metrics. Furthermore, combining pressure metrics still does not offer any insights into the critical issue of placement of boundaries.

The underlying problem is that almost all contemporary monitoring methods translate the biological assemblage into a continuous variable that can then be regressed against the pressure variable(s), whereas the WFD contains a paradox – requiring ecological status to be expressed as a (continuous) EQR on one hand while basing regulation on the (categoric) distinction between high, good, moderate, poor and bad status on the other. ECOSTAT (2005) suggests that ecological status class boundaries should be placed at points where there is a distinct discontinuity in the relationship between a biological metric and the gradient of impact. However, the nature of the pressure metrics described above is that such discontinuities do not exist. The alternative outlined in ECOSTAT (2005) is to use the 'crossover' between paired metrics (e.g. % sensitive taxa and % impact taxa), although

deciding on the meaning of the crossover point still requires an underlying 'conceptual model' that can be related back to the normative definitions.

The advantage of a robust conceptual model is that it provides a link between the normative definitions and ecological theory (note references to 'structure' and 'function' in the definition of ecological status). A qualitative 'vision' of the phytobenthos in the absence of pressures, and of how this changes along a pressure gradient can then be translated into quantitative parameters (including values of pressure metrics) that can be used to set boundaries along the EQR gradient. The disadvantage is that such a conceptual model requires a dynamic view of biofilm structure and function (Biggs *et al.*, 1998) that may be at odds with a regulator's desire for a crisp separation between ecological status classes.

1.4 Objectives

This report provides an overview of two projects that have run in parallel, to develop diatom-based tools for assessing ecological status in rivers (EMC/WP04/078 Diatoms as monitors of the ecological status of rivers) and lakes/lochs/loughs (SC030103 Development of a phytobenthos classification tool for lakes and lochs).

These projects are known by the acronyms 'DARES' – Diatoms for Assessing River Ecological Status and 'DALES' – Diatoms for Assessing Lake/Loch Ecological Status and both have, as their overall objective, to develop robust operational tool(s) to enable the prediction of ecological status based on the diatom community present at any river or standing water site in the UK. More specifically, both projects set out to:

- gather existing and new data covering benthic diatoms and associated environmental data across the complete range of still and running waters in the UK into a database;
- define the expected (reference condition) diatom community at any (river/lake/loch) site;
- develop a model for assessing ecological status (expressed in terms of quantitative deviation from the reference condition) along a nutrient/organic pollution gradient;
- develop a rationale for placing status class boundaries along this gradient;
- develop estimates of uncertainty associated with status class assessments;
- combine all of the above into a package that can be used for routine assessment of water bodies by the Environment Agency, SEPA and EHS.

A note on the text

The chapters in this report represent the work of ten authors spread between eight different institutions. Different team members co-ordinated the various work packages, each of which forms the basis for a chapter and, as the projects evolved UK TAG's Rivers Task Team and Lakes Task Team asked slightly different questions of the two projects. There are, as a result, a number of differences in style and approach between chapters. We are also aware that a report of this size is unlikely to be read in its entirety by many people and have tried to write individual chapters in such a way that they can be read with minimal cross-referencing to other parts of the report.

2 Interpreting ecological status concepts

2.1 Introduction

The WFD defines ecological status as: 'an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters, classified in accordance with Annex V'. (Article 2, paragraph 21). Some of the problems encountered in converting this definition into practice have already been described (chapter 1). The need to define five distinct status classes poses a particular problem. Anthropogenic pressures are generally interpreted as 'gradients' along which ecological changes occur gradually, often without distinct step-changes that would make obvious locations for status class boundaries (see Pollard and van der Bund, 2005). A robust system for assessing ecological status needs to reconcile this paradigm of gradual change (recognised in the WFD by the requirement to express status as an EQR) while, at the same time, allowing a categorical distinction between five status classes.

Intriguingly, while the definition of ecological status in Article 2 refers to 'structure' and 'functioning', there is no explicit reference to these in Annex V which, for phytobenthos, refers to taxonomic composition and abundance. The DARES and DALES models are built around the assumption that it is possible to infer aspects of the structure and function of an ecosystem from the taxonomic composition and relative abundance of taxa present. Moreover, we argue that it is reasonably straightforward to adapt the weighted-average metrics that are the mainstay of diatom-based monitoring, to act as EQRs. Indeed, weighted-average metrics such as the TDI (Kelly and Whitton, 1995) and Indice de Polluosensibilité (IPS: Coste in CEMAGREF, 1982) offer a convenient means of summarising information required about taxonomic change into a single value (see below). The TDI, for example, gives low scores to nutrient sensitive taxa (most likely to be encountered at high and good status) and high scores to nutrient tolerant taxa (i.e. those most likely to be found at moderate, poor and bad status), and the balance between these groups is a good reflection of the extent to which the flora has moved from 'reference conditions'. As long as reference conditions can be defined, and the flora evaluated in terms of a weighted-average metric, an EQR can be calculated very simply. Although the TDI was developed for rivers, the same concept can, with recalibration also be applied to lakes (see chapter 5). This is preferable to use of a total phosphorus (TP) transfer function as the latter does not express results in terms that are compatible with the ecological concepts at the heart of the WFD. Two problems remain:

- Weighted-average metrics assess only a single pressure (e.g. nutrients), or a suite of closely related pressures (e.g. nutrient/organic pollution) and are often insensitive to other pressures (neither the TDI nor IPS, for example, are able to detect acidification).
- These metrics provide no information on the structure and function of ecosystems, and integrate all changes caused by a pressure into a single value. There is, consequently, no *a priori* rationale for placing status class boundaries based on a weighted-average, or similar, metric.

2.2 Stream and lake flora at reference/high status

An understanding of the biota in the absence of anthropogenic pressures is central to the development of a WFD-compliant monitoring tool, as ecological status classes are all defined in terms of the relationship of the biota to this 'reference' condition. The basis for defining reference conditions in the DARES and DALES projects is outlined in chapters 4 and 5: the important point to bear in mind for the remainder of this chapter is that reference conditions are defined by the **absence** of pressure, not by the **presence** of a particular biota. The biota that is characteristic of reference conditions for a particular site defines the 'expected' biota for that site (in this project and others, the median value of metrics at reference provide the 'expected' value for EQR calculations). The biota characteristic of reference sites defines 'high ecological status' although it can, in theory, survive a slight increase in pressure from the reference state. In practice, the process of selecting reference sites is imperfect, and this project defines the high/good ecological status boundary using statistical criteria (see chapters 4 and 5 for a full explanation), in order to allow for these shortcomings. It is, therefore, possible for a site in reference condition (i.e. an absence of known anthropogenic pressures) to have a biota typical of good, or even moderate, status.

This understanding of the biota can be expressed in many ways. As the focus of the DARES and DALES projects has been development of a tool that expresses ecological status in quantitative terms, this chapter expresses this understanding in terms of descriptive and functional ecology of the entire phytobenthos in order to provide a complementary perspective.

Streams and lake littoral zones at high status in the UK are typically spatially and temporally heterogeneous, with substrata of a range of size and types. Some of these may have patches of macroalgae – as filaments, thalli, mucilaginous growths and crusts – representing several phyla (typically Cyanobacteria, Rhodophyta, Chlorophyta, Xanthophyta, though others can also be found: Figure 2.1). There will also be Bryophyta and vascular plants. The defining characteristic of this macro-view of the undisturbed stream flora, however, is that there is rarely an obvious 'monoculture' in which a single species dominates. During early spring, many water bodies have a brief 'bloom' of *Ulothrix*, and there are other exceptions (*Didymosphenia* can dominate in some streams, especially in summer) but these are either relatively rare or of brief duration.

However, as well as substrata with such growths, there are many that lack macroalgae, and which are covered, instead, by a biofilm which is often dominated by diatoms. The DARES/DALES sampling protocol focuses on collecting a representative sample of this biofilm (see chapter 3) and the model assumes that changes in its composition mirror changes in other components of the phytobenthos in a particular water body (see Kelly, 2006; Kelly *et al*., 2006).

The microflora of a stream or lake can be summarised as a list of taxa, which typically follow a pattern similar to that in Figure 2.2, with a few taxa comprising the majority of individuals in a population, accompanied by a long 'tail' of less common taxa. The less abundant taxa found in a sample will consist of species that are genuinely 'rare' constituents of the biota, along with taxa that are washed in from elsewhere in the catchment. Because diatom analyses focus on cleaned valves, it is impossible to be certain that all those taxa which form the tail represent 'signal' rather than 'noise' when trying to evaluate ecological status.

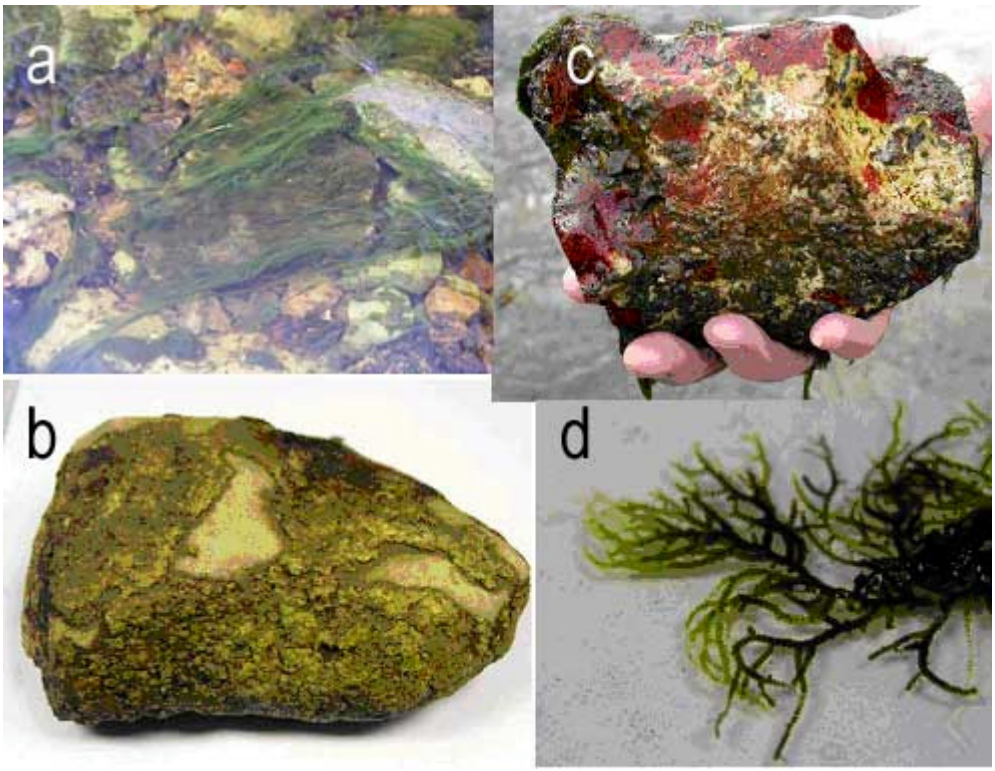


Figure 2.1. Macroalgae characteristic of high and good ecological status. (a) *Ulothrix zonata* (Chlorophyta); (b) *Homeothrix crustacea* (Cyanobacteria); (c) *Hildenbrandia rivularis*; (d) *Batrachospermum* sp. (both Rhodophyta).

A further characteristic of such samples is that those taxa that comprise the majority of individuals are drawn from a fairly small pool, so that a few taxa (e.g. *Achnanthidium minutissimum, Tabellaria flocculosa, Fragilaria capucina*) are found in almost all high status samples, although they tend not to be restricted to high status sites, but to have relatively broad niches. The balance of these 'common' taxa varies, as a series of successional changes occur within the biofilm, from the initial colonisation of a bare rock surface by algae and bacteria through to a thick biofilm composed of a diverse assemblage of algae and other micro-organisms. The trajectory that a succession will follow depends on a number of factors, but Biggs *et al.* (1998) regard resource supply as a key factor (Figure 2.3). The relevance of this conceptual model to ecological status assessment is discussed in more length in Yallop and Kelly (2006) but, in essence, it means that there is no distinct 'reference community' for any stream or lake but, instead, a cluster of possibilities, depending upon the point along a micro-successional trajectory that the sample was collected.

The early diatom colonisers in these successions at high status in circumneutral streams is usually *Achnanthidium minutissimum* (Figure 2.4a), but as the biofilm increases in thickness competition for resources becomes more intensive and long-stalked species such as *Gomphonema acuminatum* that are able to grow above the short-stalked species have a selective advantage while more loosely attached species such as *Hannaea arcus, Tabellaria flocculosa* and *Fragilaria capucina* become entangled and are able to establish (Figure 2.4b, c). Motile species are relatively rare at high status – *Navicula angusta* is one of the few that is found regularly, though rarely in great numbers. Heterotrophic organisms will also be present, particularly at later stages in the succession, but an important characteristic of biofilms at high status is that autotrophic organisms predominate and give the biofilm its microscopic structure, and that photosynthesis exceeds respiration.

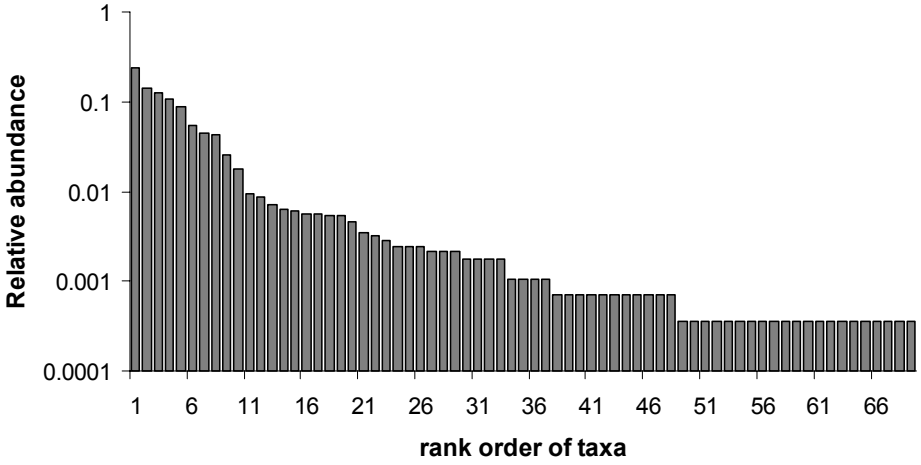


Figure 2.2. Relative abundance of diatom taxa found in the River Ribble, d/s Clitheroe STW, 17 September 2004. Only four out of the 69 taxa recorded at the site constitute more than 10% of the total count, while 58 taxa comprise < 1% of the total. The River Ribble at Clitheroe is not at good status, but this rank–abundance pattern is typical of benthic diatom samples from both rivers and lakes.

Biggs *et al*. (1998) describe the species that are able to thrive in the later stages of the micro-successions when the resource supply is low as 'stress-selected' and several of the characteristic 'S-selected' taxa that they list are organisms known to be able to fix nitrogen (e.g. *Calothrix, Tolypothrix, Epithemia*) or utilise organic phosphorus (*Draparnaldia, Batrachospermum*). Yallop and Kelly (2006) argue that slow-growing crustose algae (e.g. *Chamaesiphon, Hildenbrandia*), which are able to cope with grazing pressures and to survive scouring spates, also have a selective advantage, though they do not fit neatly into the Biggs *et al*. (1998) model.

This model has three implications for assessing ecological status. First, a change in the resource supply (e.g. an increase in nutrients due to eutrophication) will change the slope of the trajectory, but there is likely to be some overlap in the trajectories, especially during the early stages of the micro-succession (Figure 2.3b). Second, the model emphasises the importance of disturbance. This means that a number of different successional stages will be able to co-exist, as substrata of different sizes will be subject to different levels of disturbance (Figure 2.3c).

Finally, the successions are most easily explained in terms of the changes in those taxa which comprise the majority of the biomass. These tend to be the taxa on the left hand side of Figure 2.2 and these create a 'matrix' within which other taxa can live. An analogy with terrestrial flora is useful at this point: the changes described within biofilms are equivalent to the sequence of changes that occur as bare ground is colonised first to grassland and then to scrub. Within the grassland, a few common species (typically grasses – Poaceae) form the majority of the biomass, within which less common species (typically 'herbs') thrive. As competition for light increases, so taller taxa are able to invade. In the high status stream we suggest that *Achnanthidium* is the equivalent of 'grass' while the stalked diatoms such as *Gomphonema* are the 'shrubs'. Extending this terrestrial ecology analogy a little further, a phytosociologist defines types of vegetation *association* by reference to the common taxa. In the stream biofilm, we define ecological status by reference to those taxa that form the 'matrix' of the biofilm and which form the habitat within which other taxa can thrive.

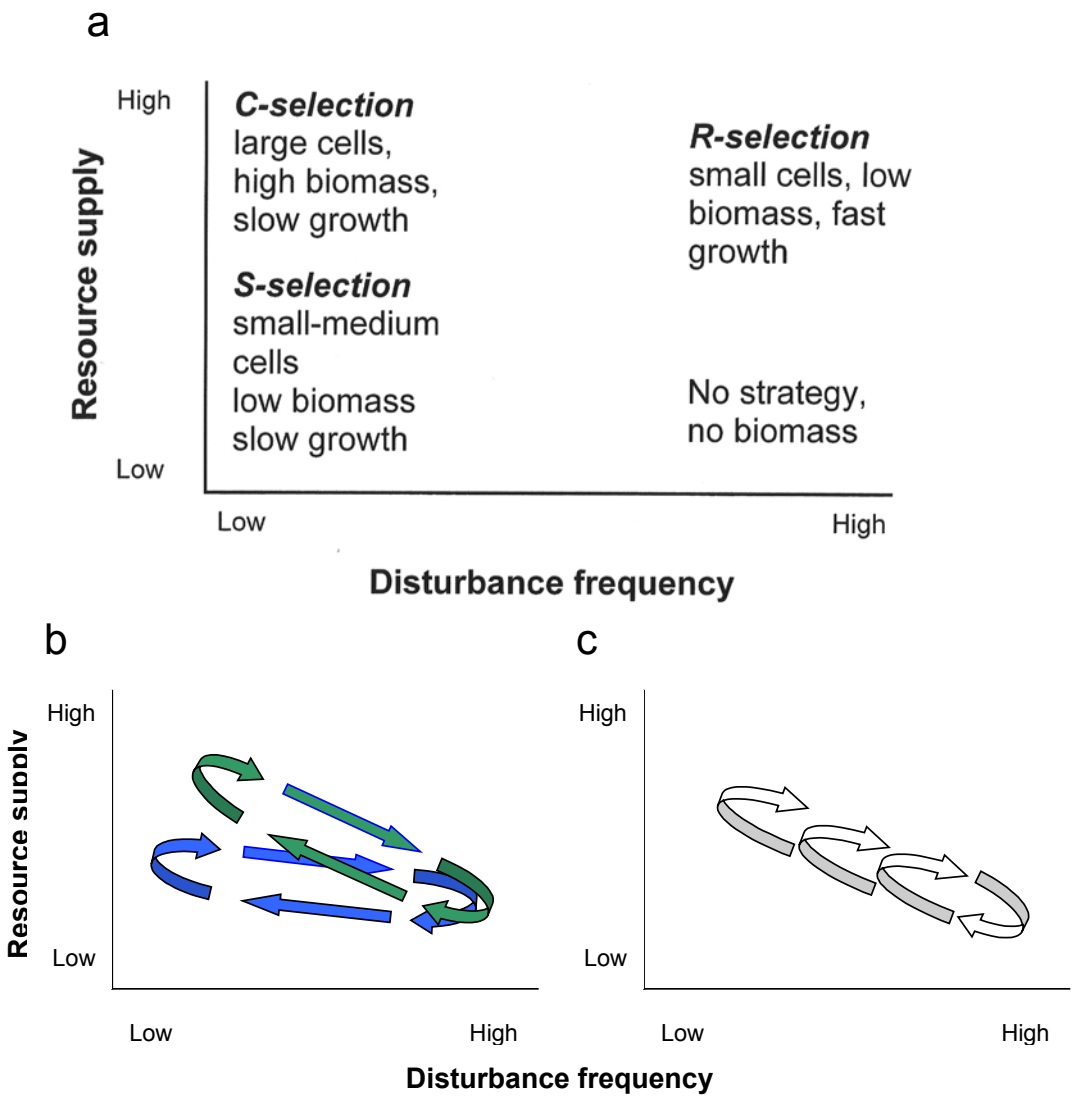


Figure 2.3. (a) Hypothesised location of taxa exhibiting three life-history strategies on a disturbance–inorganic nutrient resource supply habitat matrix: Yallop and Kelly (2006), modified from Biggs *et al*. (1998), who adopt the terminology of Grime (1979), dividing plants into three groups depending upon their ecological strategy. 'R-selected', or ruderal species are the early colonisers, S-selected, or stress-selected species are (in this context) those adapted to surviving in environments where resources are scarce, while C-selected, or competitively selected species are those adapted to outcompete other taxa in situations where resources are abundant. See Biggs *et al*. (1998) for more details. (b) Hypothesised successional trajectories of stream biofilm communities from the pioneer stage to the point at which the trajectory is truncated by a catastrophic event (e.g. an intensive flood disturbance). The trajectory can also be truncated by intensive grazers which can select in favour of taxa characteristic of more disturbed habitats and an increase in nutrient supply can adjust the slope of the trajectory, favouring competitive over stress-tolerant taxa. (c) The length of the trajectories will depend upon disturbance frequency, and smaller substrata will be disturbed more frequently than larger, leading to within-site heterogeneity of biofilm assemblages.

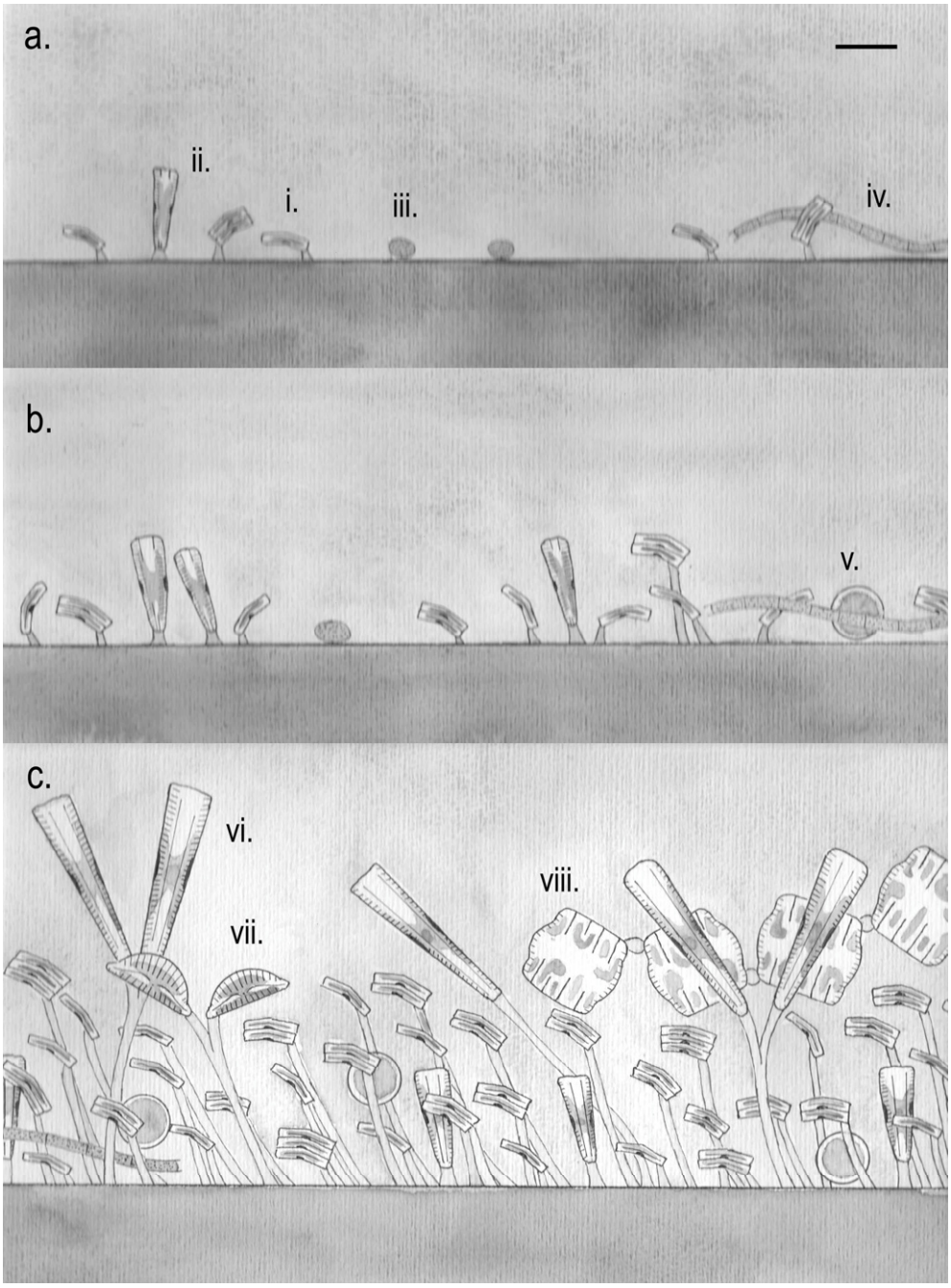


Figure 2.4. Diagrammatic representation of a diatom-dominated epilithic biofilm at three stages of the trajectory illustrated in Figure 2.3. Based on data from benthic assemblages in Wastwater in King (2000). Scale bar: 10 μm.
(a) After one week, early colonists such as *Achnanthidium minutissimum* (i), *Gomphonema parvulum* (ii), coccoid Cyanobacteria (iii) and narrow filaments of *Oscillatoria* (iv) occupy space on the upper surface of littoral rocks.
(b) After three weeks, these algae cover most of the available space on the rock, and density-dependent factors start to have a significant influence on the community (v, coccoid Chlorophyta).
(c) At six weeks, the structure of the community has changed, with long-stalked taxa such as *Gomphonema acuminatum* (vi) and *Cymbella* (vii) rising above the layer of *Achnanthidium*, while filamentous taxa such as *Tabellaria flocculosa* (viii) grow entangled with the attached algae.

2.3 Stream and lake flora at good and moderate status

The normative definitions define the composition of the phytobenthos at good ecological status (GES) to be slightly changed from that expected at high ecological status (HES), but once the composition is 'moderately changed' then the biota is at moderate ecological status (MES) and not GES. There is, therefore, a need to find a rationale to distinguish between a 'slight' and a 'moderate' change, bearing in mind that changes along pressure gradients are gradual, whereas the WFD requires the delimitation of five distinct categories of ecological status.

The option adopted in several Member States has been to set the HES/GES boundary as a percentile of values of a metric at reference (as has been done in the UK), and then to define subsequent status class boundaries as equally spaced divisions of the remaining part of the scale. This is a pragmatic solution, but it does not take account of the ecological characteristics of the reference assemblage. The normative definition is ambiguous, but our interpretation, based on the definition of ecological status in Article 2, is that GES implies a biota that is structured in a similar way to the biota at HES. This means that the *association* is the same as that found at HES, but that the taxa that live within this matrix ('herbs') might be different to those typical of HES. The term 'association' is derived from phytosociology, a discipline within ecology that has been criticised in recent years for failing to recognise that communities change gradually along environmental gradients (e.g. Smith and Smith, 2001), so there are, in fact, no clear boundaries between 'associations' as early proponents such as Clements (1916) suggested. However, analyses in chapters 3 and 4 demonstrate that, once the principal anthropogenic gradient has been removed, only a weak environmental gradient (principally alkalinity) remains and, for the purpose of the conceptual model described here, reference communities can be thought of as distinct associations, albeit highly dynamic in nature.

The biofilm at GES is, therefore, characterised as being structured and functioning in much the same way as it would at HES except that there are fewer of the most sensitive taxa while some taxa that are tolerant to increased levels of a pressure will be present. Our concept of MES is a biofilm with a significantly different structure to that found at HES. In both cases we assume that there is a relationship between the structure and functioning of an ecosystem.

This can be explained by reference to the five classes of nutrient sensitivity (s) on which the TDI is based (Figure 4.6). The taxa that are associated with 'reference conditions' tend to be found in the first three classes, but with class 2 (which includes taxa such as *Achnanthidium minutissimum* and *Fragilaria capucina*) predominating. However, as EQR decreases, so the proportion of individuals belonging to taxa in class 1 falls very steeply, and good status is characterised by a flora composed predominately of class 2 taxa, accompanied by a small number of class 1 taxa and, as the EQR decreases (and, therefore, the pressure increases), an increasing proportion of indifferent (TDI s = 3) and tolerant ($s \geq 4$) taxa. A further characteristic is that the proportion of motile taxa is low at reference conditions, but that this increases as EQR decreases. A few motile taxa (e.g. *Navicula angusta*) are characteristic of high and good status, but these are rarely abundant, whereas at lower EQR values, motile taxa often constitute > 60% of all individuals found in a sample.

These changes extend to other algae too. The most sensitive taxa found at HES may not be found at GES: examples include the heterocystous Cyanobacteria which are unlikely to have a competitive advantage after only a slight increase in load of combined nitrogen. However, a variety of macroalgae can be encountered at GES, along with an increased cover of filamentous algae such as *Cladophora*, though still not approaching a monoculture. Some macroalgae will persist into MES, but one characteristic of MES is that *Cladophora* is likely to be the most conspicuous macroalga and to cover a significant proportion of the substratum, leading to the possibility of 'undesirable disturbances'.

The reasons for these changes within the biofilm are not entirely clear, and are likely to be multi-factorial, but some possibilities include:

- Changes in land use within catchments increase sediment load in rivers, leading to more abiotic particles trapped in biofilms, favouring motile over sessile diatoms.
- Increased cover of filamentous algae has a number of implications for the diatom assemblage, including selecting in favour of shade-adapted 'understorey' taxa, favouring 'epiphytes' (e.g. *Rhoicosphenia*) and trapping sediment, which creates an environment in which motile taxa have a competitive advantage.
- Increased primary production and/or an increased load of biodegradable material encourages the growth of heterotrophic organisms within the biofilm and leads to a more open 'matrix' of filamentous bacteria rather than a matrix dominated by autotrophs found at HES and GES. Again, motile taxa have a competitive advantage over sessile taxa.
- A combination of the above.

Several of these suggestions are supported by the literature although few studies have been concerned directly with changes that occur at pressure levels between good and moderate status. The change in taxa observed in both DARES and DALES is clear; however, the relationship to structure and function within the biofilm is, to some extent, conjecture that needs to be supported by research on fundamental processes.

Locating the GES/MES boundary with respect to these changes is problematic because these changes occur gradually along a gradient. The approach adopted in these projects was to use the point at which the 'sensitive' (TDI s $\leq$ 2) and 'tolerant' (TDI s $\geq$ 4) are present in equal numbers (Figures 4.12; 5.10) as the boundary. At higher EQR values, it is sensitive taxa that predominate, while at lower EQR values, the sensitive taxa are subordinate to taxa that thrive at elevated pressure levels.

By placing the GES/MES boundary at this point, GES should be self-maintaining, especially if the catchment upstream is also at GES or HES. This will mean that incocula available to recolonise after spates will also be composed primarily of sensitive taxa and that these will have a competitive advantage over any tolerant taxa that are able to settle on bare surfaces.

2.4 Stream and lake flora at poor and bad status

No effort has been made to develop an ecological rationale to distinguish between moderate, poor and bad ecological status. Instead, the remaining EQR gradient beyond GES/MES has been divided into three equal portions. The main characteristics of poor ecological status (PES) and bad ecological status (BES) are that sensitive taxa are rare or absent and, as pressure levels increase, indifferent taxa too will disappear so that the flora is composed almost entirely of tolerant taxa. The biofilm found at PES and BES is very different to that described at HES, and it will be dominated by heterotrophic bacteria and filamentous algae such as *Cladophora*. There is usually also a relatively large content of abiotic material and most of the diatoms will be motile, rather than sessile. Several of the diatoms characteristic of high levels of organic pollution known to be capable of facultative heterotrophy may also be found (Tuchman, 1996).

3 Methods

3.1 Field and laboratory methods

3.1.1 Phytobenthos collection and identification

For rivers, five cobbles were collected from mid-stream and placed into a tray with a little stream water and the top surface of each was brushed with a clean toothbrush in order to remove the biofilm (Kelly *et al*., 1998; CEN, 2003a). For lakes, five cobbles were collected from the littoral zone away from inflow streams and obvious human impacts. Where cobbles were absent or where the bottom sediments were dominated by fine sediments with only a few larger stones, five submerged stems of a single emergent plant species such as *Phragmites australis*, *Sparganium erectum*, *Glyceria maxima* or *Typha* spp. were collected.

The resulting suspension was collected in a plastic bottle, fixed with Lugol's iodine and stored prior to analysis. Samples were either digested in a saturated solution of potassium permanganate and concentrated. hydrochloric acid (after Hendey, 1974) or digested with hydrogen peroxide in order to remove organic material, and permanent slides were prepared using Naphrax (refractive index = 1.74) as a mountant.

At least 300 undamaged valves of non-planktonic taxa were identified and counted using 1000× magnification (CEN, 2004). The primary floras and identification guides used in this study were Krammer and Lange-Bertalot (1986, 1997, 2000, 2004) and Hartley *et al*. (1996). All nomenclature was adjusted to that used by Whitton *et al*. (1998), which follows conventions in Round *et al*. (1990) and Fourtanier and Kociolek (1999). All taxa were identified to the highest resolution possible (usually species or variety). Infraspecific taxa were merged for those species where a preliminary examination of taxonenvironment scatterplots suggested that the response of infraspecific taxa were not distinguishable from that of the species. Slightly different conventions were used for lakes and rivers. For rivers, '*Achnanthidium minutissimum* type' refers to *A. minutissimum*, *A. saprophilum, A. affine* and *A. exilis* but not to *A. biasolettiana, A. microcephalum* or *A. subatomus*, all of which were more nutrient sensitive than *A. minutissimum*. *Cocconeis placentula* varieties were separated but all *Frustulia rhomboides* varieties were merged, as were *Fragilaria capucina* var. *capucina* and var. *gracilis* (although other varieties of *F. capucina* were kept separate). '*Gomphonema angustum/pumilum'* includes these two species along with *G. bavaricum* and *G. lateripunctatum.*

In the DALES dataset, as in DARES, *Frustulia rhomboides* varieties were merged; however, *Gomphonema angustum* and *pumilum* were separated but *Cocconeis placentula* varieties were not. For lakes, *Fragilaria capucina* var. *capucina,* var. *rumpens* and var. *gracilis* were merged to *F. capucina*, *Synedra tenera* was merged to *S. nana* and all *Planothidium* species that were formerly varieties and subspecies of *Achnanthes lanceolata* were merged to *P. lanceolatum*. Despite early indications in DALES that (a) coarse and fine forms of *Fragilaria vaucheriae* and (b) long, short and medium forms of *Tabellaria flocculosa* had different environmental preferences, taxon–environment scatterplots suggested similar responses, resulting in the merger of fine and coarse forms of *F. vaucheriae* and the merger of all forms of *T. flocculosa*. Small or benthic *Fragilaria (sensu lato*) refers to those *Fragilaria* spp. now considered to be separate genera: *Pseudostaurosira, Staurosira* and *Staurosirella.*

Environment Agency Technical reference Material for all methods are available on the easinet

http://intranet.ea.gov/Organisation/df/Water_Management/Conservation_and_Ecology/eat/diatoms/contents.htm or protocols http://craticula.ncl.ac.uk/dares/methods.htm.

3.1.2 Environmental data

Rivers

Environmental data linked to the new diatom samples collected in 2004 as part of this project were extracted from databases held by UK regulatory agencies. Samples in the larger DARES database that were derived from previous projects and that lacked environmental data were matched in time and space to Environment Agency/SEPA chemical data and a subset of 480 sites identified that fulfilled the criteria of (1) chemical data collected from within 200 m of the diatom site, and (2) chemical data consisting of at least six separate samples collected within one year of collection of the diatom sample.

All chemical determinands were then expressed as annual means based on between 6 and 22 samples (average 12). Much of the phosphorus (P) data had a detection limit of 20 $\mu g\ l^{-1}$; values below this were expressed as half the detection limit in the calculation of annual means to give a more accurate estimation of true P concentrations. This strategy was necessary in order to keep the dataset sufficiently large to draw robust inferences; however, it may lead to overestimates of summary statistics, particularly at low nutrient concentrations.

Diatom samples from soft water sites (total hardness < 6 mg^{-1} $CaCO_3$) were excluded because most lack P data. These will be the subject of a separate analysis looking at pH and alkalinity limits.

Lakes

Environmental data for lakes were extracted from the Access database compiled by Carvalho *et al.* (2006) for the specific purpose of metric development. The database was composed predominantly of data collected by and on behalf of the Environment Agency, CCW and SEPA.

Ideally, the 2003 and 2004 diatom data should have been matched with environmental data collected over the corresponding time periods. In many cases, 2004 data were available; however, for some sites/samples the timing of environmental data collection did not correspond with the timing of biological sampling and therefore environmental data were substituted from the nearest available time period. For some sites data were available for 2005, whereas for other sites the nearest available data were from 2000, or at worst, from as far back as 1996.

The environmental dataset comprises mean annual values. Annual averages were considered more robust than seasonal values because seasonal data were absent or patchy for a large number of lakes. The number of individual seasonal measurements from which annual means have been calculated range from 1 to 45 (average 8). Both the number of individual samples and the seasonal distribution of these samples will affect the robustness of annual mean values calculated for individual lakes.

Table 3.1 summarises the extent of missing annual mean measurements across the environmental dataset. For the majority of lakes and environmental variables there are few gaps. However, the availability of total nitrogen (TN) data is very limited, particularly for Low

Alkalinity lakes. Data for SiO_2 and total oxidised nitrongen (TON) also have many missing values.

Table 3.1. The extent of missing annual mean values for environmental variables across the dataset.

Typology	N	Depth	Alk	Chla	Cond	pH	SRP	SiO_2	TON	TN	TP
High Alkalinity	210	0	3	3	3	3	3	18	18	42	3
Medium Alkalinity	123	0	5	5	5	5	5	25	8	57	5
Low Alkalinity	244	4	13	12	13	13	13	84	27	129	13

3.2 Datasets

3.2.1 Rivers

A relational database was compiled to give comprehensive coverage of running water sites in the UK from which subsets of data could be extracted for particular purposes. The database contains over 6500 samples collected for a variety of research and monitoring purposes, mostly since 1985, along with samples collected for the present project. However, only 480 existing samples could be linked to high quality soluble reactive phosphorus (SRP) data using the criteria described in section 3.1.2. Sampling during the DARES project, therefore, focused on supplementing these datasets in order to provide comprehensive coverage of conditions along a gradient from low to high nutrients.

Additional phytobenthos samples were collected in spring 2004 from 437 sites as part of the 'General Quality Assessment' (GQA) surveys. Of these sites, 296 were re-sampled in autumn 2004 giving a total of 733 diatom samples. A number of these samples could not be matched to chemical monitoring data and, after an initial screening to remove sites with pH < 6.8, a final dataset of 571 samples with matching phosphorus (SRP) data was assembled. Over 2000 sites were visited during these surveys but diatom analyses were restricted to sites that had annual mean SRP < 100 µg l^{-1} along with an invertebrate assemblage similar to that expected in the absence of human impacts, as predicted by RIVPACS III (Wright *et al*., 2000). The criterion of annual mean SRP < 100 µg l^{-1} was used as the existing data were obtained primarily from impacted sites and there was a need to add more sites with lower nutrient concentrations. In order to develop a database that reflected conditions prevailing in the UK, the sampling strategy was stratified according to a simple typology based on the size, mean altitude and catchment geology (Annex 2, clause 1.2.1). These new diatom samples were taxonomically harmonised and merged with the 480 samples from existing data to give a combined dataset of 1051 samples from 651 sites. Geographical coverage of the dataset is shown in Figure 4.1a.

3.2.2. Lakes

The DALES dataset available for tool development consists of 576 samples taken from the littoral zones of 177 English, Scottish and Welsh lakes during 2003–2004. Table 3.2 details

the distribution of sites and samples across the different lake typologies (see chapter 5 for a description of the different lake typologies).

Site details and typologies of lakes in the DALES dataset are listed in Appendix 1 and a summary of the samples in the DALES dataset, including details of substrata and seasons, is given in Appendix 2.

Table 3.2. The total number of sites and samples in the DALES dataset along with their distribution across the different lake typologies.

Typology	Typology code	Sites	Samples
High Alkalinity, Deep	HA, D	5	18
High Alkalinity, Shallow	HA, S	24	81
High Alkalinity, Very Shallow	HA, V	34	111
Medium Alkalinity, Deep	MA, D	7	19
Medium Alkalinity, Shallow	MA, S	20	62
Medium Alkalinity, Very Shallow	MA, V	13	42
Low Alkalinity, Deep	LA, D	20	64
Low Alkalinity, Shallow	LA, S	31	112
Low Alkalinity, Very Shallow	LA, V	21	63
Low Alkalinity, Unknown	LA, U	2	4
TOTALS		177	576

Substrata

Although most phytobenthos samples were collected from rocks/cobbles (epilithon), such surfaces were scarce or absent at a number of lakes and samples were instead collected from plants (epiphyton). Figure 3.1 illustrates for each lake type the proportion of samples collected from cobble and plant substrata. In total, the dataset comprises 481 epilithon samples and 95 epiphyton samples. The majority of epiphytic samples are from HA, V lakes such as the Norfolk Broads.

Season

Figure 3.2 illustrates the seasonal distribution of phytobenthos samples across the dataset. For the purposes of DALES, 'spring' (SP) samples are classified as those collected between March and May, 'summer' (SU) corresponds to the period June to August and 'autumn' (AU) to the period September to November.

The majority of phytobenthos samples were collected during spring, summer and autumn 2004, with the largest numbers of lakes being sampled during the spring 2004 (SP04) period. A subset of English, Welsh and Scottish lakes were sampled in autumn 2003 (AU03), but only Scottish lakes were sampled in summer 2003 (SU03). Many of the Scottish lakes sampled in 2003 were not subsequently sampled in 2004.

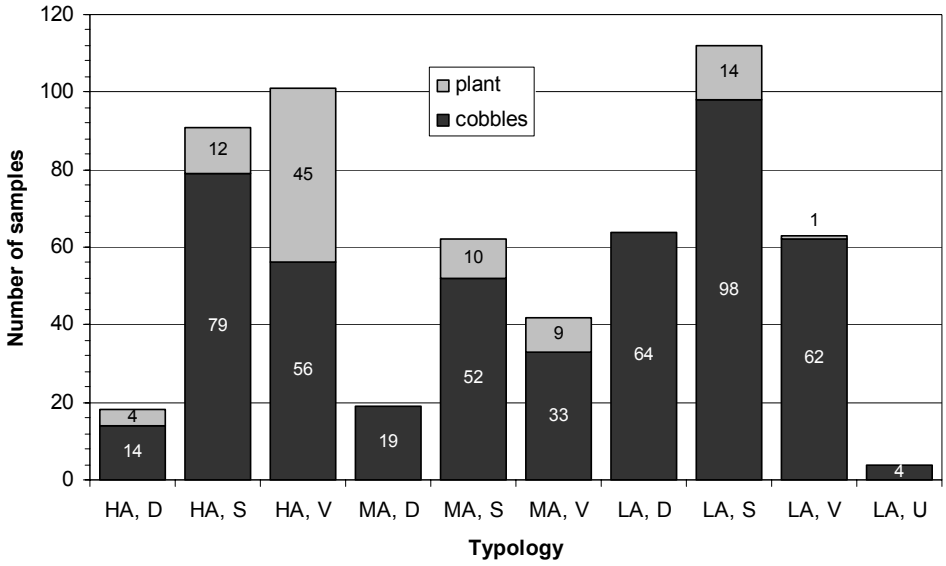


Figure 3.1. The number of cobble and plant samples from the different lake types. See Table 3.2 for typology codes.

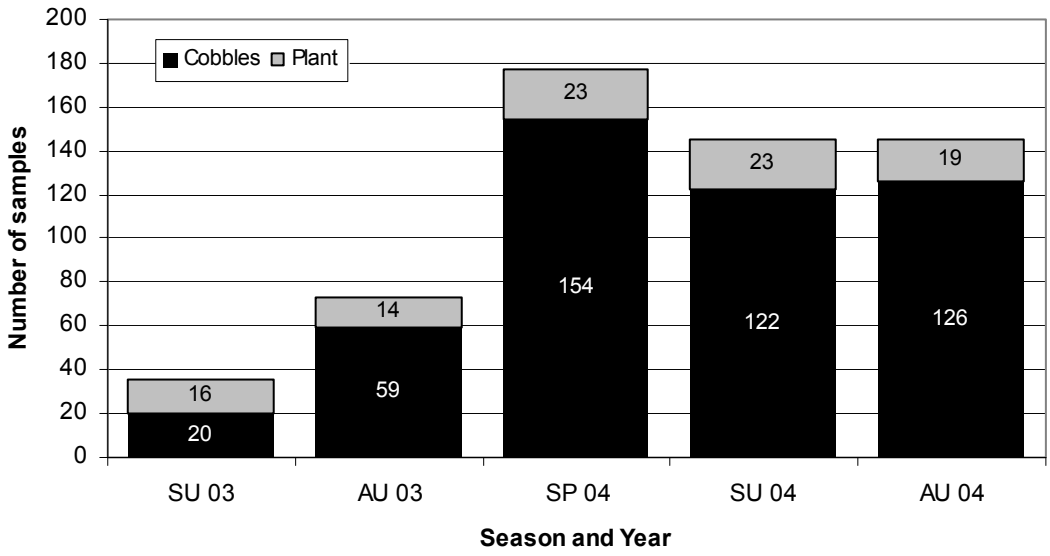


Figure 3.2. Seasonal distribution of 2003 and 2004 phytobenthos samples across the dataset. See text for key to codes for season and year.

Within the dataset there are a number of lakes where samples have been collected from three consecutive seasons. There are 31 lakes with samples from each of AU03, SP04 and summer 2004 (SU04) and 60 lakes with samples from each of SP04, SU04 and autumn 2004 (AU04).

Surface sediment diatom data

Surface sediment samples were analysed from approximately 80 lakes in the DALES dataset; however, these are not included in the current tool development. These data are available for exploration at a later stage.

4 Defining reference conditions and the expected diatom flora in rivers

4.1 Introduction

The search for 'reference conditions' in European rivers is not straightforward due to the long history of human settlement across much of the continent. In lakes, it has been possible to use palaeoecological approaches to test hypotheses about the onset of detectable human impacts and, from this, to infer the baseline state of environmental variables such as pH (Flower *et al*., 1997) and nutrients (Bennion *et al*., 2004: see also chapter 5). Except in a few cases, where it has been possible to examine the historical state either from old records and samples (Taylor *et al*., 2005) or from diatoms preserved with herbarium specimens of macrophytes (van Dam and Mertens, 1993), it is necessary to adopt 'spatial state' schemes in which the biota at sites without known anthropogenic impacts is considered to be the benchmark against which the biota at other sites is assessed. This, in turn, is based on a 'space-for-time substitution' (Pickett, 1988), which assumes that patterns observed in contemporary assemblages separated by space (e.g. a relationship between diatom assemblages and nutrients within a 'training set') can be used to infer changes at one site over time.

The underlying assumption is that a site free from anthropogenic pressures will have a 'natural' biota so, in theory, reference sites can be selected from a pool of sites lacking such pressures. Chessman *et al*. (1999) inferred these conditions from topographic maps while Rimet *et al*. (2004) used low levels of organic pollution variables as a criterion. However, such an approach has limitations. Wright *et al*. (1984), for example, selected sites that were 'by and large free from serious pollution' to ensure a classification based on natural groupings of benthic invertebrates in UK rivers. However, at the time of their study, nutrients were not considered to be 'serious' pollutants in UK rivers and Kelly and Whitton (1995), revisiting several of the sites used by Wright *et al*. (1984), found diatom floras indicative of anthropogenic enrichment.

The alternative is to infer the absence of pressures from the presence of a 'natural' biota (e.g. Gevrey *et al*., 2004; Tison *et al*., 2005). The limitation of this approach lies in knowing what this natural biota should be for any particular stream and, more especially, distinguishing between truly undisturbed sites (which may not be present in areas of high population density or intensive agriculture) and those sites that are simply the best of those available. The relationship between 'naturalness' and taxonomic criteria used in site assessments is complicated (Maitland and Morgan, 1997). There are often few reliable data on which to assess the habitat preferences of less common taxa and experts may have different perceptions of what 'reference conditions' represent. Although the use of ecological criteria for defining high ecological status is closer to the spirit of the WFD (Annex V, clause 1.2). Wallin *et al*. (2005) suggest using pressure criteria for preliminary screening of reference sites, after which ecological data should be used to corroborate this high status. Such an approach will minimise the risk of circularity in establishing reference conditions and this is the approach adopted here for establishing a 'natural' classification of benthic diatoms in UK rivers to serve as a benchmark against which deviations due to anthropogenic pressure can be assessed.

This chapter outlines the rationale for identifying reference sites for benthic diatoms in UK rivers and characterises the diatom assemblages found at these sites. It then examines two approaches by which such data can be used to define 'expected' values in EQR calculations. The assumptions underlying the 'spatial state' reference scheme are tested separately (chapter 7) by comparing contemporary diatom assemblages with those from herbarium material collected prior to 1930.

4.2 Reference sites for UK rivers

4.2.1 Identifying reference sites

The process of identifying reference sites from the DARES database was iterative, as data were screened and hypotheses tested. Guidelines from UK studies associated with the Habitats Directive (European Community, 1992) set limits no higher than 30 $\mu g\ l^{-1}$ SRP in rivers without significant anthropogenic influences (Pitt *et al*., 2002) and this value was used to filter out an initial pool of potential reference sites. A further criterion used in the first iteration was that the invertebrate biology, as evaluated by RIVPACS, had to fall into the top two classes. The precise limits varied between the Environment Agency, SEPA and EHS but all correspond, approximately, to 'good status' or better.

Following this, a further iteration (based on discussions with other experts in the UK) set a threshold of 20 $\mu g\ l^{-1}$ SRP for sites with total alkalinity < 50 mg l^{-1} $CaCO_3$. However, if the same value was applied to sites with total alkalinity ≥ 50 mg l^{-1} $CaCO_3$ too many sites were removed to permit robust calculations on those remaining and the 30 $\mu g\ l^{-1}$ SRP threshold was retained. As more sites with high resolution SRP data become available, these limits will need to be revisited.

The data were also screened to remove sites with high nitrate-N concentrations. A value of 2 mg l^{-1} nitrate-N was applied to Low Alkalinity sites while a higher value (4 mg l^{-1} nitrate-N) was applied to sites with total alkalinity ≥ 50 mg l^{-1} $CaCO_3$ for the same reasons as described above, though this will almost certainly include some slightly impacted sites. Initial analysis of the resulting reference sites showed some to have high TDI values, suggesting that even after screening using chemical criteria the reference groups still contained sites suffering from the impacts of elevated nutrient concentrations. We therefore applied a further screening and removed sites with TDI scores > 50.

The above screening identified a subset of 278 reference samples from 169 sites, from the total database of 1051 samples. Figure 4.1 shows the spatial distribution of reference and non-reference samples, and the distribution of reference samples in relation to alkalinity and altitude. Figure 4.2 summarises additional environmental characteristics of the reference samples.

Reference sites are distributed primarily around the periphery of Great Britain, with large numbers in Scotland, Wales and north and south-west England. There are almost no reference sites in the densely populated areas of the midlands and southern England. This geographic bias is also reflected in the hydrochemistry: while 661 (63%) samples in the total database are from sites with mean annual alkalinity > 50 mg l^{-1}, only 51 of the reference samples (21%) are from sites with alkalinities greater than this threshold.

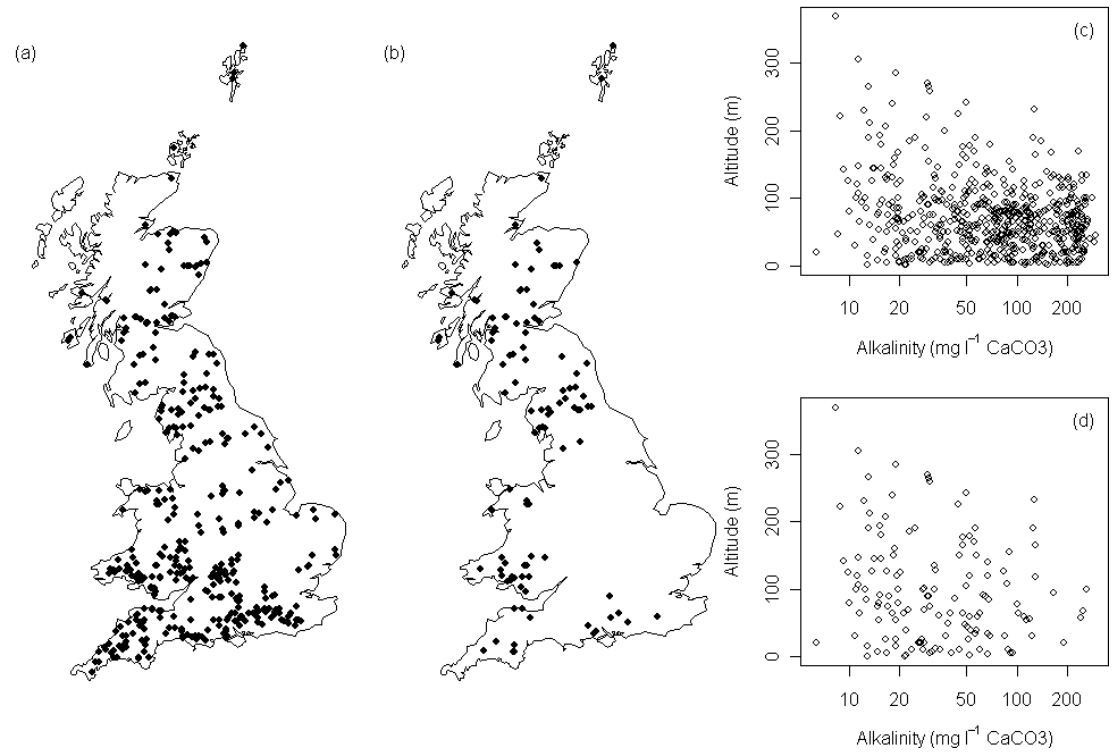


Figure 4.1. Spatial distribution of (a) all samples and (b) reference samples, and distribution of (c) all samples and (d) reference samples in relation to site altitude and mean annual alkalinity.

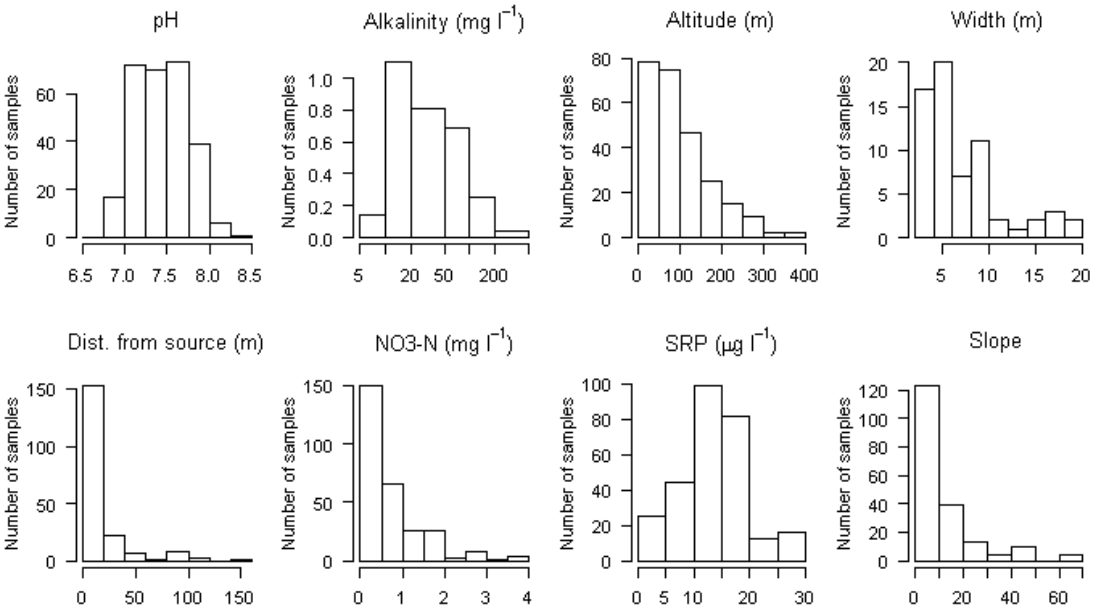


Figure 4.2. Additional environmental characteristics of the reference sites.

4.2.2 Diatom flora of reference sites

The diatom flora of samples at reference state was characterised by a high relative abundance of *Achnanthidium* spp., attached taxa such as *Gomphonema* spp. and loosely attached Fragilariophyceae but few motile taxa (Figure 4.3). *Achnanthidium minutissimum* was the most abundant taxon, across all alkalinities, although many sites also contained *A. biasolettiana* and/or *A. microcephalum* (Table 4.1). A few lower alkalinity sites were dominated by *Achnanthes oblongella* instead, and *Cocconeis placentula* was also abundant on some occasions. *Fragilaria capucina* was the most abundant of the Fragilariophyceae, but *Meridion circulare*, *Hannaea arcus* and *Tabellaria flocculosa* were all common at lower alkalinities (Table 4.1).

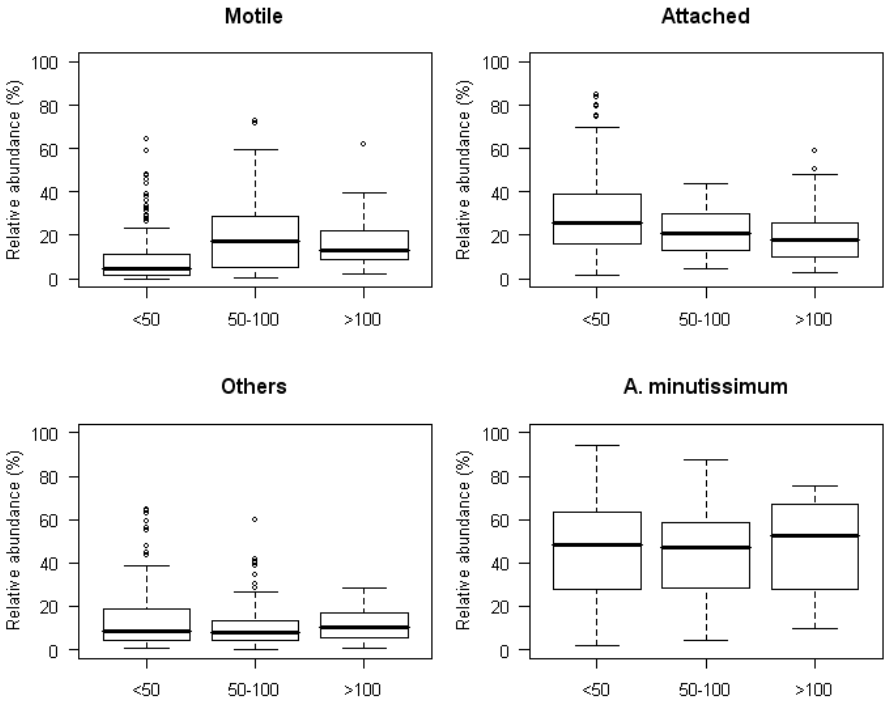


Figure 4.3. Distribution of diatom life-forms in reference samples by alkalinity (in mg l^{-1} $CaCO_3$). 'Others' refers to taxa loosely attached or associated with a substratum. *Achnanthidium minutissimum* dominates many of the reference samples and is shown separately.

Table 4.1. Diatom taxa characteristic of reference conditions in UK rivers. Figures show maximum relative abundance of each taxon in reference and non-reference samples for three alkalinity bands. Figures in parentheses give the proportion of samples in which a taxon is present.

Taxon	Alkalinity ≤ 50 mg l^{-1}		Alkalinity > 50, ≤ 100 mg l^{1}		Alkalinity > 100 mg l^{-1}	
	Reference	Non-reference	Reference	Non-reference	Reference	Non-reference
Achnanthes laevis	2.8 (17.6)	1 (10.2)	4.8 (17.2)	1.2 (6)	0.3 (3.8)	0.6 (1.3)
Achnanthes oblongella	48.4 (42)	81.2 (46.2)	35.9 (20.7)	10.8 (12.1)	0.7 (11.5)	2 (4.8)
Achnanthidium biasolettiana	54.3 (26.4)	13.3 (14.2)	30.6 (29.3)	9.6 (8.7)	13.8 (26.9)	48.3 (12.2)
Achnanthidium microcephalum	37.3 (30.6)	8.8 (9.6)	15.5 (25.9)	4.1 (5.3)	21 (34.6)	1.2 (2.6)
Achnanthidium minutissimum type	94.3 (100)	86.3 (99.5)	87.5 (100)	83.8 (98.5)	75.7 (100)	82.3 (98.4)
Achnanthidium subatomus	9.7 (6.2)	6.3 (4.6)	1.5 (5.2)	3.6 (1.9)	0 (0)	10.6 (1.6)
Brachysira vitrea	21.6 (13.5)	1.6 (7.1)	0.6 (5.2)	1.5 (3)	3.7 (15.4)	0.6 (0.6)
Cocconeis placentula	43.5 (35.2)	28.4 (34)	12.3 (29.3)	13 (14)	1 (19.2)	3.4 (10.6)
Cymbella affinis	4.4 (11.9)	0.3 (2)	0.3 (5.2)	0.5 (0.8)	2.3 (19.2)	1.8 (5.1)
Cymbella delicatula	2.3 (2.1)	0.3 (1)	2.1 (1.7)	0.3 (1.1)	0.6 (15.4)	0.3 (0.6)
Cymbella microcephala	22 (8.8)	0.9 (1)	0.3 (3.4)	1.3 (3)	1 (23.1)	2.5 (2.9)
Diatoma mesodon	11.2 (23.8)	16.7 (23.4)	1.4 (8.6)	2.6 (3.8)	1 (7.7)	0.6 (0.3)
Diatoma moniliformis	21.6 (11.9)	29.9 (5.6)	3.6 (10.3)	4.3 (2.6)	5.1 (15.4)	4.2 (2.6)
Diatoma problematica	12.8 (6.7)	14.4 (7.6)	1.1 (3.4)	8.5 (1.1)	4.3 (15.4)	1.1 (1.9)
Diatoma tenue	10.5 (17.1)	19.8 (11.2)	9.4 (20.7)	3.4 (8.3)	14 (19.2)	31.3 (4.8)
Encyonema gracile	3.9 (8.8)	0.8 (5.1)	0 (0)	0.3 (0.4)	0 (0)	0 (0)
Encyonema minutum	19.7 (63.7)	43.3 (47.7)	34.9 (65.5)	15.2 (46.8)	7.5 (50)	17.8 (34)
Encyonema silesiacum	10 (47.7)	13.7 (44.7)	7.2 (51.7)	8.9 (47.2)	6.9 (34.6)	40.9 (29.8)
Eucocconeis flexella	2.2 (8.8)	0.6 (3.6)	1 (8.6)	1.3 (0.8)	0.7 (11.5)	0 (0)
Fragilaria capucina	68.5 (85.5)	60.1 (73.1)	29.7 (82.8)	35.9 (52.1)	13.6 (61.5)	30.7 (29.5)

Taxon	Alkalinity ≤ 50 mg l^{-1}		Alkalinity > 50, ≤ 100 mg l^{1}		Alkalinity > 100 mg l^{-1}	
	Reference	**Non-reference**	**Reference**	**Non-reference**	**Reference**	**Non-reference**
Fragilaria perminuta	16.9 (9.8)	7.9 (5.1)	6.1 (17.2)	0.3 (1.1)	1.3 (7.7)	0 (0)
Fragilaria vaucheriae	51 (79.3)	41.3 (75.1)	15.1 (75.9)	71.8 (58.1)	13.8 (69.2)	40.4 (53.8)
Gomphonema acuminatum	1.6 (9.3)	0.9 (3.6)	0.7 (3.4)	0.5 (4.2)	2.3 (7.7)	2.9 (2.6)
Gomphonema clavatum	2.2 (5.2)	0.3 (3.6)	0.2 (1.7)	0.3 (0.4)	0.5 (3.8)	1.1 (2.2)
Gomphonema gracile	2.9 (6.2)	1 (1.5)	0.3 (3.4)	0.3 (0.8)	0 (0)	0.3 (0.3)
Gomphonema olivaceoides	30.3 (56.5)	28.5 (40.1)	7 (62.1)	15.2 (17.7)	6.5 (15.4)	1.6 (5.4)
Gomphonema parvulum var. *exilissimum*	16.6 (4.1)	3.7 (3.6)	1.6 (3.4)	1.4 (0.4)	0 (0)	1.4 (1)
Hannaea arcus	60.5 (39.4)	19.6 (13.7)	7.4 (27.6)	0.9 (6.4)	2.7 (7.7)	0.3 (0.3)
Meridion circulare	32.5 (35.2)	8.7 (46.7)	3.9 (53.4)	9.1 (34.7)	4.6 (42.3)	6.7 (21.5)
Meridion circulare var. *constrictum*	11.3 (9.3)	5 (8.6)	0.3 (1.7)	1 (2.6)	0 (0)	0.6 (0.3)
Psammothidium grishunun f. *daonensis*	17.8 (17.6)	4.1 (15.7)	7.1 (12.1)	1.4 (4.9)	0 (0)	0.3 (0.3)
Psammothidium subatomoides	9.3 (26.4)	5.8 (25.9)	1.7 (17.2)	1.4 (14.7)	0.3 (3.8)	1.6 (2.2)
Tabellaria flocculosa	60.5 (33.2)	7.7 (28.9)	1.3 (17.2)	1.8 (4.9)	2.2 (11.5)	1.2 (1.3)

4.3 Deriving a metric for pressure assessment

The reference site approach to ecological status requires the use of a metric or metrics that converts some aspect of the biology at a site to a simple numerical score representing its location along a pressure gradient. Previous analysis has already shown that the Trophic Diatom Index (TDI) developed by Kelly and Whitton (1995) is sensitive to nutrient enrichment (e.g. Kelly, 2001, 2003).

The TDI is based on the weighted-average equation of Zelinka and Marvan (1961):

$$index = \frac{\sum_{j=1}^{n} a_j s_j v_j}{\sum_{j=1}^{n} a_j v_j}$$

where a_j = abundance or proportion of valves of species j in sample, s_j = pollution sensitivity (1–5) of species j and v_j = indicator value (1–3). Values of sensitivity (s) are as follows:

1 = favoured by very low nutrient concentrations
2 = favoured by low nutrient concentrations
3 = favoured by intermediate concentrations of nutrients
4 = favoured by high concentrations of nutrients
5 = favoured by very high concentrations of nutrients

In addition, a few taxa have TDI sensitivity values of zero. These include taxa that are relatively rare in freshwaters and whose ecological preferences are not well defined, along with planktonic taxa, which are routinely excluded from calculations.

Calculating this equation gives the 'weighted mean sensitivity' ('WMS') of the taxa present in the sample. This varies from 1 (for sites with very low nutrient concentrations) to 5 (for sites with very high nutrient concentrations). TDI is the WMS expressed on a scale from 0 to 100. It is calculated as follows:

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

In its original formulation TDI scores were only derived for genera and a limited number of diagnostic species. In order to extract the maximum sensitivity from the data TDI scores were revised at the species and lower taxonomic level using the DARES dataset in an iterative weighted-averaging procedure similar to that described by M. Hill *et al*. (2000) and Walley *et al*. (2001). This simple procedure assigns scores to unknown taxa (i.e. those not classified in the original TDI) on the basis of their distribution in relation to taxa with known scores. If it is assumed that the primary gradient in the full dataset is a nutrient-related pressure gradient then the new scores will reflect this gradient more faithfully.

Table 4.2 shows the relationship between the original TDI scores and rescaled scores for the 513 taxa in the DARES dataset. Overall, only 152 taxa (30%) retain the same score in the 'original' and 'revised' versions of the index while 131 (26%) change TDI score by more than one unit.

In order to validate the revised TDI scores and test the assumption that the main gradient in the DARES dataset is related to nutrient pressure we performed a canonical correspondence analysis (CCA) of the DARES dataset with SRP and NO_3-

N as constraining variables. Both SRP and NO_3-N account for significant portions of variance in the diatom data ($p < 0.001$, Monte Carlo permutation test): thus the first axis of the resulting CCA reflects the positioning of sites and taxa along an aggregate N/P pressure gradient.

Both original and rescaled TDI scores have a very high correlation with the CCA sample scores, indicating that the TDI scores faithfully reflect the underlying nutrient pressure gradient (Table 4.3). The original TDI taxon scores have only a weak correlation ($r = 0.33$) to the CCA taxon scores. This improves to 0.72 after rescaling, as the scores of many rarer taxa, lumped at the generic level in the original TDI, are now more accurately represented. The improvement in the correlation of TDI and CCA sample scores after rescaling (from 0.87 to 0.93) is modest – probably because many of the key diagnostic and numerically dominant taxa are already distinguished in the original TDI. Nevertheless, the above rescaling procedure both validates the original TDI scores and extends their use to the species-level taxonomic indicator system derived here.

The WFD requires that ecological status assessments are presented as an Ecological Quality Ratio (EQR) with a scale from 0 to 1 where high ecological status is indicated by values close to one and bad ecological status by values close to zero. An EQR is calculated from the revised TDI site score using the equation EQR = O/E, where O = 100 – measured TDI and E = 100 – expected TDI. The rescaling (100 – n) is necessary as the TDI is a nutrient index where low values imply 'good' and high values 'bad', whereas the WFD requires high values to indicate high status (implying low nutrient concentrations) and low values to indicate poor or bad status. EQR values >1 are set to 1.0 in the final tool.

The expected TDI for a site has been calculated using two different approaches based on type-specific and site-specific reference conditions respectively. These are described in the following sections.

Table 4.2. Comparison of original and revised taxon TDI scores after rescaling.

Original score	New score				
	1	**2**	**3**	**4**	**5**
1	20	26	13	5	9
2	16	17	17	5	7
3	17	27	28	20	19
4	4	24	40	66	55
5	6	3	19	29	21
Total	3	97	117	125	111

Table 4.3. Correlations between CCA sample and species scores and TDI taxon/site scores for original and rescaled TDI metric.

	Original TDI scores	Rescaled TDI scores
Species scores	0.33	0.72
Sample scores	0.87	0.93

4.4 Type-specific reference conditions

4.4.1 Derivation of reference site typology

Results of an early iteration of 'reference sites' in the General Quality Assessment (GQA) dataset was used to derive a typology using multivariate regression trees (MRT: De'ath, 2002). This technique provides a site-based classification of the biological data, with splits between clusters defined by a simple rule based on environmental values, and chosen to minimise within-cluster dissimilarity. Various MRTs were produced, with different combinations of driving variables. A deliberately simple typology was chosen (Table 4.4), giving four 'types', based on alkalinity (above and below 50 mg l^{-1} $CaCO_3$ total alkalinity) and altitude (above and below 80 m).

Table 4.4. Characteristics of four UK river types defined using multivariate regression trees (MRTs).

Type	Total alkalinity (mg l^{-1} $CaCO_3$)	Altitude (m)
1	< 50	< 80
2	< 50	≥ 80
3	≥ 50	< 80
4	≥ 50	≥ 80

Figure 4.4 shows the spatial distribution of the four types in the DARES database and the environmental properties of the reference sites are shown in Figure 4.5. Types 3 and 4, with ≥ 50 mg l^{-1} $CaCO_3$ have higher pH values than the Types 1 and 2. Width and distance from source are low in most cases, reflecting the location of unimpacted sites in low order streams. Type 1 is the exception, with water bodies exhibiting a wider range of these physical properties, as this type includes areas of Scotland underlain by hard rock geology.

Taxa belonging to TDI group 2 (i.e. nutrient sensitive taxa) were most abundant at all four types, with TDI group 1 taxa (very nutrient sensitive) also abundant at types 1 and 2 sites but relatively scarce at types 3 and 4 sites. TDI groups 4 and 5 (nutrient tolerant and very nutrient tolerant respectively) had low relative abundances in types 1 and 2 but TDI group 4, in particular, formed up to about 30% of the total valves recorded in types 3 and 4 (Figure 4.6). Similarly the proportion of motile taxa is also low in types 1 and 2 with a mean of 11 and 8% respectively, rising to a mean of 20 and 18% in types 3 and 4.

Figure 4.4. Map of diatom sites, coded by type (see text for details). (a) all DARES GB sites; (b) reference sites.

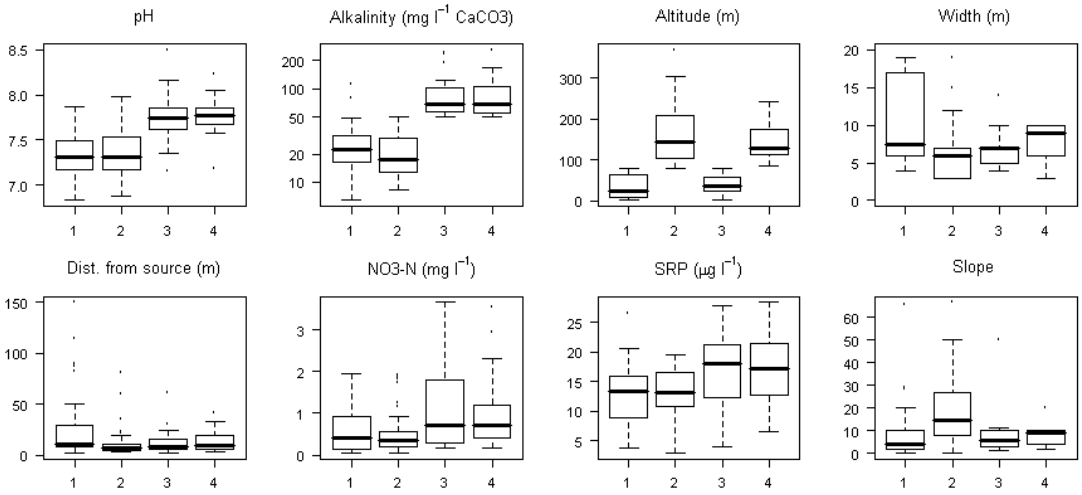


Figure 4.5. Environmental characteristics of UK river types 1–4

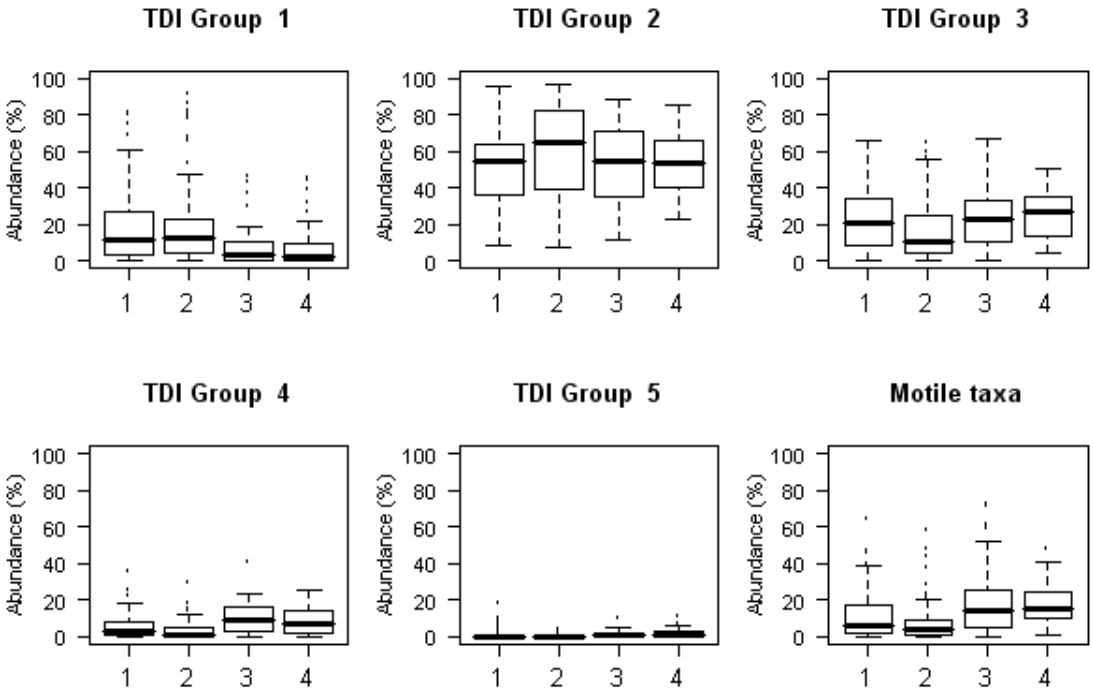


Figure 4.6. Relative abundance of TDI sensitivity classes (1 = most sensitive to elevated nutrients; 5 = most tolerant to elevated nutrients) and motile valves characteristic of diatom assemblages at reference conditions in the four running water types found in UK river types 1–4.

4.4.2 Calculation of expected TDI score at reference conditions

Figure 4.7 shows the distribution of TDI scores for the reference samples for the four types defined in the previous section. Median TDI values were 29.0 and 25.7 for types 1 and 2, but were considerably higher (36.9 and 36.2 respectively) for types 3 and 4, although there was a broad spread of values around these medians for all four types (Table 4.5).

Table 4.5. DARES dataset: number of samples, number of reference samples and median TDI of reference samples for each river type.

Type	Number of samples	Number from 'reference sites'	Median TDI of reference samples
1	265	105	29.0
2	165	94	25.7
3	403	44	36.9
4	218	35	36.2

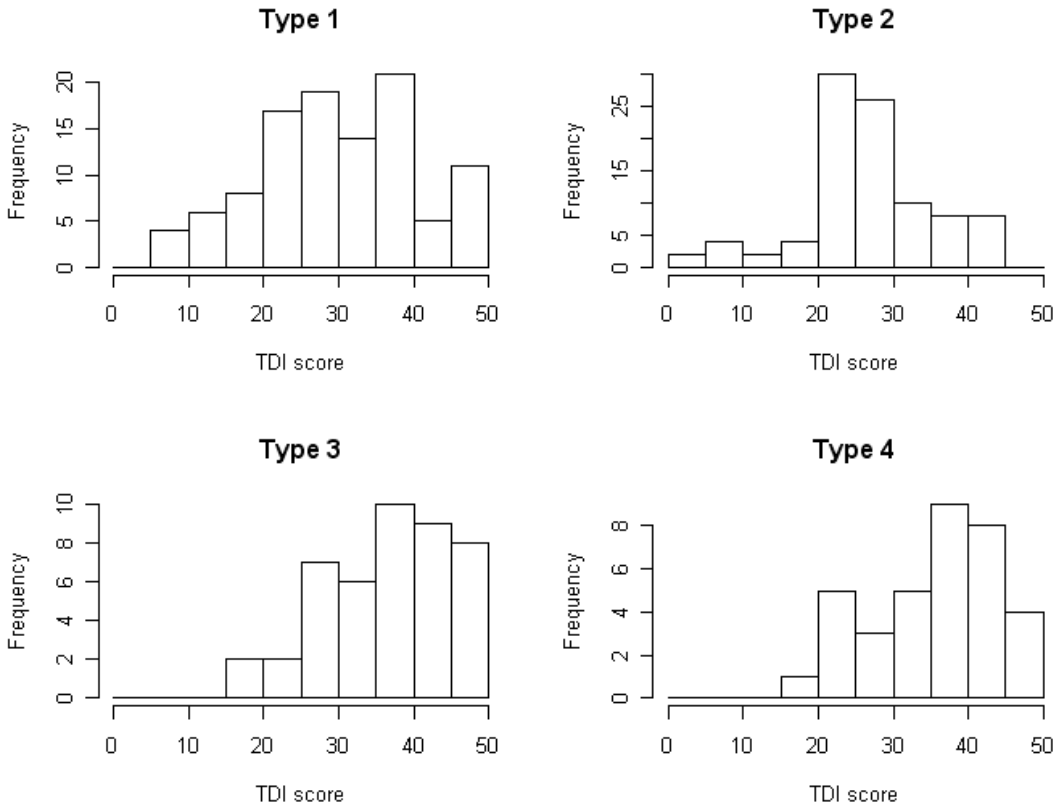


Figure 4.7. Distribution of TDI values for reference sites in types 1–4.

4.5 Site-specific reference conditions

Section 4.4 outlined a method for calculating a set of TDI values representing each 'type' in a more-or-less pristine state which can then be used to set the 'expected' value in EQR calculations. However, such 'type-specific' reference conditions are relatively crude, imposing a categorical scheme onto a system which, in reality, shows continuous variation. An alternative to the use of 'type-specific reference conditions' is to use environmental variables not directly related to the pressure gradient to predict an 'expected' value for each site. This 'site-specific' prediction provides a more elegant solution that, at the same time, bypasses the need for a typology and reduces the uncertainty in estimates of the expected flora for those types with few reference sites.

The potential for site-specific predictions was explored by correlating TDI against non-pressure-related environmental variables for the reference sites (Table 4.6). Initial analysis comparing seasonal samples from the same site suggested that late summer and autumn samples had a slightly higher TDI score than spring and early summer samples. The effect of season as a predictor was also explored by including it as a binary predictor coded as spring/early summer (March–June) or late summer/autumn (July–November).

Table 4.6. Pearson product moment correlations between TDI scores and selected non-pressure environmental variables for reference samples.

Variable	Pearson product moment correlation	p-value
Calcium (log_{10})	0.43	< 0.001
Alkalinity (log_{10})	0.50	< 0.001
pH	0.37	< 0.001
Altitude	-0.20	0.003
Slope	-0.15	0.048
Width	0.11	0.42
Distance from source	0.04	0.63

Results indicate that the TDI of the reference samples is moderately correlated with alkalinity (or its correlates), and weakly negatively correlated with site altitude (Figure 4.8). Plots of TDI against alkalinity and other potential predictors show substantial scatter and a number of outliers. We therefore used median regression, also known as least absolute deviation regression, to relate TDI to non-pressure predictors as this is a robust regression technique that is relatively resistant to outliers. Median regression models were fitted using the rq function for quantile regression described in Koeneker (2005). A series of regression models were fitted using alkalinity and altitude as predictors and are summarised in Table 4.7. Model performance is assessed using the squared correlation ('coefficient of determination') between the observed and fitted scores as measure of a variance in TDI scores accounted for by the model, and the root mean squared error (RMSE), a measure of prediction error (Wallach and Goffinet, 1989). A linear regression with alkalinity as a sole predictor (Model 1) was highly significant but tended to over-predict expected values, particularly in soft waters. The inclusion of a quadratic term for alkalinity improved the fit and produced a small but significant increase in the explained variance (Model 2). Altitude did not account for any additional variance in TDI and, as it led to an increase in prediction error, was therefore omitted (Model 3). The inclusion of

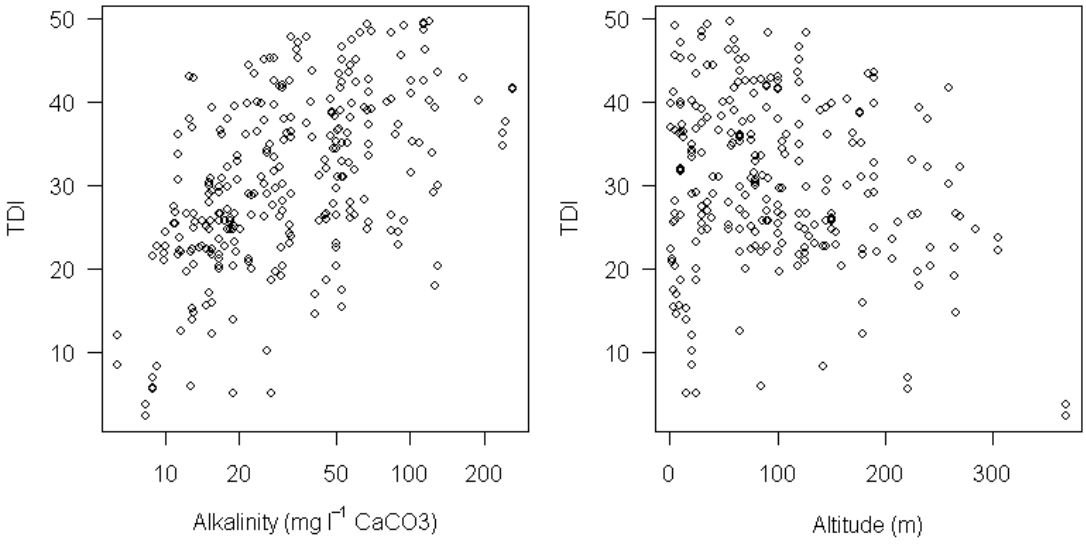


Figure 4.8. Relationship between TDI score and alkalinity (left) and altitude (right).

Table 4.7. Summary of regression models for predicting TDI at reference sites based on different non-pressure environmental variables.

Term	Model 1	Model 2	Model 3	Model 4
Intercept	5.18	-21.32	-26.29	-25.36
Log_{10}(Alkalinity)	16.96	52.86	63.81	56.83
Log_{10}(Alkalinity)^2		-11.41	-15.05	-12.96
Altitude			-0.32 (p = 0.12)	
Season				3.21 (p = 0.008)
r^2	0.29	0.33	0.30	0.35
Root mean squared error (RMSE)	8.47	8.25	8.37	8.10

All terms significant at $p \leq 0.005$ unless indicated.

sampling season in the model led to small but significant improvement in model fit (Model 4). The regression coefficient for season suggests that, on average, late summer and autumn samples have a TDI score ~3.2 units higher than spring early summer samples. The modelled seasonal effect is thus small and although the reduction in prediction error is also modest we retain this variable to allow for more complex seasonal effects in future revisions of model as more data become available.

Regression models including other non-pressure variables listed in Table 4.6 and their interaction terms (with alkalinity) were also explored but none improved the fit over Model 4. The inclusion of a quadratic term for alkalinity implies a non-linear relationship between alkalinity and TDI score. We also attempted to model this relationship using a back propagation neural network but this approach yielded a model with similar prediction errors to the simpler regression models. We therefore adopt Model 4 on the basis of parsimony.

The relationship between TDI score and alkalinity for the reference samples is shown in Figure 4.9, with regression lines added for spring/early summer and late summer/autumn models. Calibration samples for the model lie between alkalinities of 6 and 260 mg l^{-1} $CaCO_3$, although the relationship is poorly constrained above alkalinities of 150 mg l^{-1} $CaCO_3$. To avoid spurious extrapolation we set new samples outside the calibration range to these limits. With alkalinity measured in mg l^{-1} $CaCO_3$ the complete algorithm for site-specific predictions of the expected TDI score becomes:

If alkalinity < 6 then set alkalinity = 6;

If alkalinity > 150 then set alkalinity = 150;

If sample date is after December and before July set season = 0 else set season = 1;

Expected TDI =

$-25.36 + 56.83 \log_{10}(\text{Alkalinity}) - 12.96 \log_{10}(\text{Alkalinity})^2 + 3.21 \text{ season}$.

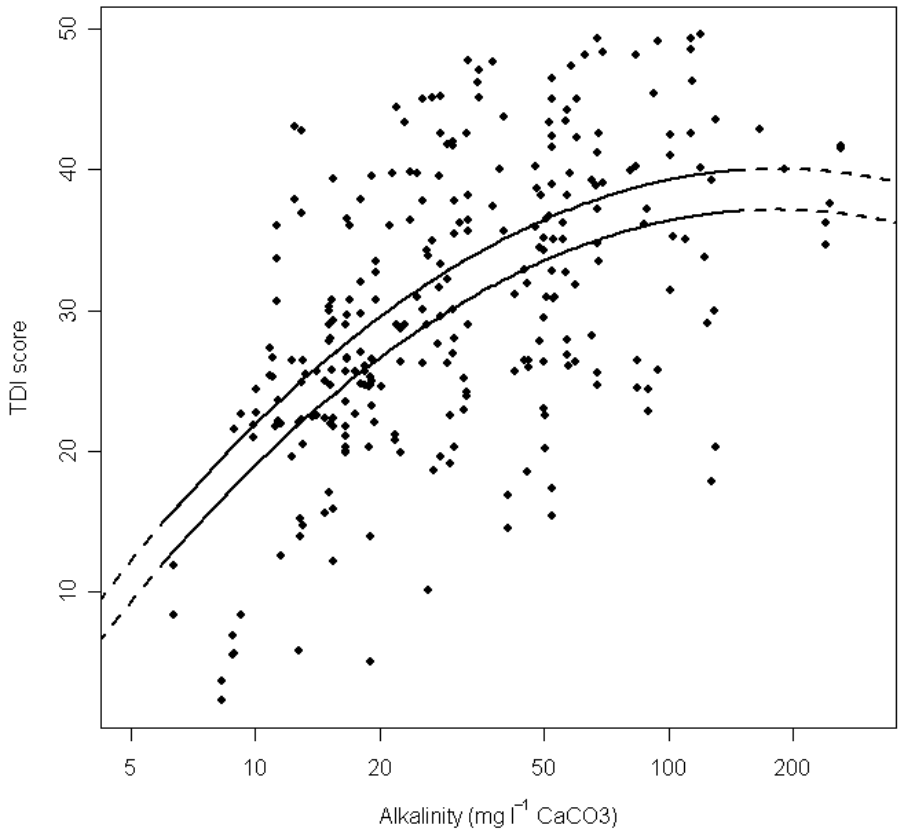


Figure 4.9. Relationships between TDI score and alkalinity for the reference samples, showing the fitted quadratic regression models for spring/early summer (bottom) and late summer/autumn (top).

The relationship between observed and predicted TDI using the above Model 4 is shown in Figure 4.10. Although highly significant the model only explains about 32% of the variation in observed TDI at reference sites. The reason why such models are relatively weak may be related to the rapid biological changes that occur within biofilms (described more fully in chapter 2), leading to the hypothesis that the 'expected' TDI of a biofilm may vary depending on the stage of a micro-succession that the biofilm had achieved. In particular, a thick, 'mature' biofilm may be expected to have a more diverse flora and to offer niches to a wider range of diatoms than a pioneer biofilm. Incorporation of information on the number and relative abundance of 'pioneer' versus 'climax' taxa may allow us to improve predictions in the future.

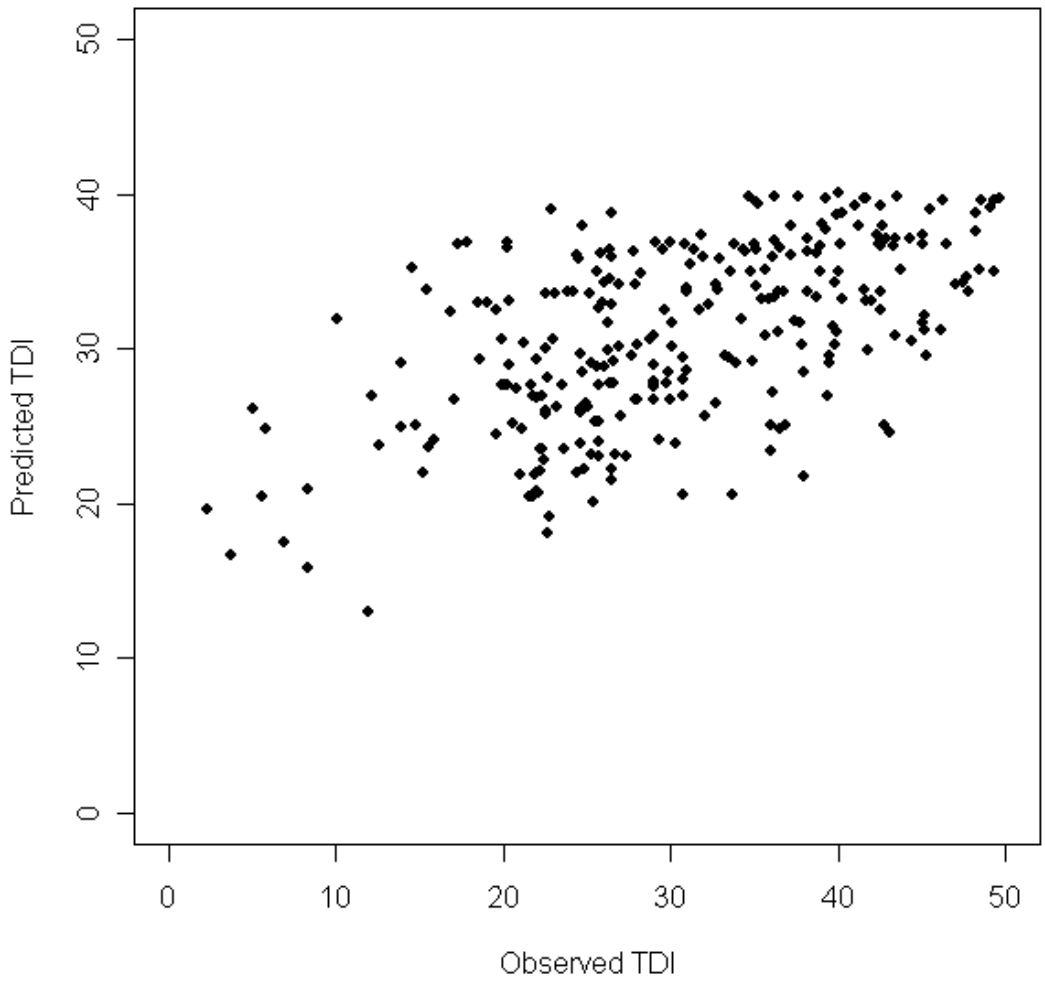


Figure 4.10. Plot of observed versus predicted TDI score for reference sites using Model 4 (see Table 4.7).

4.6 Choice of approach – type-specific or site-specific predictions of reference conditions?

Site-specific predictions of reference conditions using the model developed in section 4.5 above have a number of advantages over type-specific predictions. The first, and most compelling, is that the site-specific approach yields significantly better predictions of the expected TDI score at reference sites (Table 4.8). Secondly, the site-specific model also incorporates information on the significant effect of sampling season, and while this could in theory be incorporated into the type-specific approach the current small sample size of types effectively precludes a robust estimate of seasonal effects for all types. Thirdly, the 'type-specific' approach applies a single reference TDI value to all sites within a type despite the considerable within-type variation in alkalinity and reference TDI score. The site-specific approach thus avoids the need for what is a rather arbitrary classification and eliminates the artificial step-changes in predictions that accompany spatial comparisons of sites. Fourth, by

including all reference sites in a single regression model we reduce the uncertainty in estimates of the expected TDI score for those types with few reference sites. Finally, the site-specific approach is more easily modified and extended to include other predictors as more data on reference sites become available. For these reasons we use site-specific predictions in subsequent analyses.

Table 4.8. Summary statistics comparing the predictive ability of type-specific and site-specific approaches.

Model type	Variance explained (r^2)	Prediction error (RMSE)
Type-specific	12%	9.77
Site-specific	33%	8.10

4.7 Provisional status class boundaries

Figure 4.11 shows the distribution of EQR values, calculated using the site-specific model described in section 4.5, for reference sites. Due to the presence of a flora suggesting enrichment at some putative reference sites, the high/good status class boundary is placed at the 25th percentile of this distribution (0.93).

To define the position of the good/moderate boundary we pooled diatom taxa from TDI groups 1 and 2 and groups 3 and 4 to form 'nutrient sensitive' and 'nutrient tolerant' categories for reference and non-reference samples. Diatom taxa from TDI group 3 formed a final category of taxa that were either indifferent to nutrients or which had a preference for intermediate nutrient concentrations.

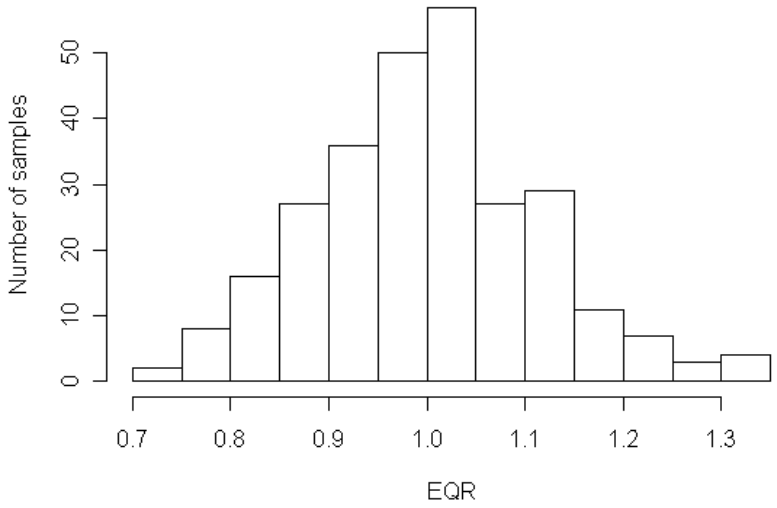


Figure 4.11. Distribution of EQR values for reference sites based on site-specific predictions of the expected flora.

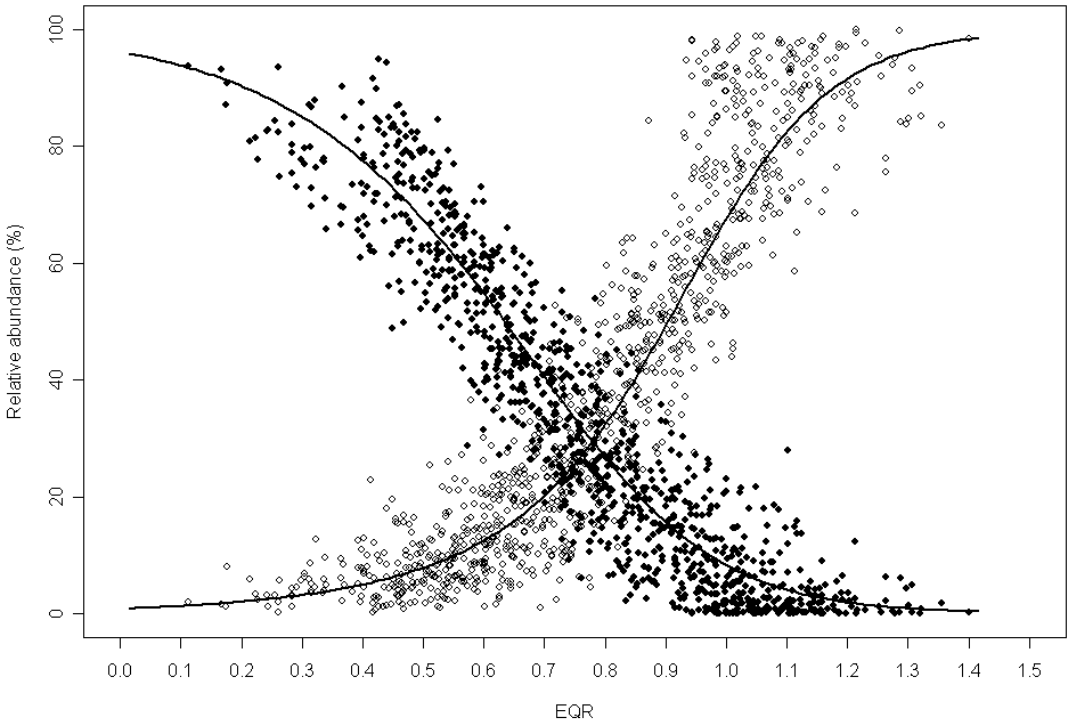


Figure 4.12. Variation in the relative abundance of nutrient sensitive (closed circles) and nutrient tolerant taxa (open circles) with EQR for UK rivers. Lines are fitted regression models derived using a generalised additive model (GAM) with logistic link and binomial error term (Faraway, 2006).

The proportion of valves belonging to nutrient sensitive taxa decreased as EQR decreased while the proportion of nutrient tolerant taxa increased (Figure 4.12). In terms of valve numbers, taxa such as *Achnanthidium* and *Fragilaria capucina* constitute a large part of the 'sensitive' group, while taxa such as *Amphora pediculus, Navicula* and *Nitzschia* spp. provide many of the nutrient tolerant valves. The shift from an assemblage dominated by *Achnanthidium* and *Fragilaria* to one dominated by nutrient tolerant and, often, motile taxa, along with epiphytic species such as *Rhoicosphenia abbreviata* and *Cocconeis pediculus*, suggests that there are also functional changes in the biofilm along the EQR gradient which provide an ecological justification for the position of the GES/MES boundary. The transition between the two states is, however, not sharp and, consequently, the boundary has been placed at an EQR of 0.78, which is the point ('crossover') at which numbers of sensitive and tolerant taxa, predicted by generalised additive model regressions, are equal. Good ecological status is, therefore, defined as samples whose EQR values fall between the good/moderate status boundary and the high/good boundary.

The moderate/poor and poor/bad boundaries are set at equal points below the good/moderate boundary. The final boundary positions are given in Table 4.9.

Table 4.9. Provisional diatom status class boundaries for UK rivers.

Boundary	EQR
High/good	0.93
Good/moderate	0.78
Moderate/poor	0.52
Poor/bad	0.26

Although the WFD sets targets in terms of ecological criteria, effective management of catchments will necessitate an understanding of the nutrient concentrations that will support these. The approach adopted by UK TAG has been to use the 90th percentile of mean annual SRP concentrations for those sites whose diatom-derived EQR values correspond with a particular status class to set the regulatory standards for that class. Table 4.10 shows 90th percentile of the SRP values for each type/class for three models: (1) type-specific model described in section 4.4 with original dataset used to derive values in Duncan *et al*. (2006), (2) type-specific model based on fourfold typology but using a slightly expanded dataset (new data), and (3) site-specific predictions based on Model 4 (Table 4.7) with the new dataset. Table 4.10 also shows the bootstrap 95% confidence intervals for the 90th percentile SRP values.

For high status samples the 90th percentile SRP values are very similar for type-specific and site-specific models. Bootstrap confidence intervals (CIs) are rather narrow for types 1 and 2 but are much wider for types 3 and 4, reflecting the relatively small number of samples in these types and hence, the large uncertainty attached to the 90th percentile SRP limits (see Figures 4.13 and 4.14). For good status samples, the 90th percentile SRP values are also similar between type- and site-specific models for types 1–3 but is lower for the latter for type 4 although the bootstrap CI is large (54–149 µg l^{-1} SRP), reflecting the large uncertainty due to the small sample size. The wide CIs for some estimates and the large overlap in the CIs between the type- and site-specific models suggests that the SRP limits derived from the two models are not significantly different from each other.

Table 4.10. SRP limits (µg l^{-1}) for high and good status, derived using type-specific and site-specific models.

Type	Type-specific model		Bootstrap 95% CI of 90th percentile	Site-specific model	Bootstrap 95% CI of 90th percentile
	Original data	New data		New data	
	High status				
1	34	39	30–43	38	31–39
2	21	23	20–32	26	22–32
3	43	42	27–49	42	27–48
4	50	50	38–68	59	37–68

Type	Type-specific model		Bootstrap 95% CI of 90th percentile	Site-specific model	Bootstrap 95% CI of 90th percentile
	Original data	New data		New data	
	Good status				
1	45	45	39–65	44	35–51
2	37	36	29–42	32	27–37
3	59	59	52–111	59	54–110
4	116	100	61–149	96	70–149

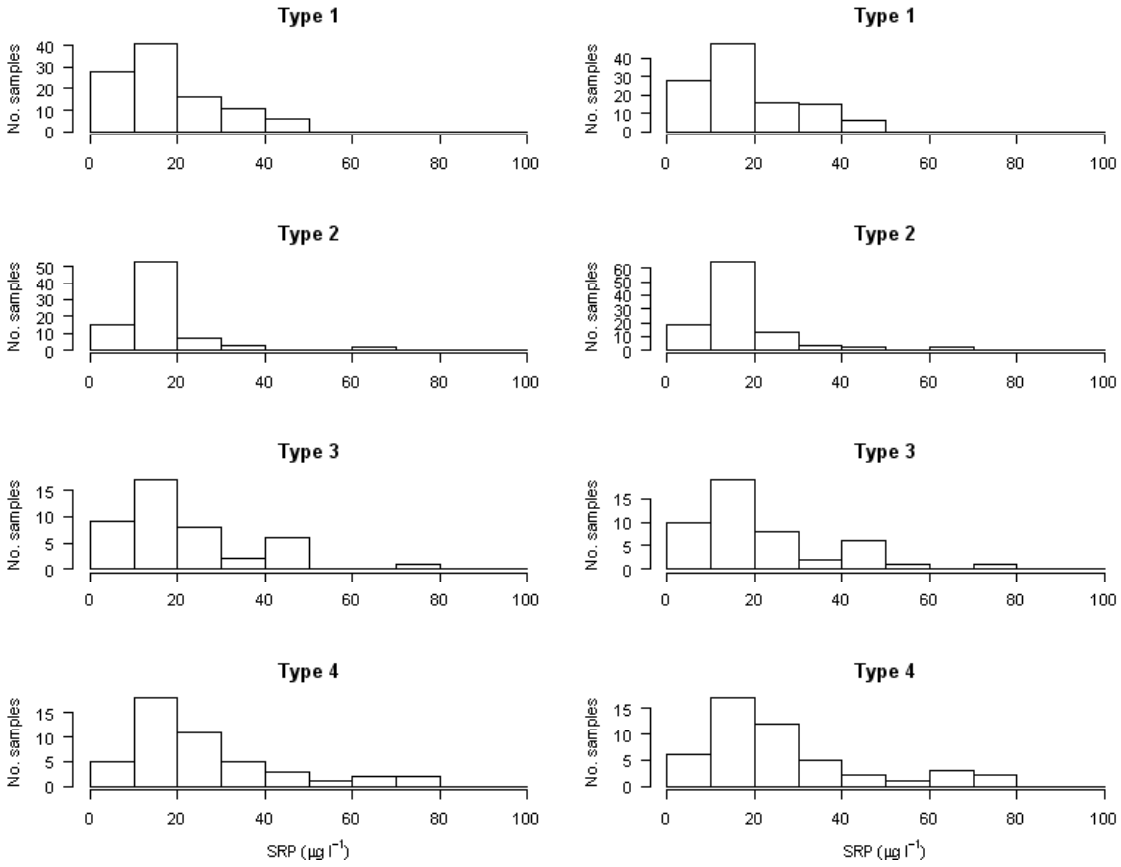


Figure 4.13. Histograms of SRP values by type for samples predicted to have high status. Left: type-specific model; right: site-specific model.

A further note of caution to add to these values is that the use of annual mean SRP concentrations based on, typically, monthly samples is unlikely to be a good predictor of the response of phytobenthos to enrichment when nutrient concentrations are low, as other forms of phosphorus (e.g. organic-P) and intermittent pulses of nutrients that are likely to be missed by monthly samples may play a large role in shaping the phytobenthos.

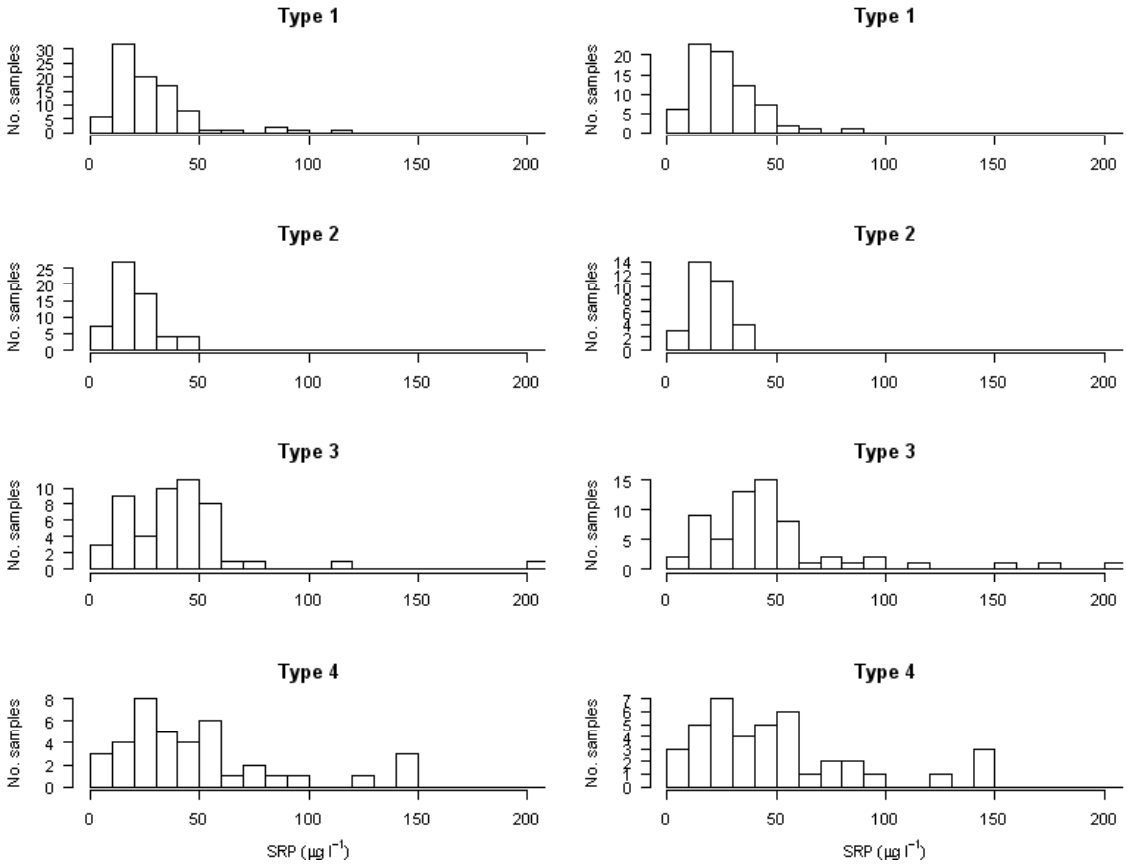


Figure 4.14. Histograms of SRP values by type for samples predicted to have good status. Old model left, new model right.

5 Defining reference sites and the expected flora in lakes

5.1 Establishing a typology

5.1.1 The lake typology for Great Britain

The reporting typology for ecoregion 18 (Great Britain – GB) divides lakes into potentially 18 types based on the base status of their catchment geology and their mean depth (Phillips, 2003 – Table 5.1). For geology the area of each rock type listed on the 1:625,000 solid geology map was determined for each water body catchment (with a catchment area > 1 ha) using GIS and catchment polygons derived from a digital terrain model (Bennion *et al.,* 2003). These types were aggregated into either calcareous or siliceous types following guidance from the British Geological Survey and were subsequently modified where measured alkalinity data were available. Mean lake depth data were taken from the GB lakes database (Hughes *et al*., 2004). It should be noted however that full bathymetric surveys have not been carried out at all lakes and therefore for many water bodies the mean depth is estimated or modelled based on the relationship between maximum depth and mean depth.

5.1.2 Lake typology used in DALES

For the purposes of the DALES project, a simplified version of the GB typology was used. This classifies the lakes based on the geology criteria only into three broad types: Low, Medium and High Alkalinity. There were two principal reasons for not incorporating mean depth: (i) the phytobenthos samples at the lake margins are unlikely to reflect differences in mean lake depth, (ii) there were very low numbers of lakes in some types if the sites were classified according to depth as well as geology. The first of these reasons was tested by performing a detrended correspondence analysis (DCA) of the diatom data for each lake type There was considerable overlap in the taxa present in the three depth classes for all lake types, as shown in Figure 5.1 for High Alkalinity lakes, indicating that the phytobenthos does not discriminate between deep, shallow and very shallow lake types.

The locations of the lakes are shown in Figure 5.2 and the environmental characteristics of each of the three lake types in the DALES dataset are shown in Figure 5.3 and summarised in Table 5.2. Each type has its own environmental characteristics that differentiate it from the other types. For instance, the Low Alkalinity group has the lowest pH and conductivity values and generally has low concentrations of nutrients and chlorophyll a. At the other end of the spectrum, the High Alkalinity lakes have the highest pH and conductivity values and the highest concentrations of nutrients and chlorophyll a. The values for the Medium Alkalinity group are intermediate between those for the Low and High Alkalinity types.

Table 5.1. Reporting typology for lakes in Great Britain (Phillips, 2003).

(a) geology

Geology	Code	Catchment	Alkalinity		Conductivity	Colour
			µeq l^{-1}	mg $CaCO_3$ l^{-1}	µS cm^{-1}	mg Pt l^{-1}
Organic	P	> 75% peat	< 200	< 10		> 30
Siliceous	LA	> 90% siliceous solid geology			< 70	≤ 30
	MA	> 50% siliceous solid geology	200–1000	10–50	71–250	
Calcareous	HA	> 50% calcareous geology	> 1000	> 50	251–1000	
	Marl	> 65% limestone				
Brackish	B				> 1000	

(b) depth type

Depth	Code	Mean depth (m)
Very shallow	VSh	≤ 3
Shallow	Sh	3–15
Deep	D	> 15

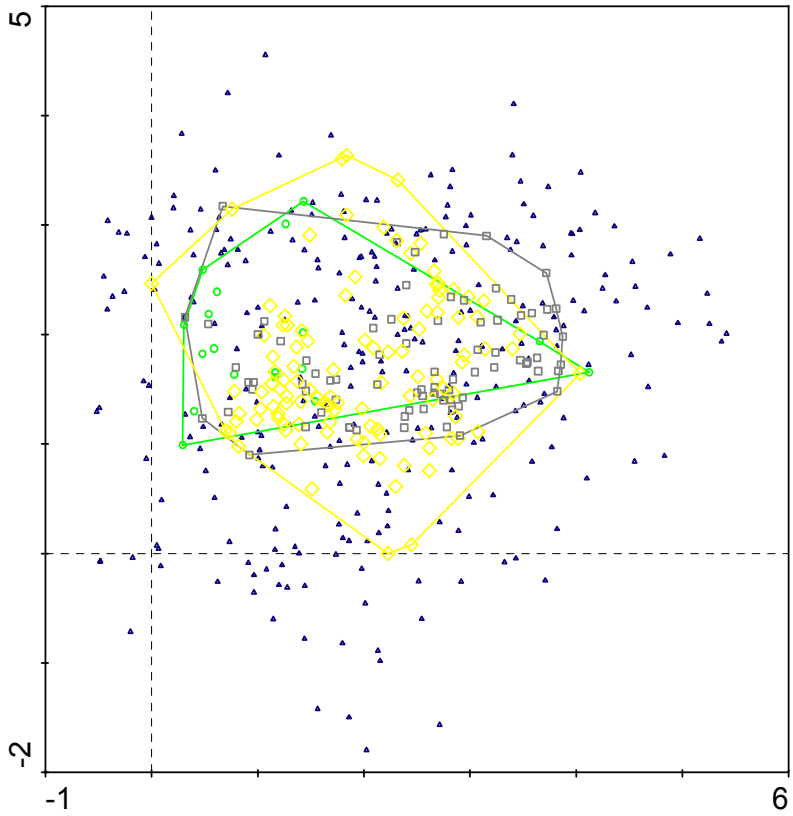


Figure 5.1. Axes 1 and 2 of a DCA of the diatom data (273 taxa and 210 samples) for High Alkalinity lakes. The polygons represent the three lake depth classes (green = deep, grey = shallow, yellow = very shallow).

5.2 Defining reference conditions

5.2.1 Reference lakes

The WFD requires that biological, hydromorphological and chemical elements of water quality should be based on the degree to which present day conditions deviate from those expected in the absence of significant anthropogenic influence, termed reference conditions. The WFD states that, in the absence of long-term data, reference conditions can be derived using a number of methods including spatial state schemes, expert judgement and modelling. For the latter, hindcasting methods such as palaeolimnology (the study of the lake sediment record) are given as one such technique (Pollard and Huxham,1998; European Union, 2000).

In order to identify a set of reference sites to assist in tool development, a combination of the above methods were employed. One data source was the set of reference lakes identified in June 2005 by the phytoplankton classification project, following discussion with both SEPA and the Environment Agency, to support the development of a GB-calibrated morphoedaphic index model (MEI). The lakes are assumed to have no significant anthropogenic sources of phosphorus (P) and thus represent high status

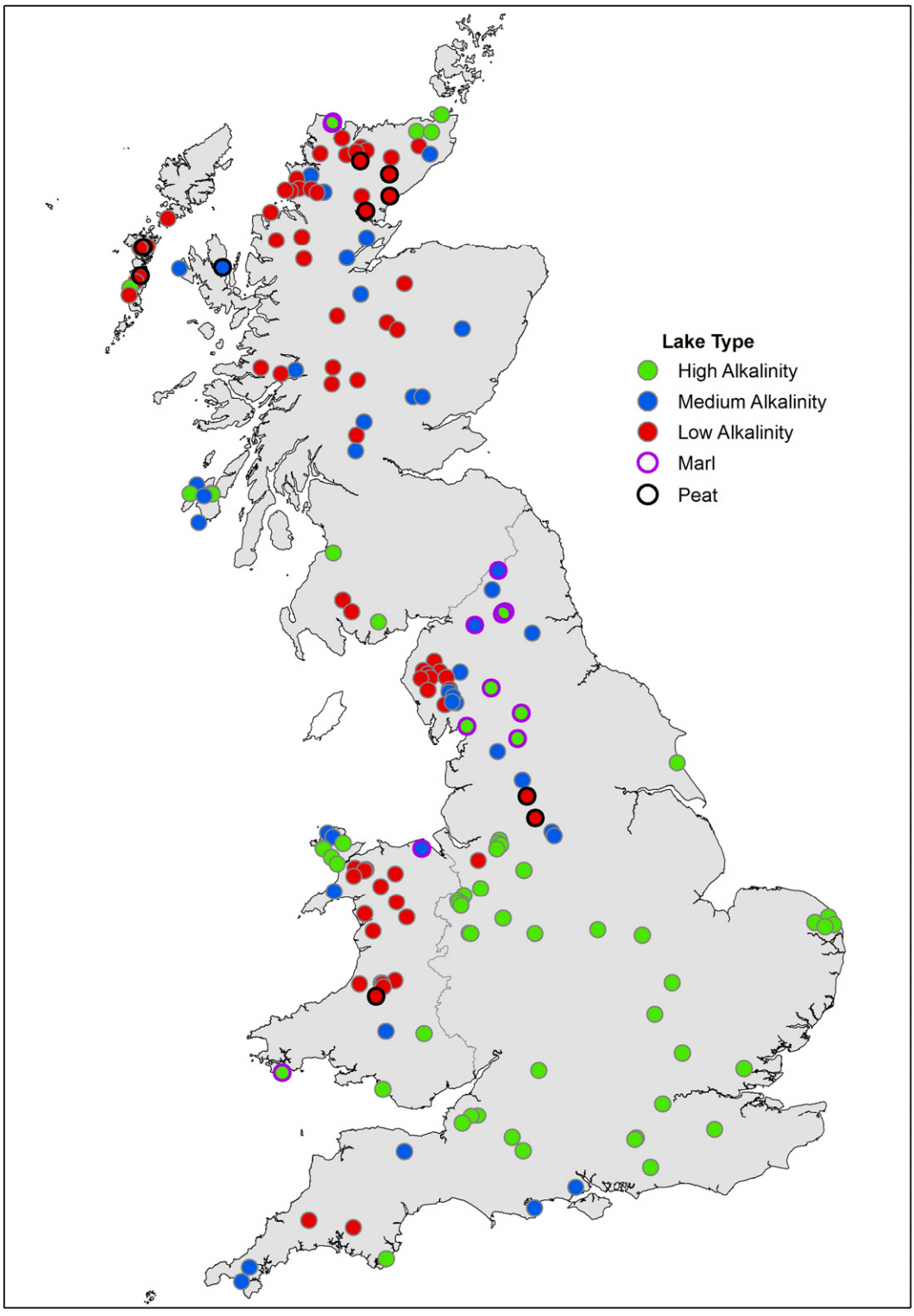


Figure 5.2. Map showing the location and type of lakes in the DALES dataset.

Table 5.2. Summary environmental characteristics of the three lake types in the DALES dataset (see Figure 5.3 for units).

		Mean	Median	Min	Max
HA	**Alk**	2.52337	2.45	1.152	4.30572
	Chla	19.448	15.2644	0.59	117.113
	Cond	443.267	373.625	139.9	5109.5
	pH	8.05183	8.05833	7.3525	8.797
	SRP	78.9823	27.4259	0.995	781.722
	SiO_2	4.56555	3.23125	0.305	16.9375
	TON	1.22662	0.54369	0.05	7.175
	TN	2.27793	1.94875	0.085	7.92571
	TP	126.952	68.95	3	1026.35
MA	**Alk**	0.51308	0.44775	0.13315	0.99244
	Chla	8.88351	7.09125	0.98333	30.8375
	Cond	115.185	97.8757	41.3667	231.125
	pH	7.42923	7.42778	6.44167	8.33125
	SRP	14.6678	7.38542	1.42857	128.5
	SiO_2	2.20119	1.87966	0.4	6.68
	TON	0.48507	0.16675	0.0311	4.1
	TN	1.22052	0.85145	0.43325	4.53833
	TP	27.5404	21.5792	3	81.0625
LA	**Alk**	0.09471	0.07657	0.005	0.2
	Chla	4.18423	2.825	0.77768	50.3222
	Cond	87.2676	51.8	20.5	2360.5
	pH	6.56654	6.6675	3.93333	7.80167
	SRP	8.38752	6.11111	1.13333	52
	SiO_2	1.38009	1.169	0.255	4.548
	TON	0.14356	0.1	0.015	0.4471
	TN	0.47685	0.45092	0.04	1.285
	TP	12.2645	8	0.75	137

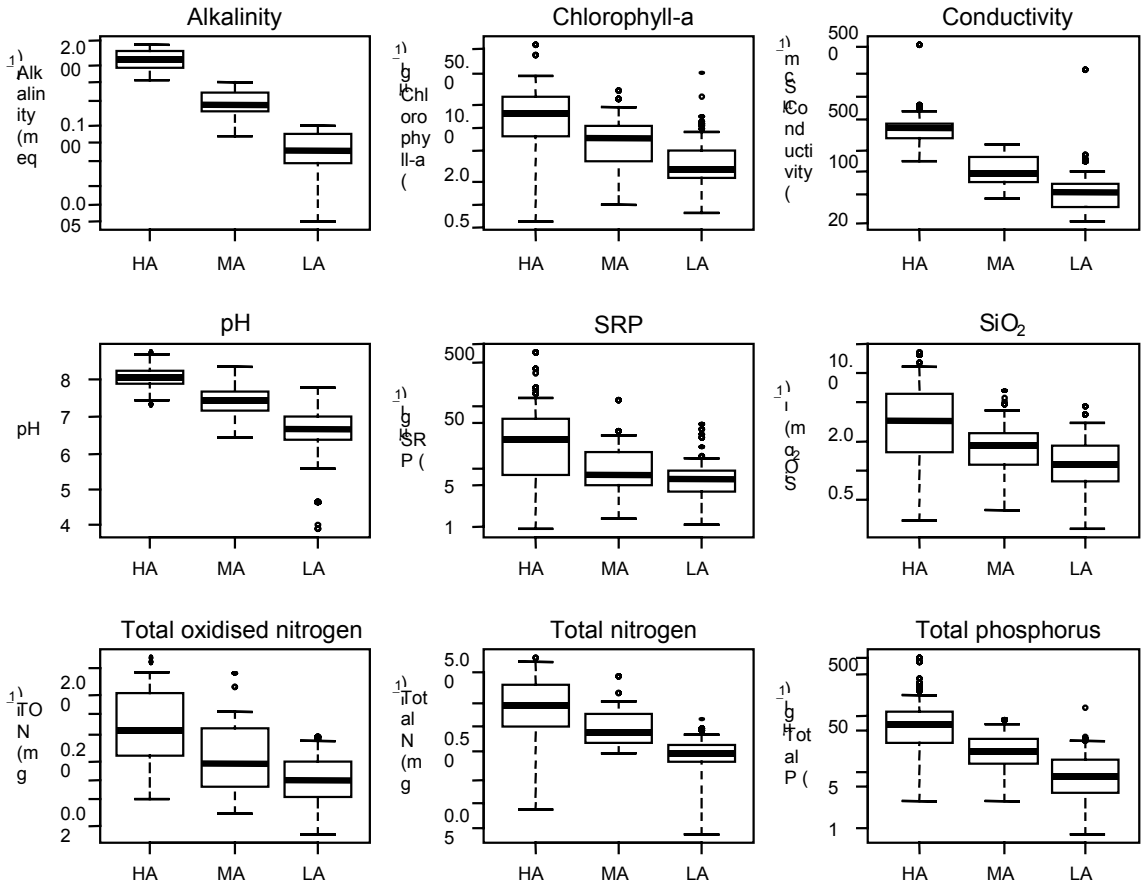


Figure 5.3. Boxplots showing the environmental characteristics of the three lake types in the DALES dataset.

lakes in the context of their total P (TP) concentration. A second set of reference lakes was identified for the EU Rebecca project by the Centre for Ecology and Hydrology (CEH) based on an analysis of reference conditions for TP and chlorophyll a. This list is being used as a basis for the identification of intercalibration reference lakes for the Northern Geographical Intercalibration Group (GIG). A further set of high alkalinity reference lakes has been identified by the Central GIG on the basis that they have no point sources of P, < 10% non-natural land use and < 10 inhabitants km^2. A list of the lakes used for each of the above purposes is documented in an Excel spread sheet (LTT_106a_GP_QRY_RefList_Mar06) and further details are given in TAG/LTT 106 (Phillips, 2006).

A further body of data for identifying potential reference lakes is the palaeoecological database held by the Environmental Change Research Centre (ECRC). Data were collated from all UK lakes where palaeoecological diatom studies have been undertaken. The 'top and bottom' approach was adopted whereby the top and bottom samples of a sediment core are assumed to represent the present day and reference conditions respectively (Cumming *et al*., 1992). This methodology has been successfully applied by the US Environmental Protection Agency's (USEPA) Environmental Monitoring and Assessment Program for Surface Waters (EMAP-SW: Dixit *et al*., 1999), in Canada to infer changes in southeastern Ontario lakes (Reavie *et al*., 2002) and to assess ecological change in UK lakes (Bennion, 2004; Bennion *et al*., 2004). For the UK, it is generally agreed that approximately AD 1850 is a suitable date against which to assess impacts for lakes as this represents a period prior to major industrialisation and agricultural intensification (Battarbee, 1999; Fozzard *et al*.,

1999). However, because aquatic systems have been subjected to anthropogenic impacts over much longer time-scales, these reference conditions are unlikely to equate to a natural or pristine state. Nevertheless, the core sample dated to about AD 1850 for each lake was taken to represent the reference sample and for undated cores the lowermost (i.e. oldest) sample was selected (e.g. Burgess *et al*., 2005).

The degree of floristic change between the reference and present day sample for each site was assessed using a squared chord distance coefficient (Overpeck *et al.,* 1985) implemented in the statistical software R (R Development Core Team, 2004). This is preferred to other dissimilarity measures as it maximises the signal to noise ratio, it performs well with percentage data and has sound mathematical properties (Overpeck *et al.,* 1985). The scores range from 0 to 2 whereby 0 indicates that two samples are exactly the same and 2 that they are completely different. Scores less than 0.29, 0.39, 0.48 and 0.58 indicate insignificant floristic change at the 1st, 2.5th, 5th and 10th percentiles respectively (Simpson, 2005; Simpson *et al.,* 2005). The 2.5th percentile (score < 0.39) was used to define sites with low floristic change between the bottom and top sample and thereby to identify a reference site. This is more stringent than the 5th percentile (score < 0.48) used in previous similar studies (e.g. Bennion *et al*., 2004) and reflects revised thinking on the use of the chord distance statistic. This revision follows closer examination of sediment sample data from over 200 UK lake cores held in the ECRC's in-house AMPHORA database whereby unimpacted sites typically have chord distance values of < 0.4 (in many cases < 0.3) between core top and bottom samples (e.g. Bennion, 2004).

The chord distance measure is useful for estimating degree of change. However, it should not be used in isolation but instead as part of a suite of tools for identifying potential reference lakes. In a number of cases the sediment cores were relatively short (< 30 cm) and in the absence of dating it cannot be guaranteed that the base of the core is sufficiently old to represent pre-impact conditions. The surface sediments of shallow, high alkalinity are often dominated by non-planktonic *Fragilaria* taxa and, in these cases, the chord distance may underestimate broader ecological change at the site, so caution should be exercised when using this measure for defining reference sites for this lake type. The chord distance scores applied here are based on floristic change in the diatom community alone and therefore may not reflect the degree of change in other biological groups.

A summary of the reference sites in the DALES dataset identified using the various datasets above is given in Appendix 3. A total of 28, 10, and 5 reference sites were generated for Low, Medium and High Alkalinity lakes respectively. Unfortunately, there are few examples of High Alkalinity reference lakes but this might be expected given the long history of impacts and productive nature of their catchments.

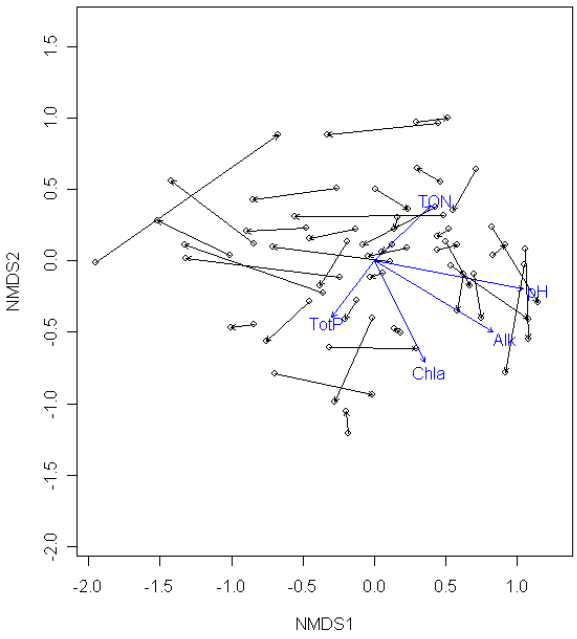
5.2.2 A priori status classification

In addition to helping define a set of reference lakes, the palaeoecological data can be used, where available, to develop an *a priori* status classification for the lakes in the DALES dataset based on the deviation from reference condition. Initially the chord distance scores, which provide an estimate of degree of floristic change (see previous section), were employed to define whether lakes were in good status or less than good status. However, the scores are not able to differentiate those sites where the change is driven principally by nutrient pressures from those where the species shifts may be explained by other factors. Hence, in order to support the chord distance scores, ordination plots for each lake type were produced to assess direction and magnitude of floristic change at each site and therefore improve our *a priori* ecological classification.

This was achieved by performing non-metric multidimensional scaling (nMMDS) of Bray-Curtis distances between the top and bottom samples of each core to represent distances in two dimensions. The modern environmental data were superimposed on the ordination plots to provide a qualitative interpretation of the between-sample distances and to identify the axis that best represented the nutrient gradient for each lake type. The distance along the 'nutrient axis' was subsequently used to estimate the amount of floristic change attributed to nutrients. Figure 5.4 illustrates the results for the three lake types. In each set of plots, the left panel shows the sample distance between core bottom and top with the arrow pointing towards the core top and with the chemical data overlain, and the right panel shows the lake codes positioned at the core top for that site. For the Low and Medium Alkalinity lakes (Figure 5.4a, b) nutrients are clearly represented on axis 2, while for the High Alkalinity lakes (Figure 5.4c) nutrients are associated with both axes but most strongly with axis 1.

The nature and magnitude of change can now be determined from the plots. For example, in the Low Alkalinity group, Lake Bala (BALA) and Llyn Padarn (PADA) have arrows moving in the direction from top to bottom of the plot and have high sample distances on axis 2 associated with nutrient enrichment. However, the arrows for Loch Dee (LDE), Loch Doilet (DOI), Llyn Bodlyn (BODL) and Loch Laidon (LAI) all point toward the left in the direction of decreasing pH on axis 1 and therefore the diatoms are responding to acidification rather than enrichment. In the High Alkalinity group, Betton Pool (BETT) and Crose Mere (CROS) have long arrows moving from right to left of the plot and hence have high sample distances on axis 1 associated with nutrient enrichment. In contrast, Broomlee Lough (BROL) has a short arrow pointing towards the right of the diagram and thereby has a low distance score on axis 1 indicating little response along the nutrient gradient. This analysis therefore enabled identification of those lakes where the diatom changes represented a response to enrichment, those where the response was to acidification, and those where the changes were due to other factors.

A combination of the chord distance scores, the new nutrient distance scores, existing environmental and ecological data and expert judgement were used to assign a best estimate of status class to each lake (see Appendix 1). This classification serves as a useful dataset with which to compare the classifications produced by the DALES tool and helps to guide decisions during development of the pressure metric and boundary setting. It is referred to as the *a priori* classification in the following sections.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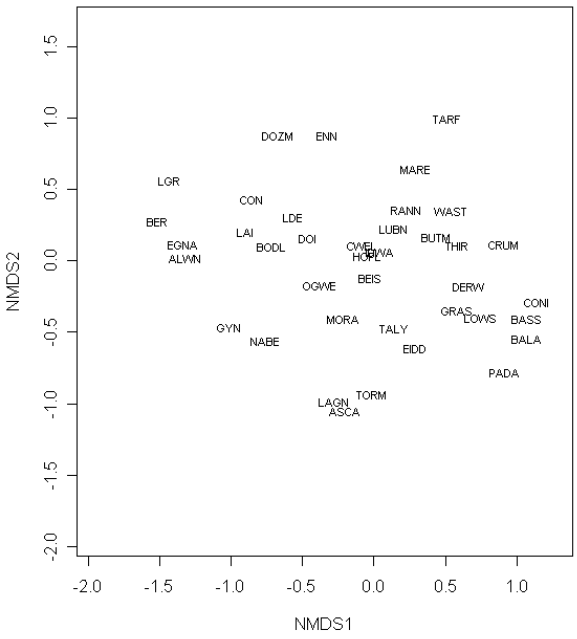
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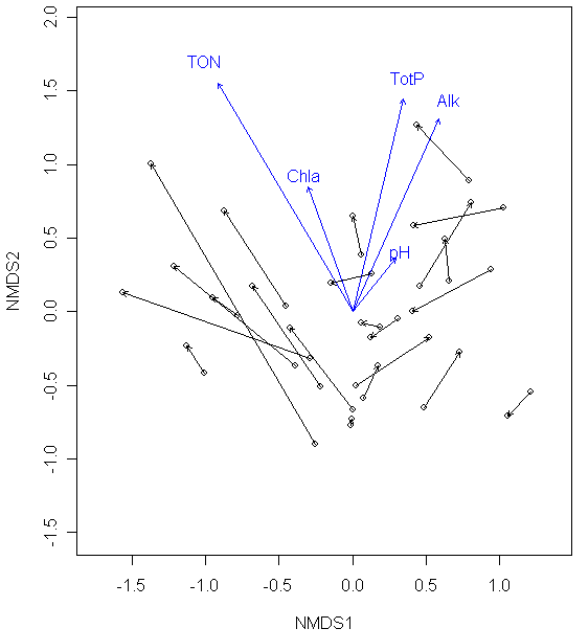
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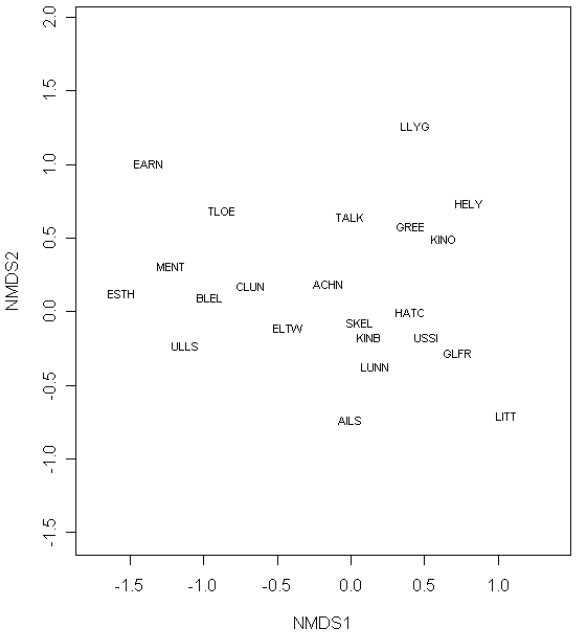
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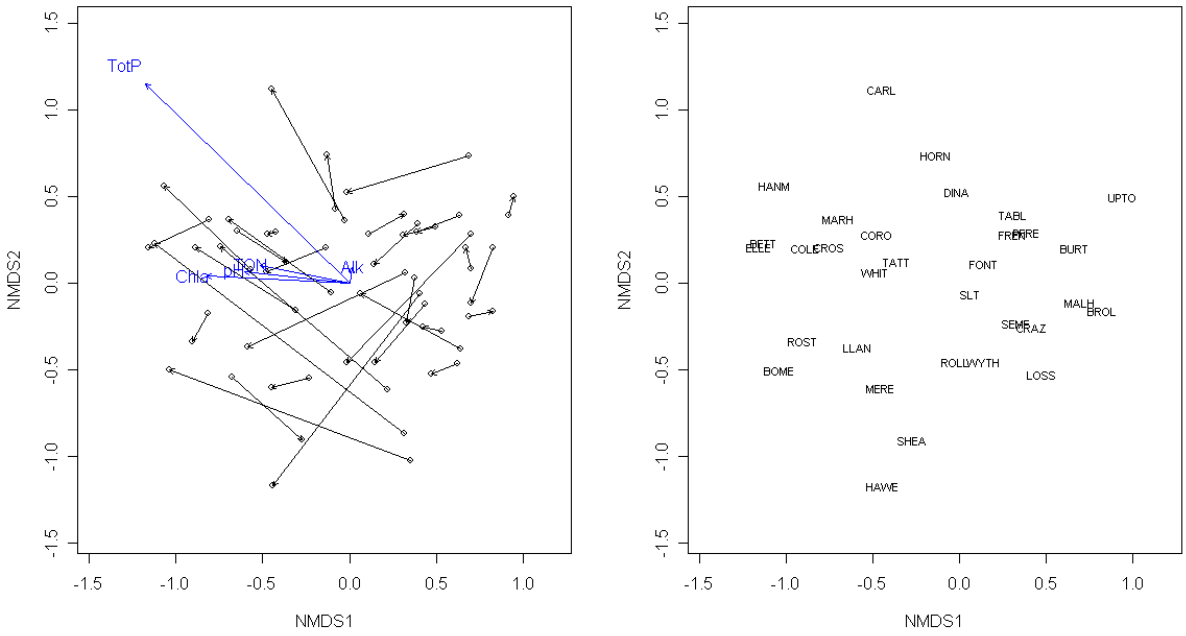


Figure 5.4. Biplots showing sample distances between core bottom and top for each lake type: (top) Low Alkalinity, (middle) Medium Alkalinity, (bottom) High Alkalinity. The left panels show the sample scores on axes 1 and 2 with arrows pointing in the direction of the core top and chemical data overlain. The right panels show the lake code positioned at the core top.

5.3 Deriving a pressure metric: Lake Trophic Diatom Index (LTDI)

5.3.1 The Lake Trophic Diatom Index (LTDI)

The Trophic Diatom Index (TDI) developed for rivers (Kelly and Whitton 1995) was taken as the starting point for deriving a pressure metric for lakes as it is an existing expert system for phytobenthos in UK waters, it is sensitive to the pressure of interest (nutrients), it does not require calibration with the Environment Agency–SEPA environmental datasets, and it is more in keeping with the ecological structure and function concepts of the WFD than diatom transfer function models which focus on inference of chemical variables (e.g. Bennion *et al*., 1996).

As a first step, a rescaling algorithm was used to assign scores to any lake taxa absent from the rivers dataset and to 'adjust' taxa to the DALES gradient. The resulting index is termed the Lake Trophic Diatom Index (LTDI). As for rivers, five groups of taxa were derived for each lake type (Figure 5.5):

- Groups 1 (blue) and 2 (green) – nutrient sensitive;
- Group 3 (yellow) – indifferent;
- Groups 4 (orange) and 5 (red) – nutrient tolerant.

The Low Alkalinity group (Figure 5.5a) contains a number of acid sites which affects the rescaling in that TDI group 1 becomes dominated by acid taxa and hence circumneutral, nutrient sensitive taxa are pushed into TDI group 2. This problem was solved by removing sites with pH < 7 from the rescaling database. Nevertheless group 1 with LTDI scores generally < 20 is comprised largely of acid tolerant taxa including *Brachysira* spp. (BR001A, BR003A, BR006A, BR010A), *Eunotia exigua* (EU009A), *Frustulia rhomboides* (FU002A) and *Synedra nana* (SY009A). Group 2 includes *Achnanthidium minutiissimum* (AC013A), *Eunotia incisa* (EU047A) and *Tabellaria flocculosa* (TA001A). Groups 4 and 5 are comprised of circumneutral taxa including *Encyonema minutum* (CM031A), *Fragilaria intermedia/vaucheriae* (FR007A) and *Gomphonema parvulum* (GO013A). In the Medium Alkalinity group (Figure 5.5b), group 1 taxa include *Cymbella microcephala* (CM004A) and *Gomphonema angustum* (GO073A) with LTDI scores mostly < 30. Group 2 comprises circumneutral taxa such as *Achnanthidium minutissimum* (AC013A) and *Gomphonema pumilum* (GO080A). Groups 4 and 5 occur at LTDI scores > 30 and include several benthic *Fragilaria* (*sensu lato*) spp., *Navicula* spp. and *Nitzschia* spp. Finally, in the High Alkalinity group (Figure 5.5c), group 1 and group 2 are similar to those for the Medium Alkalinity lakes, the former comprises largely *Cymbella* and *Encyonema* spp. and *Gomphonema angustum* (GO073A), and the latter containing *Achnanthidium minutissimum* (AC013A) and *Gomphonema pumilum* (GO080A). Groups 4 and 5 had a larger membership than in the other two lake types and occurred principally at LTDI scores > 40. Taxa in these groups include *Amphora pediculus* (AM012A), many benthic *Fragilaria* (*sensu lato*) taxa, *Navicula* spp. and *Nitzschia* spp. as well as planktonic taxa from the small, centric genera *Stephanodiscus*.

The assumption is that if the primary gradient in the DALES dataset is a nutrient pressure then this should be reflected by the LTDI scores. In order to test this assumption a canonical correspondence analysis (CCA) was performed on the whole dataset with nutrient parameters as the only environmental variables. A comparison of axis 1 species scores and sample scores with LTDI scores provides an assessment of how well the new metric reflects the nutrient pressure. Results show a strong correlation ($r = 0.82$) between the CCA axis 1 species scores and LTDI scores, and a very strong correlation ($r = 0.97$) between the CCA axis 1 sample scores and LTDI sample scores. These high correlations suggest that LTDI does reflect the nutrient pressure gradient across the dataset as a whole and validates its use as a nutrient pressure metric.

The next step was to explore the relationship between the LTDI groups and the nutrient gradient for each lake type separately to determine whether the metric reflected a nutrient pressure at the type-specific scale. In Figure 5.6, the relative abundances of the major taxa in the five LTDI groups are plotted along the nutrient gradient for each lake type which, for simplicity, is expressed as total phosphorus (TP).

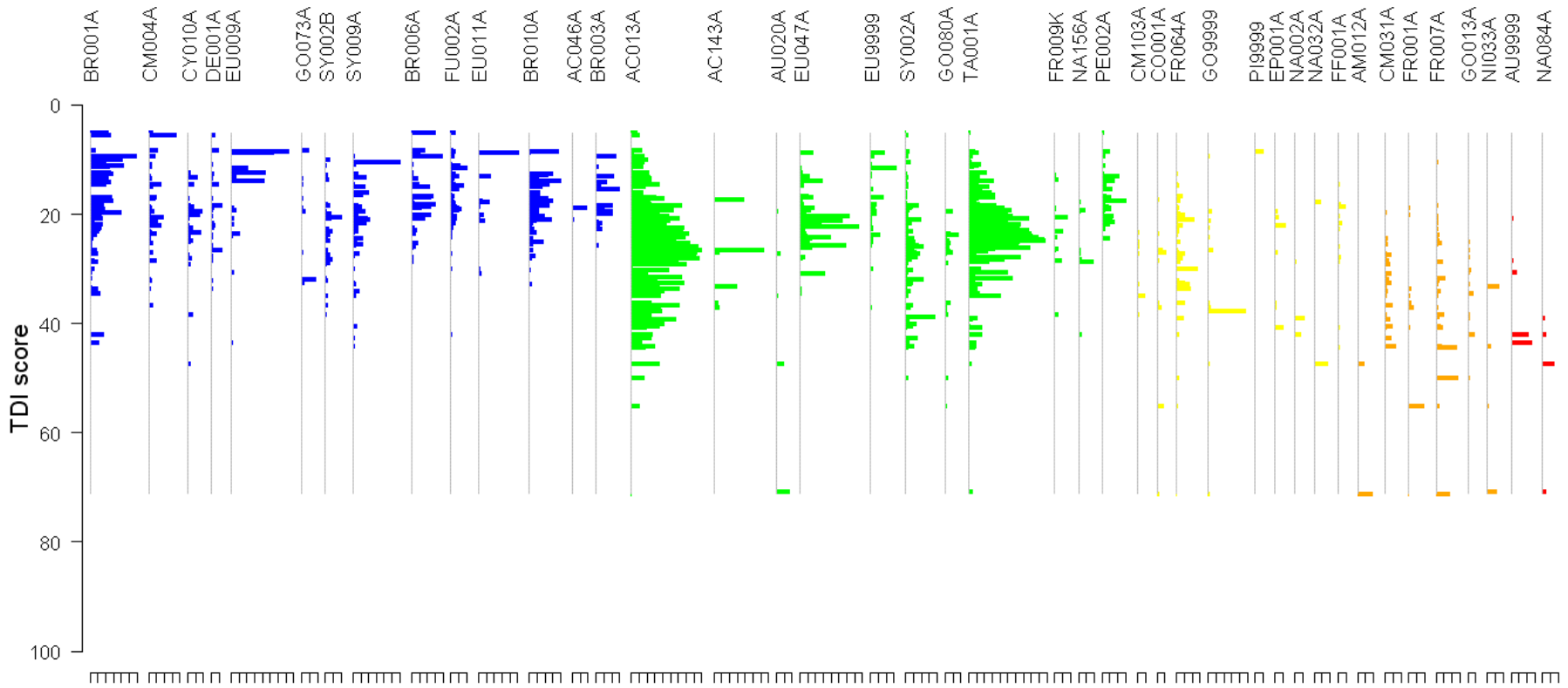


Figure 5.5a. Distribution of taxa belonging to the five LTDI groups for Low Alkalinity lakes. Bars are coded according to the TDI groups: group 1 (blue), group 2 (green), group 3 (yellow), group 4 (orange) and group 5 (red).

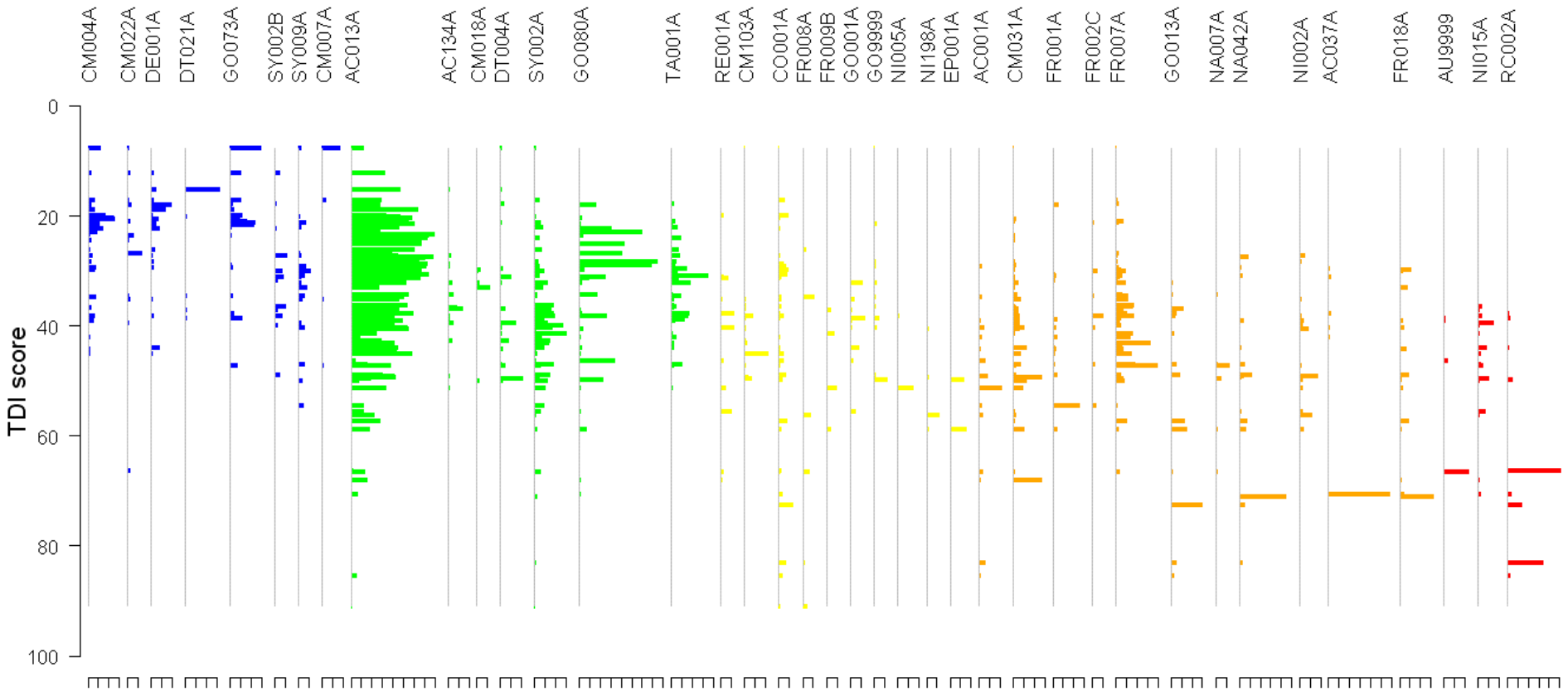


Figure 5.5b. Distribution of taxa belonging to the five LTDI groups for Medium Alkalinity lakes. Bars are coded according to the TDI groups: group 1 (blue), group 2 (green), group 3 (yellow), group 4 (orange) and group 5 (red).

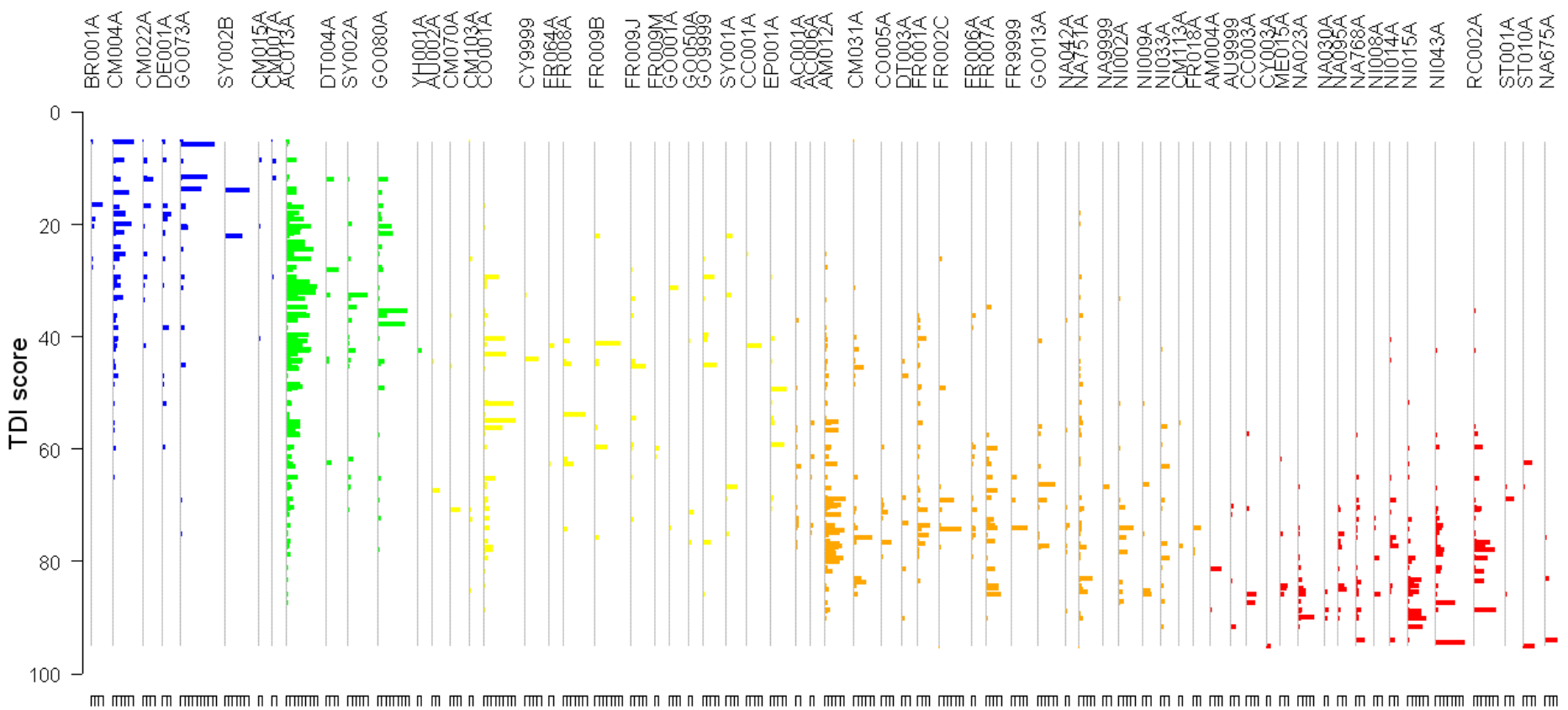


Figure 5.5c. Distribution of taxa belonging to the five LTDI groups for High Alkalinity lakes. Bars are coded according to the TDI groups: group 1 (blue), group 2 (green), group 3 (yellow), group 4 (orange) and group 5 (red).

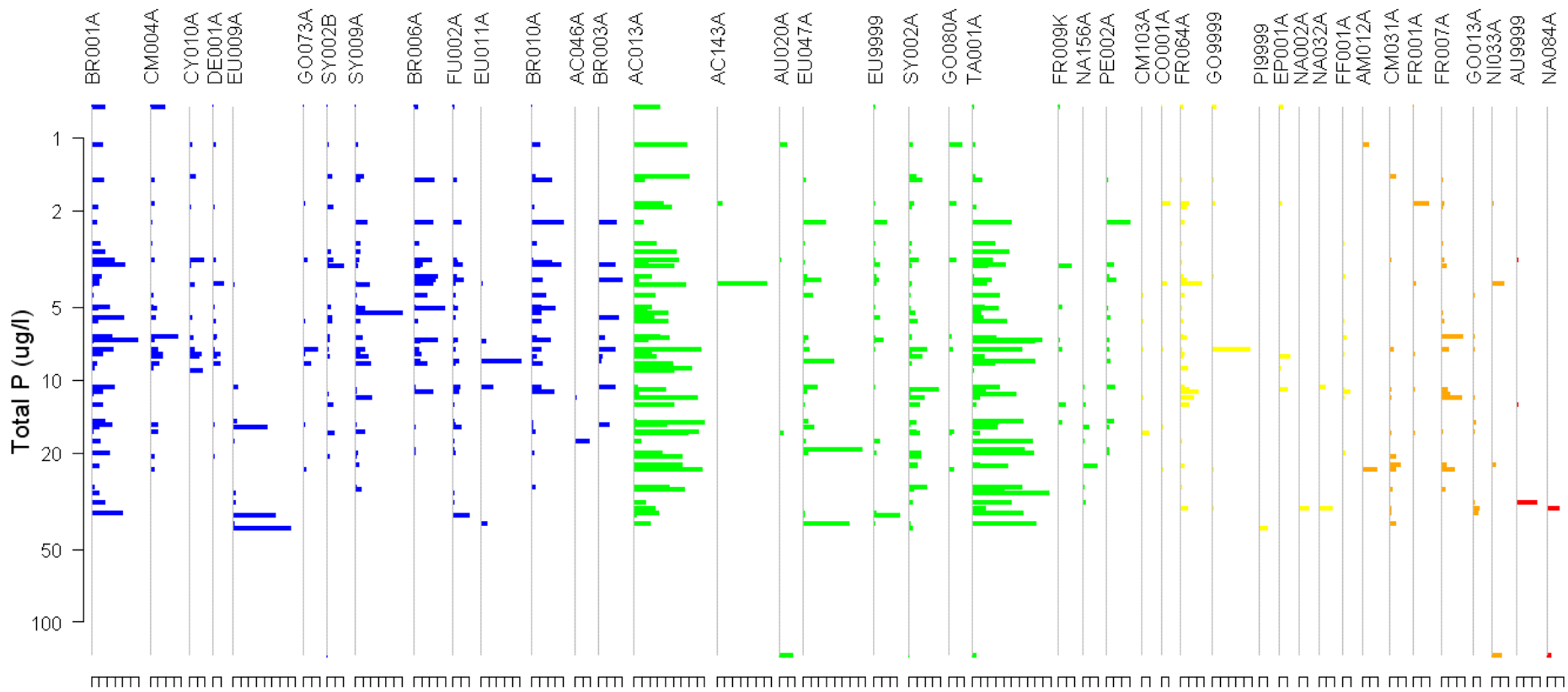


Figure 5.6a. Distribution of taxa belonging to the five LTDI groups for Low Alkalinity lakes plotted along the TP gradient. Bars are coded according to the TDI groups: group 1 (blue), group 2 (green), group 3 (yellow), group 4 (orange) and group 5 (red).

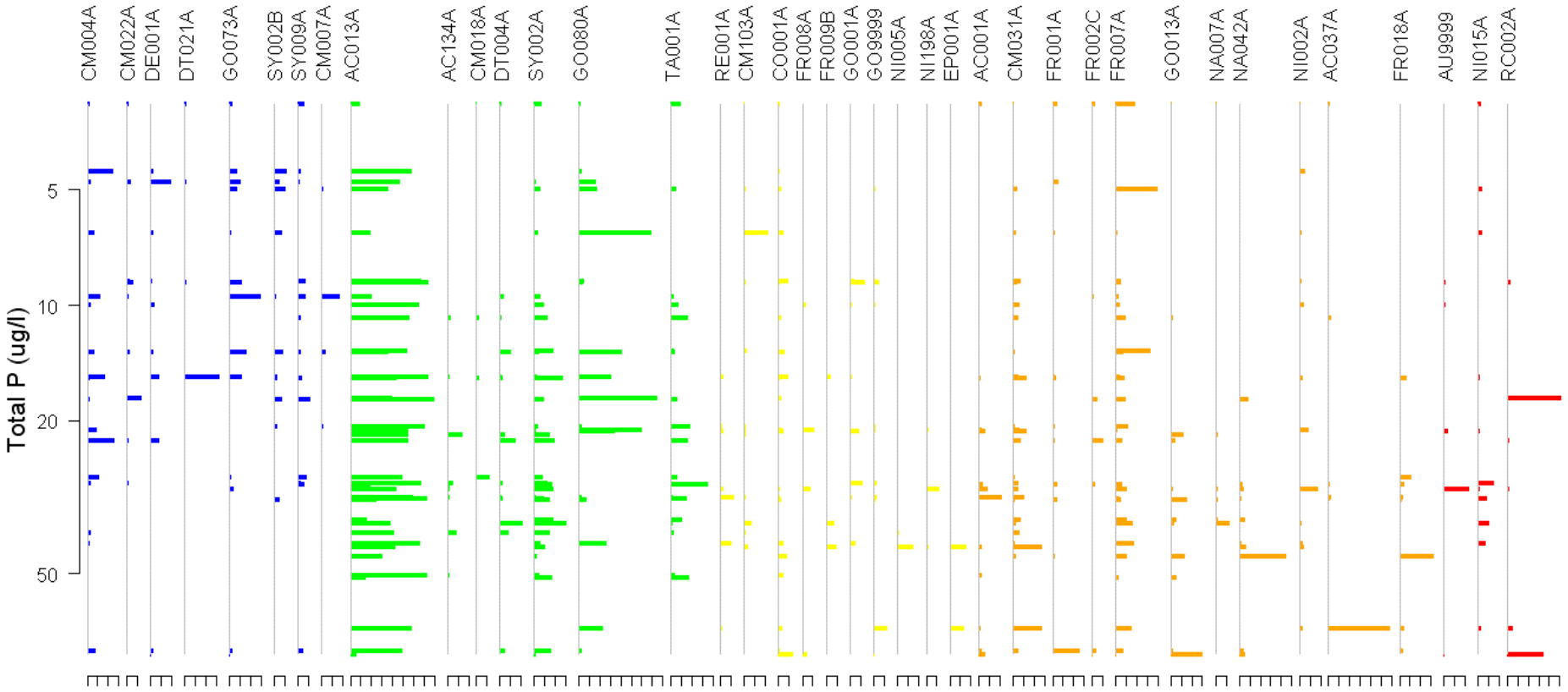


Figure 5.6b. Distribution of taxa belonging to the five LTDI groups for Medium Alkalinity lakes plotted along the TP gradient. Bars are coded according to the TDI groups: group 1 (blue), group 2 (green), group 3 (yellow), group 4 (orange) and group 5 (red).

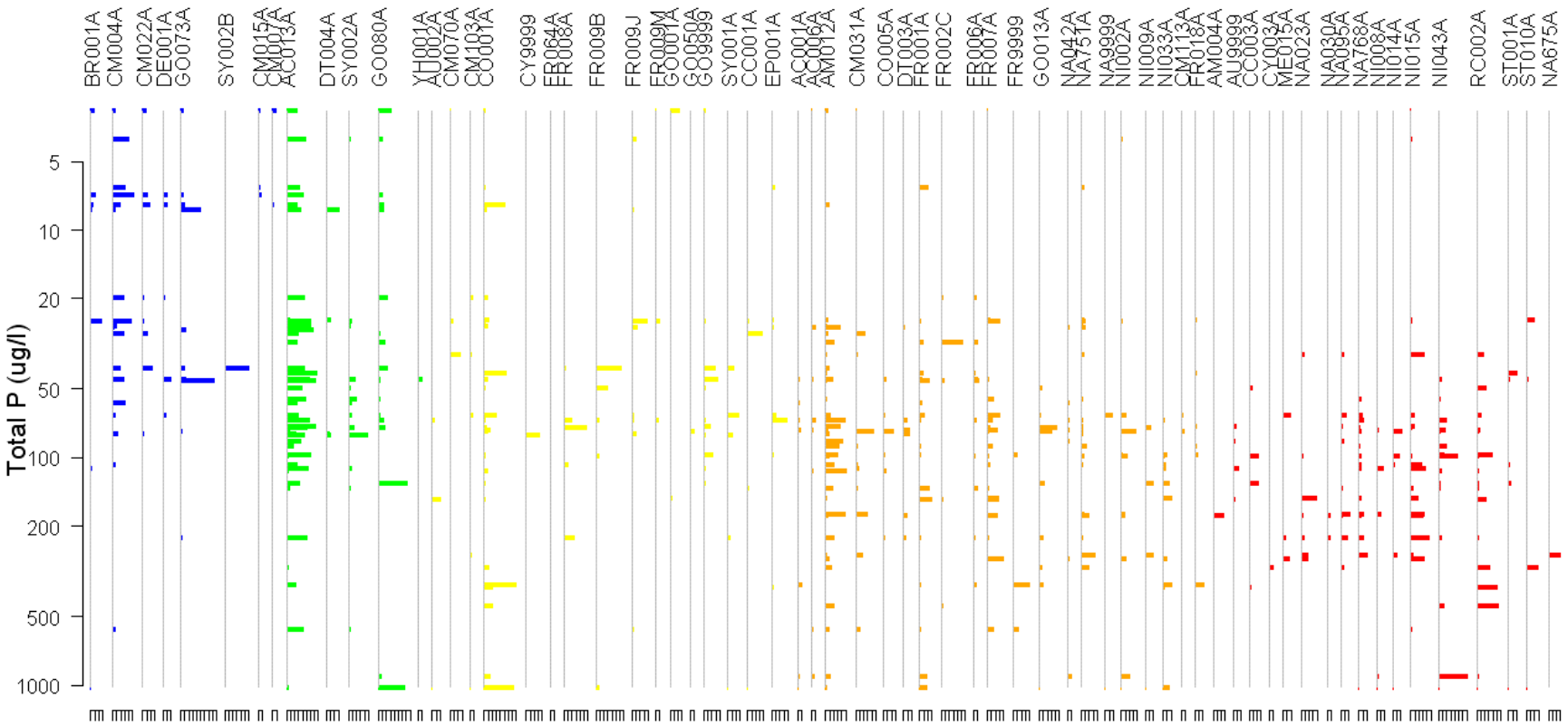


Figure 5.6c. Distribution of taxa belonging to the five LTDI groups for High Alkalinity lakes plotted along the TP gradient. Bars are coded according to the TDI groups: group 1 (blue), group 2 (green), group 3 (yellow), group 4 (orange) and group 5 (red).

Figure 5.6a illustrates that for the Low Alkalinity group the diatoms exhibit little response along the TP gradient. *Achnanthidium minutissimum* (AC013A) was abundant across the whole gradient. *Brachysira vitrea* (BR001A) and *Tabellaria flocculosa* (TA001A) also occurred in high relative abundances particularly at TP concentrations > 2 µg l^{-1}. However there were no taxa with a strong preference for higher TP values. The benthic diatoms, therefore, do not appear to be sensitive to changes in TP at relatively low concentrations (< 50 µg TP l^{-1}). Palaeoecological work at Low Alkalinity lakes such as Lochs Lomond and Awe indicates that it is subtle shifts in the diatom plankton that suggest enrichment and there are not marked changes in the non-planktonic forms (Bennion, 2004). Similarly in diatom surface sediment training sets the main response along the TP gradient is in habitat shifts (i.e. a switch from benthic to planktonic forms) or in composition of the planktonic community such as a decrease in oligotrophic *Cyclotella* spp. and increase in mesotrophic taxa (*Asterionella formosa* and *Fragilaria crotonensis*) (e.g. Bennion, 1995). It seems that in these systems the *Achnanthidium minutissimum–Brachysira* spp.-*Tabellaria flocculosa* assemblage can absorb some degree of enrichment before it gives way to other associations. The sheer numbers of inocula of these taxa in a lake littoral zone would serve to 'buffer' it against change and within the DALES dataset the Low Alkalinity lakes do not cover a long enough nutrient gradient for us to see any marked species turnover. The result is that the DALES classification tool based on the benthic diatom community is likely to underestimate degree of ecological change attributed to nutrient enrichment. The palaeoecological studies and other ecological data suggest that Loweswater (LOWS), Bassenthwaite (BASS), Bala (BALA) and Grasmere (GRAS), for example, are not in their former oligotrophic state and have experienced enrichment. However, this impact is not reflected in the benthic diatom community.

The Medium Alkalinity group (Figure 5.6b) was also dominated by *Achnanthidium minutissimum* (AC013A), and *Gomphonema pumilum* (GO080A) was equally abundant in several lakes. There was a little more distinction along the TP gradient than for the Low Alkalinity lakes with a notable decrease in TDI groups 1 and 2 taxa and a relative increase in TDI groups 4 and 5 taxa above ~20 µg l^{-1}. The latter included *Gomphonema parvulum* (GO013A), *Navicula minima* (NA042A), small *Fragilaria* (*sensu lato*) spp. (FR018A) and *Nitzschia dissipata* (NI015A). The most marked response along the nutrient gradient was exhibited by the High Alkalinity group (Figure 5.6c) where taxa in TDI groups 3, 4 and 5 occurred almost exclusively at TP concentrations > 30 µg l^{-1}.

The relationship between the LTDI scores and nutrients for each lake type was further explored via correlation. The correlation statistics between the LTDI score for each sample and the key chemical variables (Table 5.3) clearly show that LTDI reflects nutrients in the Medium and High Alkalinity lake types but has a weak relationship with nutrients in the Low Alkalinity group where the metric is more closely associated with alkalinity. This further explains why little taxon differentiation across the TP gradient was observed in Figure 5.6a. This presents a major problem for the development of the classification tool for the Low Alkalinity group as the dominance of the alkalinity gradient makes it difficult to separate the effects of alkalinity and nutrients. This problem is not evident in the DARES dataset as the Low Alkalinity river type does not include many soft waters and probably covers the equivalent of both Low and Medium Alkalinity lake types.

Table 5.3. Correlations between LTDI score for each sample and key chemical variables (log_{10} transformed except pH), by lake type. Bold indicates high correlations.

	Alk	pH	Cond	Chl a	PO4	TP	TON	TN	SiO2
LA	**0.4**	0.35	0.08	0.38	0.11	0.3	0.31	0.05	0.01
MA	0.19	0.17	0.25	0.37	0.2	**0.53**	0.31	**0.58**	0.39
HA	0.16	0.15	0.21	0.36	0.7	**0.69**	0.42	**0.48**	0.02

5.3.2 Diatom assemblages at reference condition (high status)

The diatom assemblages at reference condition for each lake type can now be defined by examining the dominant taxa in the reference lakes identified in section 5.2.1 and comparing these with the taxa present in the impacted lakes identified in the *a priori* classification in section 5.2.2.

One difficulty in defining the reference diatom community is that there are so few reference lakes for the High Alkalinity group. A further difficulty is that the overriding alkalinity gradient in the Low Alkalinity group makes it almost impossible to identify a set of nutrient sensitive taxa. *Achnanthidium minutissimum* occurred both in reference lakes and in sites classed as moderate in the Low Alkalinity group. Nevertheless, the assemblages of the reference sites were characterised by high numbers of *Achnanthidium minutissimum, Tabellaria flocculosa* and *Brachysira* spp. but very few taxa from the genera *Navicula, Nitzschia* or *Amphora*. For the Medium Alkalinity lakes, the diatom flora of samples at reference were characterised by an *Achnanthidium minutissimum–Cymbella* spp. association, and as with the Low Alkalinity group, *Navicula* and *Nitzschia* spp. were largely restricted to sites classed as less than good status. For the High Alkalinity group the lakes classed as reference or good status were characterised by an *Achnanthidium minutissimum–Cymbella* spp. association, as seen for the Medium Alkalinity group. The benthic *Fragilaria* taxa, *Amphora* spp., motile *Navicula* and *Nitzschia* spp., and planktonic taxa were present in negligible amounts in the samples from reference and good status sites but were abundant in sites deemed to be at less than good status. In summary, the dataset does allow diatom assemblages at reference condition to be identified for each type although the strong alkalinity gradient in the Low Alkalinity group results in a weak response of the taxa to nutrients and the lack of reference sites for the High and Medium Alkalinity types means that there are relatively few samples on which to base the definitions.

5.3.3 Effect of season and substratum on LTDI

The majority of lakes in the DALES dataset have been sampled at different seasons (spring, summer and autumn) over the course of 2003 and 2004. The majority of samples have been collected from the epilithon but at some sites epiphyton and/or epilithon samples have been collected (see Table 5.4). Variability in sampling season and habitat raises the important question of the effect of these variables on LTDI. This effect is explored below.

Table 5.4. DALES sample distribution by season and habitat

Season	Habitat	
	Epilithon	Epiphyton
Spring	155	23
Summer	142	39
Autumn	185	33

In order to examine seasonal effects we extracted data from all sites with spring, summer and autumn samples (121) and calculated the mean LTDI score for each season at each site. We then used an analysis of variance coupled with Tukey's Honestly Significant Difference (HSD) *post hoc* test to compare differences in mean LTDI between seasons (Quinn and Keough, 2002). Results of the test reveal a small, non-significant effect ($p > 0.05$), with autumn samples having a slightly higher LTDI than summer or spring.

To examine the effect of substratum (sample habitat) on LTDI we extracted samples for sites with both an epilithon and epiphyon sample (33 sites) and calculated the mean LTDI score for each substratum at each site. Figure 5.9 shows the mean LTDI score for the 33 paired samples.

There is a tendency for epilithon samples at higher LTDI sites to have to have slighter higher LTDI scores than epiphyton samples although overall the mean difference between the paired samples of 2.2 LTDI units is not significant ($p = 1.84$, paired t-test).

These analyses reveal small seasonal and substratum effects of up to three LTDI units. However, these effects are not significant ($p > 0.05$) and we therefore do consider these variables in subsequent model development.

5.4 Calculation of EQR and status class boundary setting

5.4.1 Expected LTDI at reference conditions

Having described the diatom assemblages at reference condition in a qualitative manner, the next step is to quantify this by deriving expected LTDI at reference condition. The relationship between the LTDI metric and the nutrient pressure (expressed as the TP gradient) was further explored by plotting the LTDI scores for

each lake type against TP, coded by the *a priori* estimate of status where blue represents reference or high status lakes, green is good, orange is moderate, red is poor/bad and white is unknown as palaeoecological data are not available for all sites (Figure 5.7). For the High Alkalinity sites there is a clear boundary between sites classed as good and those deemed to be less than good at ~50 µg TP l^{-1} and a maximum LTDI of ~40–50. For the Medium Alkalinity lakes the boundaries are less clear although lakes considered to be at reference all have LTDI scores < 40 and TP concentrations < 30 µg TP $l^{-1.}$ As expected there is no clear boundary for the Low Alkalinity group with reference sites present along the entire Total P gradient and covering a broad range of LTDI scores.

To examine the distribution of LTDI values more closely, histograms of the LTDI scores for each type broken down by the *a priori* status classification are presented in Figure 5.8. The numbers in the header strips are the median TDI values of that subset. Another way of examining the data is to summarise the range of LTDI values within each *a priori* quality class for each lake type using boxplots (Figure 5.9). On the basis of these distributions, and bearing in mind the LTDI scores for good, moderate and poor status, this would suggest the following expected LTDIs at reference condition:

Low Alkalinity = 20

Medium Alkalinity = 25

High Alkalinity = 25

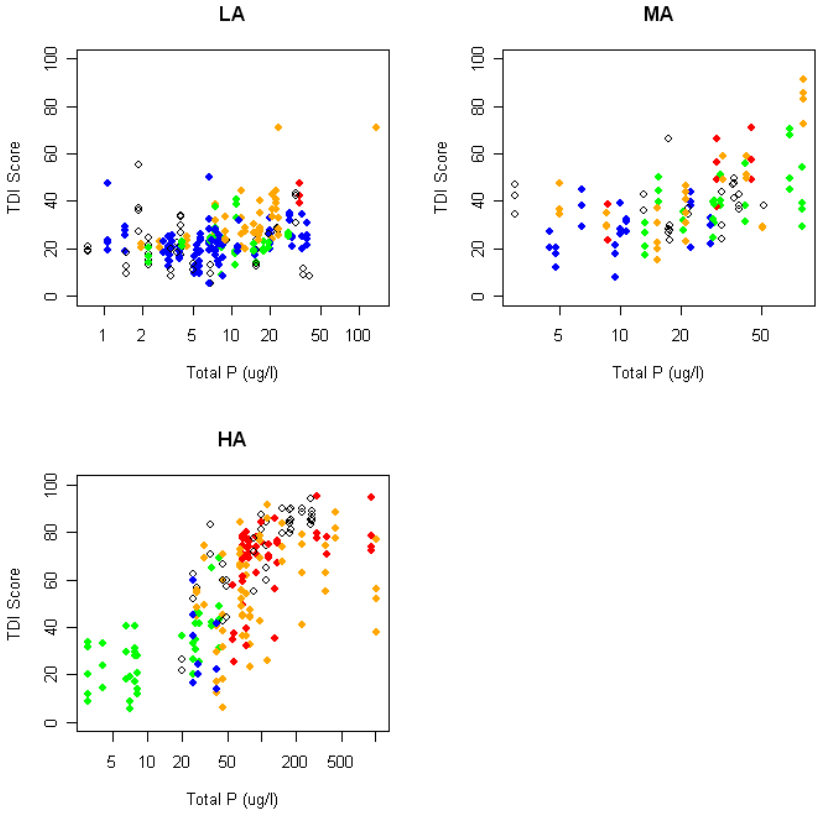


Figure 5.7. Scatterplots of the LTDI scores for each lake type against TP, coded by the *a priori* status classification. Reference/high (blue), good (green), moderate (orange), poor/bad (red), unknown (white).

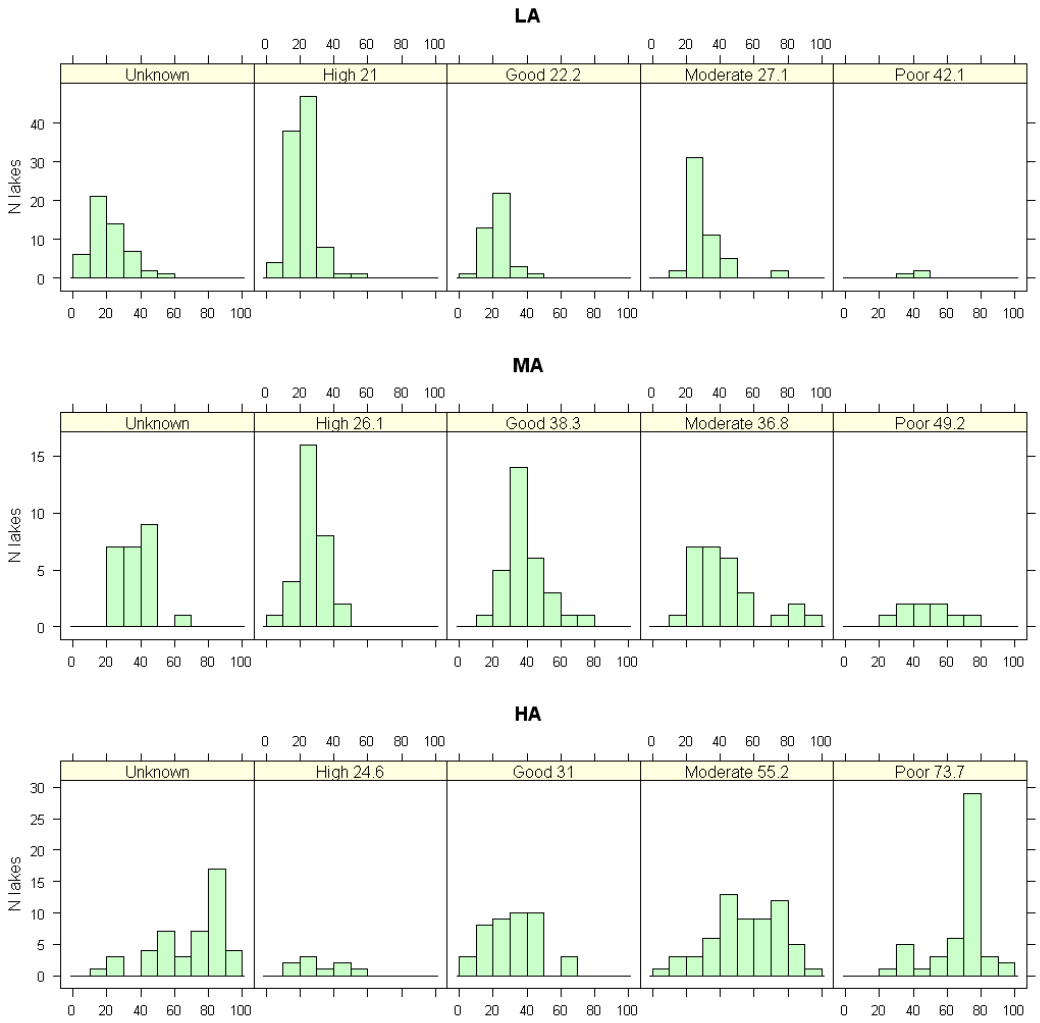


Figure 5.8. Histograms of the LTDI scores (x axis) for each lake type broken down by the *a priori* status classification. The numbers in the header strips are the median LTDI values of that subset.

5.4.2 Defining the class boundaries

The procedure for defining the class boundaries follows that of the DARES approach for rivers. Firstly the LTDI scores were converted to EQR scores based on the expected LTDIs at reference condition defined above.

High Alkalinity type

Table 5.5 gives the quantiles of the EQRs for each quality class in the *a priori* classification. If, as in DARES, the high/good boundary is placed at the 25th percentile of the EQR values for reference sites (high status) in each type then an EQR of 0.78 would define the high/good boundary for the High Alkalinity group. Figure 5.10 shows a scatterplot of the percentage relative abundance of LTDI species groups 1 and 2 (nutrient sensitive), and groups 4 and 5 (nutrient tolerant).

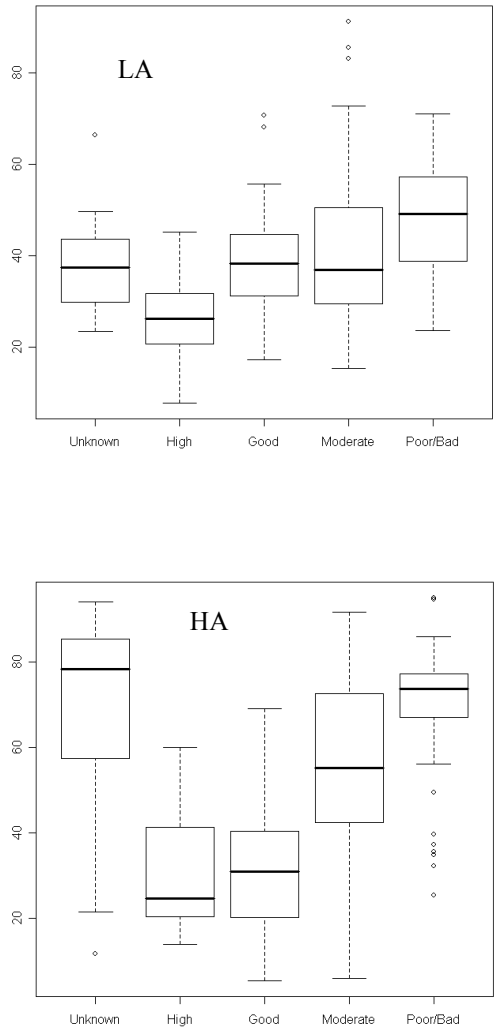


Figure 5.9. Boxplots of the LTDI scores for each lake type broken down by the *a priori* status classification.

The crossover between the two groups occurs at an EQR of 0.66, and if we follow the criteria used for rivers in chapter 4 then this is taken to represent the good/moderate boundary. However, due to the small number of reference sites, it is not possible to use the 25th percentile approach adopted for rivers to define the high/good boundary in lakes. Instead, we have worked backwards and chosen the midpoint between the good/moderate boundary (0.66) and an EQR of 1.0, which would be 0.83. This still seems low compared to values derived for rivers (see chapter 4) although the reference LTDI value is also lower (25). In light of this and the lack of reference sites for High Alkalinity lakes, the boundaries set for the Medium Alkalinity lakes (see below) are proposed such that an EQR of 0.90 represents the high/good boundary, an EQR of 0.66 represents the good/moderate boundary and the gradient is then divided evenly into the remaining classes, as follows:

Table 5.5. Quantiles of EQRs for each quality class in the *a priori* classification for High Alkalinity lakes.

	0	25	50	75	100
Unknown	0.08	0.2	0.29	0.56	1.18
High	0.53	0.78	1.01	1.06	1.18
Good	0.41	0.8	0.92	1.06	1.26
Moderate	0.11	0.37	0.6	0.76	1.25
Poor/bad	0.07	0.3	0.35	0.43	0.99

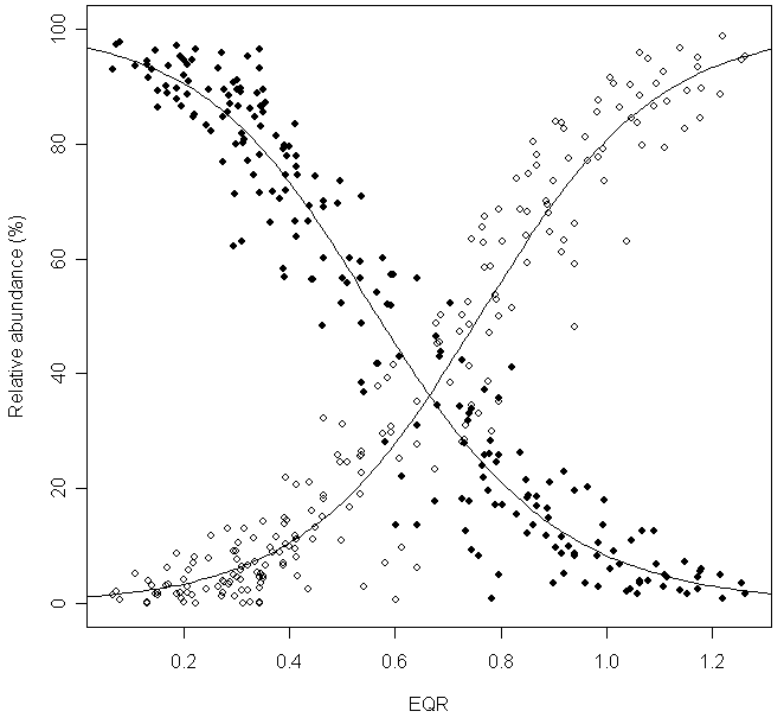


Figure 5.10. Scatterplot of % relative abundance of LTDI groups 1 and 2 (open circles) and groups 3 and 4 (black circles) against EQR using an expected reference LTDI value of 25 for High Alkalinity lakes.

- H/G EQR = 0.90
- G/M EQR = 0.66
- M/P EQR = 0.44
- P/B EQR 0.22

Medium Alkalinity type

Table 5.6 gives the quantiles of the EQRs for each quality class in the *a priori* classification. If the high/good boundary is placed at the 25th percentile of the EQR values for reference sites then an EQR of 0.91 would define the high/good boundary for the Medium Alkalinity group. Figure 5.11 shows a scatterplot of the percentage relative abundance of LTDI species groups 1 and 2 (nutrient sensitive), and groups 4 and 5 (nutrient tolerant). The crossover between the two groups occurs at an EQR of 0.66, the same as for the High Alkalinity group, and if we follow the criteria used in DARES then this is taken to represent the good/moderate boundary. An EQR of 0.9 is therefore proposed to represent the high/good boundary, an EQR of 0.66 is chosen to represent the good/moderate boundary and the gradient is then divided evenly into the remaining classes, as follows:

- H/G EQR 0.90
- G/M EQR 0.66
- M/P EQR 0.44
- P/B EQR 0.22

Table 5.6. Quantiles of EQRs for each quality class in the *a priori* classification for Medium Alkalinity lakes.

	0	**25**	**50**	**75**	**100**
Unknown	0.45	0.76	0.83	0.93	1.02
High	0.73	0.91	0.98	1.06	1.23
Good	0.39	0.74	0.82	0.92	1.1
Moderate	0.12	0.67	0.84	0.94	1.13
Poor/bad	0.39	0.57	0.68	0.82	1.02

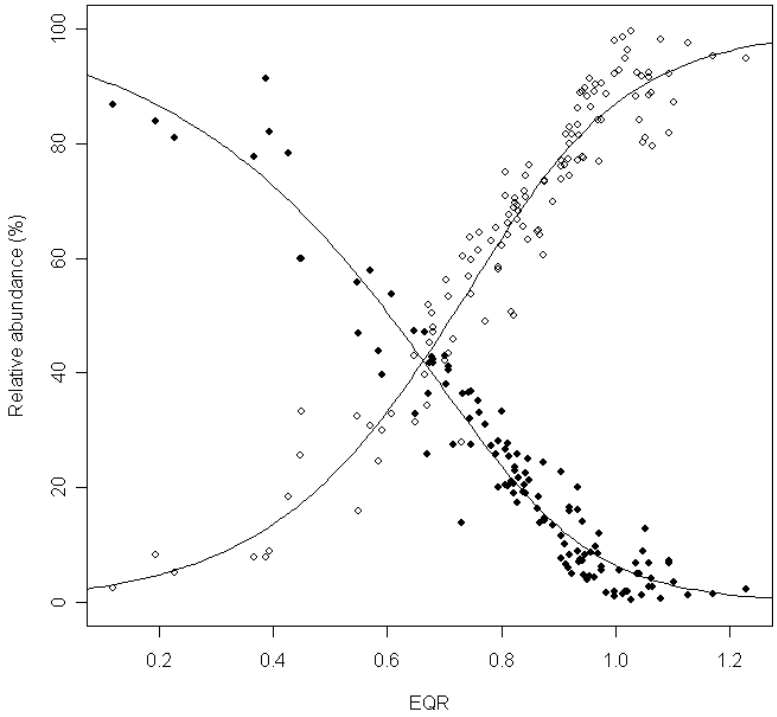


Figure 5.11. Scatterplot of % relative abundance of LTDI groups 1 and 2 (open circles) and groups 3 and 4 (black circles) against EQR using an expected reference LTDI value of 25 for Medium Alkalinity lakes.

Low Alkalinity type

Table 5.7 gives the quantiles of the EQRs for each quality class in the *a priori* classification. If the high/good boundary is placed at the 25th percentile of the EQR values for reference sites then an EQR of 0.93 would define the high/good boundary for the Low Alkalinity group. Figure 5.12 shows a scatterplot of the percentage relative abundance of LTDI species groups 1 and 2 (nutrient sensitive), and groups 4 and 5 (nutrient tolerant). The crossover between the two groups occurs at an EQR of 0.63 which, following the criteria used in DARES, would represent the good/moderate boundary. In light of this and the boundaries set for the Medium and High Alkalinity lakes (see above), an EQR of 0.9 is proposed to represent the high/good boundary, an EQR of 0.63 is chosen to represent the good/moderate boundary and the gradient is then divided evenly into the remaining classes, as follows:

- H/G EQR 0.90
- G/M EQR 0.63
- M/P EQR 0.44
- P/B EQR 0.22

Table 5.7. The quantiles of the EQRs for each quality class in the *a priori* classification for Low Alkalinity lakes.

	0	25	50	75	100
Unknown	0.56	0.91	1.01	1.08	1.18
High	0.62	**0.93**	0.99	1.02	1.18
Good	0.74	0.94	0.97	1.01	1.14
Moderate	0.36	0.83	0.91	0.95	1.02
Poor/bad	0.66	0.69	0.72	0.74	0.76

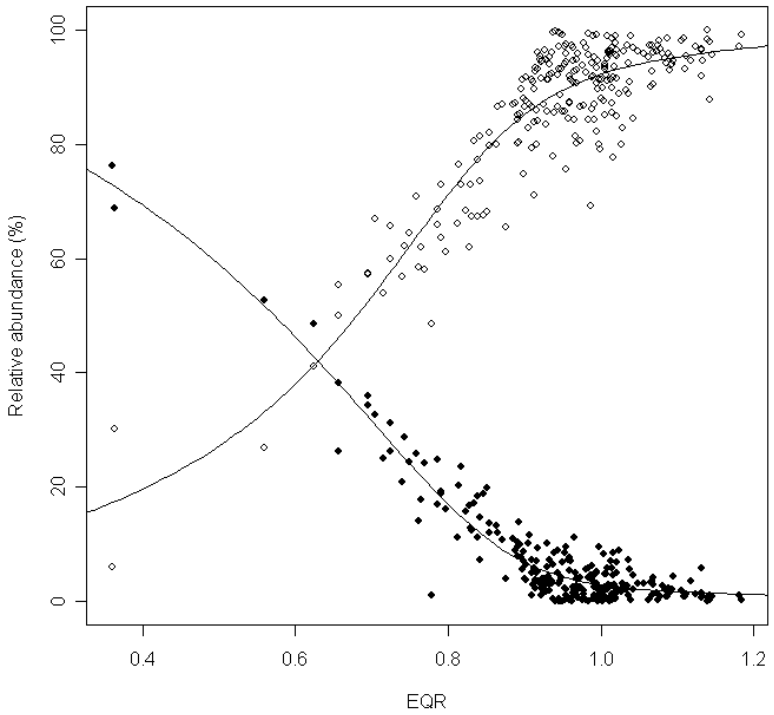


Figure 5.12. Scatterplot of % relative abundance of LTDI groups 1 and 2 (open circles) and groups 3 and 4 (black circles) against EQR using an expected reference LTDI value of 20 for Low Alkalinity lakes.

5.5. Predicted class status

The predicted class status for each sample in the DALES dataset based on the boundaries proposed above is given in Appendix 1. A summary of the *a priori* status classes (vertical) versus model predictions (horizontal) for each lake type is presented in Table 5.8. This gives the predictions for a) each individual sample and b) for each site by averaging the scores for multiple samples from that site.

For Medium and High Alkalinity lakes, the model appears to provide a reasonable match with the *a priori* status classes. For example, of the samples predicted as high status using the DALES model for High Alkalinity lakes, 5 are classed as high, 24

good, 8 moderate and 2 poor/bad based on the *a priori* classification; for those predicted as good status 3 samples are classed as high, 25 good, 18 moderate and 5 poor/bad based on the *a priori* classification; and for those predicted as moderate status one sample is classed as high, 2 good, 15 moderate and 5 poor/bad based on the *a priori* classification. For the Medium Alkalinity lakes, of the samples predicted as high status using the DALES model 24 are classed as high, 12 good, 10 moderate and 1 poor/bad based on the *a priori* classification; for those predicted as good status 7 samples are classed as high, 14 good, 11 moderate and 4 poor/bad based on the *a priori* classification; and for those predicted as moderate status no samples are classed as high, 3 good, 3 moderate and 3 poor/bad based on the *a priori* classification.

For Low Alkalinity lakes, the predictions provide a poor match with the *a priori* classification. Almost all lakes are classed as high status including 35 samples that were deemed to be moderate in the *a priori* classification. This is not surprising given the problems with the Low Alkalinity dataset described above, namely the apparent lack of sensitivity of the benthic diatoms to the nutrient gradient and the confounding relationship with alkalinity.

It should also be recognised that this is a rigorous test of the model and goes beyond the assessments of model performance employed in the other classification tool projects. The boundaries should also be considered as provisional and may change as and when more data become available. For example, within the uncertainty module there is potential to extract the benthic signal only from the surface sediment dataset and to test whether the apparent lack of response observed in the epilithic diatom assemblages is also evident in the surface sediment assemblages.

Table 5.8. Predicted class versus *a priori* status class for samples (a) and sites (b) in each lake type.

	Low Alkalinity lakes – Predicted class				
	Bad	**Poor**	**Moderate**	**Good**	**High**
a) samples					
Unknown	0	0	1	11	39
High	0	0	2	12	85
Good	0	0	0	5	35
Moderate	0	2	0	20	29
Poor/bad	0	0	1	2	0
b) sites					
Unknown	0	0	0	4	12
High	0	0	0	3	28
Good	0	0	0	1	11
Moderate	0	1	0	6	7
Poor/bad	0	0	0	1	0

	Medium Alkalinity lakes – Predicted class				
	Bad	**Poor**	**Moderate**	**Good**	**High**
a) samples					
Unknown	0	0	1	16	7
High	0	0	0	7	24
Good	0	2	3	14	12
Moderate	2	2	3	11	10
Poor/bad	0	1	3	4	1
b) sites					
Unknown	0	0	0	7	3
High	0	0	0	2	7
Good	0	0	1	5	4
Moderate	1	0	2	3	3
Poor/bad	0	0	2	0	1

	High Alkalinity lakes – Predicted class				
	Bad	**Poor**	**Moderate**	**Good**	**High**
a) samples					
Unknown	17	11	10	5	4
High	0	0	1	3	5
Good	0	1	2	15	24
Moderate	5	16	15	18	8
Poor/bad	4	34	5	5	2
b) sites					
Unknown	6	4	4	1	2
High	0	0	0	1	2
Good	0	0	1	3	7
Moderate	1	6	5	4	2
Poor/bad	0	11	3	1	1

6 Uncertainty in ecological status assessments using diatoms

6.1 Introduction

Chapters 3 to 5 describe the development of models for assessing ecological status in running and standing waters in the UK. While the rapid response of diatoms to environmental change makes them very useful indicators of water quality (Lowe and Pan, 1996) it also means that diatom communities are inherently variable both in space and time (King *et al*., 2006). As the WFD focuses on the need for a potentially expensive Programme of Measures in water bodies that do not achieve good ecological status, it is important to understand the consequences of the inherent variability of diatom assemblages for ecological status assessments. A sample provides information on the condition of the biology at a point in time and, while this is of potential interest to regulators, a water quality planner will wish to ask broader questions about ecological status and, more specifically, the risk of misclassification at a sampling station. In particular:

- what is the risk of a 'false positive' – a downgrade in status class due to samples that were influenced by short-term variability in the condition of the water chemistry and/or biology? (in statistical terms, a 'Type I 'error)? and;
- bearing the 'precautionary principle' in mind, what is the risk of a 'false negative' a water body that is no better than moderate status being classified as 'good status' (a 'Type II' error)?

These questions relate primarily to the accuracy of status assessments (i.e. the closeness of agreement between the status assessment and the 'true' status of a water body) but are entangled with issues that relate to the 'precision' of status assessments (closeness of agreement between independent results obtained under stipulated conditions).

Many sources of variability can be controlled by ensuring adherence to rigorous protocols via training and quality control programmes. Prygiel *et al.* (2002) carried out a diatom inter-comparison exercise involving 24 diatomists on the River Loup (France). Collection of the samples in the field was found to be a critical stage. Inter-operator variability was greater than intra-operator variability and the main source of variability involved misidentification. They advocated implementing inter-comparison exercises, internet exchanges and reference material collections to reduce the variation. Alverson *et al.* (2003) focused on laboratory sources of error for sampling algal communities. Variability in cell densities were found to be associated with non-random distribution of diatoms on the coverglass though this did not affect species composition. The overriding concern is to ensure comparability between water bodies, which is best achieved by a set of simple guidelines applicable with only slight modification to all water bodies. However, even if these precautions are followed, natural spatial and temporal variability remains and needs to be understood and incorporated into models as error terms.

Sources of variability include temperature, light, nature of substratum, nutrient availability, space, grazing and hydromorphological regime (Vinson and Rushforth, 1989; Allan, 1995; Borchardt, 1996; Burkholder, 1996; Hill, 1996). While diatoms collected from the surface layers of lake sediment cores provide an 'integrated' sample incorporating the spatial and temporal variability within that lake (Battarbee *et al*., 2001), littoral regions of lakes and stream/river beds are highly dynamic and samples taken on one occasion provide a shorter 'environmental history' (Biggs, 1995; King *et al*., 2006, Yallop and Kelly, 2006). Theoretically, a composite sample where phytobenthos from several locations within the sampling station are pooled should remove most of the effects of local spatial variability (over scales of metres); however, there are few quantitative studies to support this.

Diatom assemblages vary in time as well as in space, even in the absence of known perturbations. In rivers, nutrient concentrations, in particular, are often related to discharge, and a single sample taken after a period of prolonged low flows may imply lower status than a sample taken at another time. The long-term average condition of the biology provides a sound basis for managing a water body but raises further questions about the number of samples required and how these should be spaced through the classification period. A balance needs to be struck between a sampling intensity that provides a robust classification in a practical time period and the need to avoid any risk of pseudo-replication (Hurlbert, 1984; Hairston, 1989) or for seasonality to confound interpretation.

In addition to the inherent variability among samples there is also variation due to operator sampling in the field and stages in the processing of the sampling including cleaning and enumeration of samples (Table 6.1).

While many studies have demonstrated spatial and temporal variation in benthic diatom communities (reviewed in King *et al*., 2006), few of these have quantified sources of natural variation in relation to water quality indices or ecological status assessments. Our own observations suggest that the scale of variation in metrics such as the TDI can also vary from site to site, which means that it is difficult to draw generalisations from studies such as Prygiel *et al*. (2002) that focus on a single site. However, including all possible sources of variation in a single study would require an impossibly large and unwieldy nested sampling design. For this reason, we have made some assumptions at the outset of the project, drawing upon previous research work, and have used best judgement to measure the errors that we feel are likely to cause the greatest variation. While variation associated with identification and enumeration should not be discounted, this study focuses on uncertainty associated with sampling (see Prygiel *et al*., 2002).

In this chapter we ask two questions:

- What is the uncertainty associated with a single sample as an estimate of ecological status on the day that the sample was collected?
- How well does this sample reflect the long-term average condition of the biology?

These questions are addressed separately. The former uses a nested analysis of variance that examines variation in metrics associated with variability on a slide nested within variability at a site. In order to cover a range of water body types (lakes and rivers), no attempt has been made to separate (natural) spatial variability from variability introduced by the operator but the latter sources of error were minimised by use of standard protocols.

Table 6.1. Errors associated with processing or samples for diatom enumeration.

Step	Action	Potential errors
Sample	Collect at least five cobbles	Natural spatial heterogeneity of biofilm
	Brush upper surfaces using toothbrush	Selection of appropriate microhabitats
		Selection of suitable substrata
	Pour suspension into sample bottle	Contamination of toothbrush
Cleaning	Remove sub-sample from bottle	Incomplete mixing within raw sample (vigorous shake)
	Digest using oxidising agents	Incomplete removal of organic matter; incomplete separation of colonies and frustules
Slide	Remove one or two drops from cleaned sub-sample	Incomplete mixing within suspension (vigorous shake)
	Evaporate liquid from suspension	Diatoms strewn too densely; edge effects

The second question relied on data already available within the DARES and DALES databases for sites with $n \geq 6$ records, spanning at least a three-year period.

6.2 Variability associated with a single sample

6.2.1 Study design and statistical analyses

Diatom samples were collected from four lakes (Bassenthwaite, Blagdon, Betton Pool and Crummock Water) and four rivers (Ribble, Loddon, Wylye and Ely) during the summer of 2005. Each sample was collected by an experienced operator according to a standard agreed protocol (chapter 3). Three samples were collected from each site, each composed of the pooled biofilm from five cobbles (except for Betton Pool where the samples were composed of the pooled biofilm from five stems of *Typha angustifolia*). Cobble and macrophyte samples were collected from Blagdon and the Ribble. Samples were either preserved in the field or immediately on return to the laboratory. In the laboratory a sub-sample from each sample was digested and three separate slides were prepared according to the standard protocol. At least 300 valves were counted for each of the nine slides examined by a single analyst.

A nested analysis of variance model was used to evaluate variability associated with:
a) slide making;
b) spatial variability at a site.

The Level 1 model (i.e. that for the slides) was:

$$y_i = \beta_0 + r_i$$

where:
y_i = the value of a variable for slide i;
β_0 = the mean value for a sample; and,
r_i = the individual error associated with slide i.

Rather than being fixed, the β-term is treated as a random variable that takes different values for the different samples (a set-up that is different from the usual ANOVA model). The β-term is defined by a second linear equation which expresses the variation due to sampling at different sites within the same river (the Level 2 model):

$$\beta_0 = \gamma_{00} + \gamma_{01}(\bar{x}_j) + u_k$$

where:
γ_{00} and γ_{01} = constants;
$\bar{x}_j$ = mean for site j; and,
u_k = error for sample k.

A combined model was fitted using HLM6 (Raudenbush *et al.,* 2005). This allows for independent estimation of the error variance at Level 1 (the slide-making variance) and Level 2 (the sampling variance) as well as testing for dependence of the Level 1 errors on values at Level 2. Empirical Bayes estimators were used to determine confidence intervals for each sample.

These analyses are performed on both TDI values and EQRs. However, when interpreting the latter, it is important to bear in mind that the use of ratios in statistical monitoring is problematic for a number of reasons. First, there is a loss of information in that variation in the ratio may result from variation in either the numerator or denominator. Secondly, errors in the numerator and denominator are compounded resulting in loss of precision. Thirdly, ratios tend to have unusual probability distributions which complicates the estimation of standard errors, confidence intervals and other elements of inference. Finally, non-independence can be induced in error variances so that, for example, variance at one level of a nested design may become a function of variance at another level. For these reasons, results based on EQRs should be treated with caution.

6.2.2 Results

Estimates of variability in the TDI

Level 1 variance (sigma squared) = 1.797 (SE 0.519) and the Level 2 variance (tau) was 16.862 (SE = 7.131). The differences between TDI values of samples taken from the same river were relatively large in some cases (e.g. Wylye), indicating high local-scale heterogeneity, whereas the three TDI values from the River Ely showed little variability (Figure 6.1). The individual Level 1 variances fell within a narrow range between 0.78 and 5.10. The main source of error in estimation of the TDI was therefore the variation due to sampling at different places in the river.

The variance at Level 1 was not a function of sample mean or river mean. There was sufficient variation between samples taken from the same river for two rivers to be recorded as having the same or different TDI score depending on which of the three samples was taken from each. All the rivers fell within a similar range of TDI values spanning a mean of 68.7–85.9.

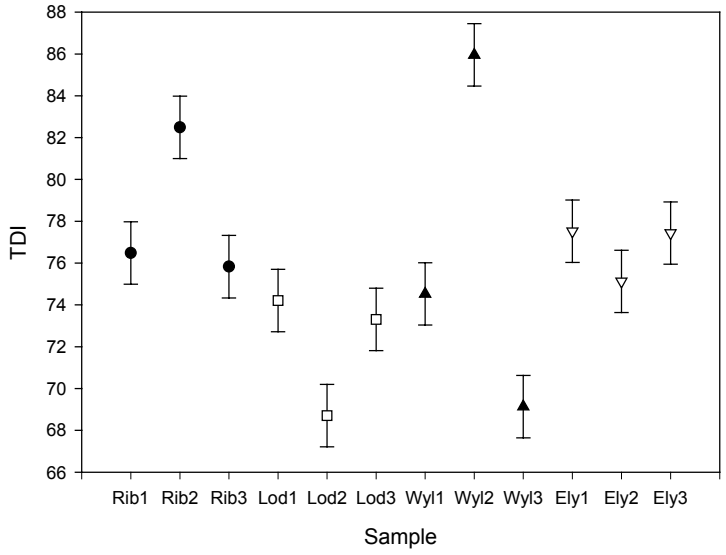


Figure 6.1. Sample TDI values (n = 3) for each river (n = 4) with 95% confidence limits. Rib, River Ribble; Lod, River Loddon; Wyl, River Wylye; Ely, River Ely.

Estimates of variability in the LTDI

Level 1 variance (slides) was 2.708 (SE 0.782) and Level 2 variance (samples) was 3.664 (SE 1.882). Slide variance was not a function of the sample mean or the lake mean. There was a greater range of TDI values in the lakes compared with the rivers varying from a TDI of 26.2 from one sample in Crummock Water to 79.5 in one sample from Blagdon Lake. Intra-lake sample differences were lower than those for rivers and sample estimates were, with one exception, consistent within a lake (Figure 6.2). Slide variance ranged between 0.05 and 7.05, of a similar order to that in rivers and considerably lower than that for the samples where variance ranged from 0.002 (Betton Pool) to 12.77 (Bassenthwaite Lake). Significant differences between the TDI values for all lakes were observed.

Estimates of variability in the EQR (rivers)

The variance at Level 1 was not a function of sample mean or river mean. Level 1 variance (sigma squared) = 0.00044 (SE = 0.00013) and the Level 2 variance (tau) was 0.00413 (SE = 0.00175). The range of EQR values within the rivers was relatively small spanning from 0.197 to 0.461. The main source of error in estimation of the EQR was therefore the variation due to sampling at different places in the river. In one river, there was a significant difference in the EQR of each sample. In two of the other three rivers, one sample differed significantly from the other two. The

differences between samples taken from the same river are relatively large in some cases (Figure 6.3) indicating high local-scale heterogeneity.

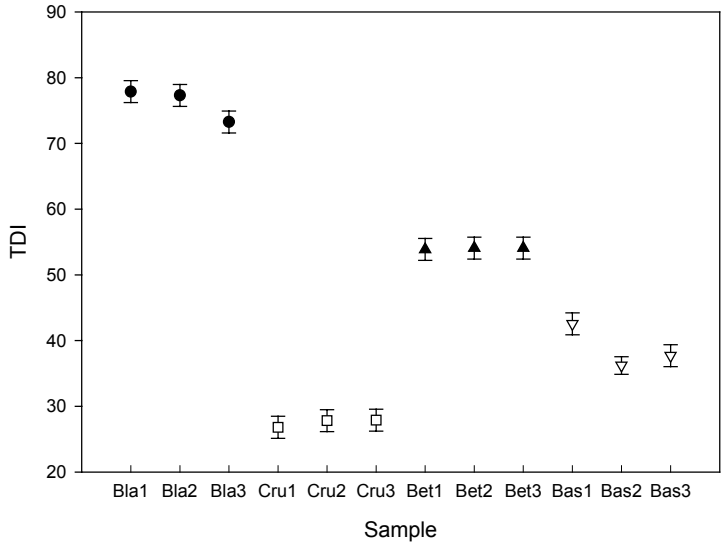


Figure 6.2. Sample TDI values (n = 3) for each lake (n = 4) with 95% confidence limits. Bla, Blagdon Lake; Cru, Crummock Water; Bet, Betton Pool; Bas, Bassenthwaite Lake.

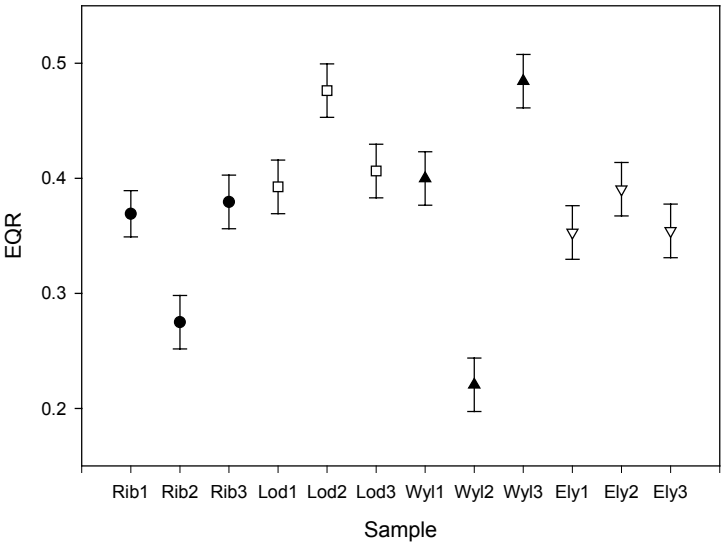


Figure 6.3. Sample EQR values (n = 3) for each river (n = 4) with 95% confidence limits. See Figure 6.1 for explanation of abbreviations.

Estimates of variability in the EQR (lakes)

Level 1 variance (slides) was 0.00048 (SE = 0.00014) and Level 2 variance (samples) was 0.00065 (SE = 0.00033). Slide variance was not a function of the sample mean or the lake mean. There was a greater range of EQR values in the four lakes sampled, compared with the rivers, ranging from 0.273 in one sample from Blagdon Lake to 0.954 in one of the samples from Crummock Water. All lakes are, however, significantly different from each other and this would not depend on which of the three samples had been chosen (Figure 6.4).

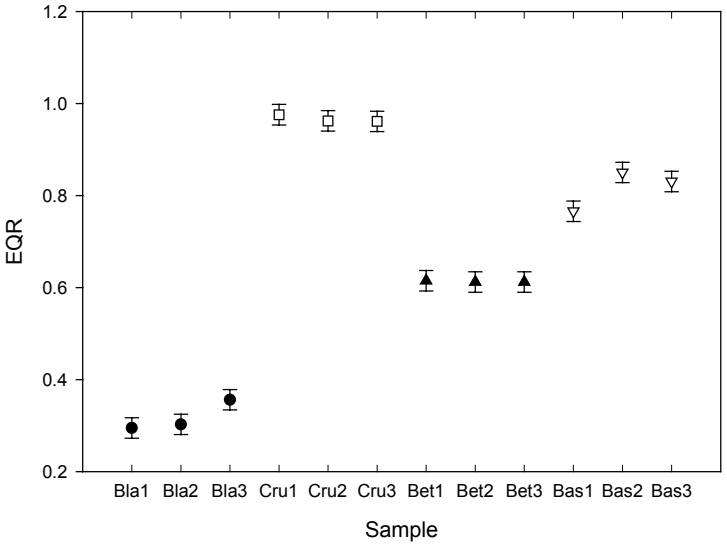
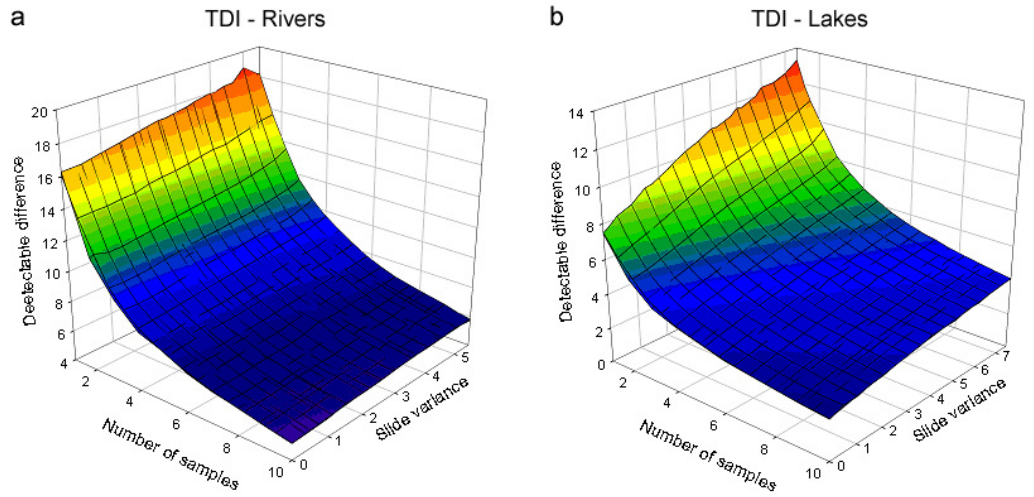


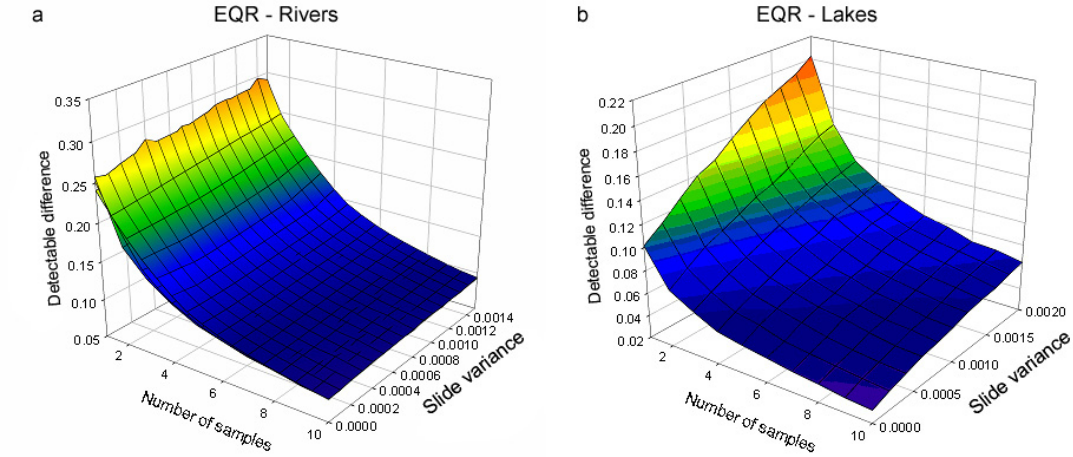
Figure 6.4. Sample EQR values (n = 3) for each lake (n = 4) with 95% confidence limits. See Figure 6.2 for explanation of abbreviations.

Simulations of repeated sampling

Using the additive model (i.e. modelling total variance as a linear sum of variance at Level 1 and Level 2), estimates of variance obtained from preliminary data and an assumption of Gaussian distributions for the variance parameters, a program was written to simulate repeated sampling from water bodies with control over the number of samples taken and the variance expected at this and the slide-making stage. This program enabled the simulation of large (n = 10,000) numbers of replicates from a given water body and the production of slides from these. This allowed estimates of the population level distribution of scores expected in sampling from water bodies for each combination of sample number and slide variance. By estimating the 97.5% quantile of the resulting distribution and multiplying this by two, it was possible to estimate the minimum difference between two water bodies that could be detected using a given diatom-related measure at $\alpha = 0.05$ and with a two-tailed alternative hypothesis (Figures 6.5 and 6.6). These figures emphasise the importance of sample size in rivers and lakes that have large-scale local heterogeneity.



(1)



(2)

Figure 6.5. Simulations of minimum detectable difference in (1) TDI and (2) EQR depending on the number of samples collected and the variance estimates at each level for (a) rivers and (b) lakes.

6.3 Confidence of class and risk of misclassification

6.3.1 Introduction

The analyses above provide an indication of the scale of variability associated with diatom sampling, and indicate that 'spatial variability' (in which variation in the naturally heterogeneous biofilm and variation due to sampling are combined) can be a significant source of uncertainty when using diatoms to assess EQR. However, spatial variability needs to be considered within a framework of temporal changes at a site: both within a year and between years. Results in chapter 4 indicate that seasonality accounts for only a small part of the variation in the model; however,

other factors can influence composition and, therefore, the value of metrics. Long periods of low flow in the summer, for example, are likely to lead to quite different assemblages from those found in summers when the weather is less settled.

The details of how ecological status classifications are to be used are still being finalised, but a likely scenario is that the classification will relate to the mean status within a three-year monitoring period. Within this period there will be fluctuations in observed status due in part to natural changes and in part to short-term alterations in water quality. In many cases it will be difficult to distinguish between these but if the scale of variation likely to be encountered at a site over the time period used to classify a water body is known, then it should be possible for the uncertainty associated with an estimate of ecological status to be calculated. This, in turn, allows the 'risk of misclassification' to be known as well as to indicate the extent to which further sampling may improve the confidence of predictions.

The ideal means of assessing such variation would be a nested design with levels accounting for all the factors that contribute to this. However, the complexity of the design would mean that results would not be available until the entire three-year monitoring period had elapsed. A simpler approach has been adopted here, making use of data that are already available from rivers monitored as part of the Urban Waste Water Treatment Directive and Habitats Directive programmes. In many cases, samples have been collected over a period of three years or longer and, as there is no reason to assume a systematic bias in how samples were collected over that period, these data can be assumed to encompass all sources of variability operating at a site, albeit in a way that cannot be decomposed to show the effect of individual components.

6.3.2 Methods

Separate analyses were performed to evaluate uncertainty associated with spatial and temporal variability.

For the study of temporal uncertainty in rivers the DARES database was queried to extract all data for sites with at least six samples. This yielded a large number of sites, although a number were subsequently removed either because samples were collected over a relatively short period of time (i.e. < 1 year) or because there were known changes within the catchment during the period of data collection (typically due to installation of nutrient stripping at sewage works upstream of the site). However, over 100 sites remained in the dataset used for further analyses, mostly representing one or two samples per year over a period of at least three years (some sites had three samples per year but none had more). In order to encompass as much of the variation observed at sites as possible, sites where samples had been collected over periods > 3 years were not excluded from the analyses. All sites were assigned to their appropriate DARES type and EQR values were calculated. These values were then used to calculate mean and standard deviations of EQR values for each site.

For lakes, the dataset collected in 2004–2006 as part of the DALES project was used. Only ten lakes had at least six samples collected over the three-year period. In all cases, we assumed that there were no significant changes due to anthropogenic causes during the sampling period.

The studies of spatial variability followed the same sampling pattern as section 6.2, with three replicate samples collected from each site (although the second level –

analysis of within-sample variability) was dropped. In total, 15 lakes and 22 river sites were sampled.

The method for estimating confidence of class and risk of misclassification is described in detail for temporal uncertainty in rivers, but the same principles apply to all the analyses described in this chapter. More details are given in Ellis (2006).

Standard deviations of EQR values for each site were plotted as a function of mean EQR and a polynomial function was then fitted to the data, constrained by two 'anchor points' at EQR = 0 and EQR = 1. Ideally, these should be based on replicate datasets with EQR values close to 0 and 1 but, as these were not available, plausible estimates were made by visual examination of the data. The polynomial function makes it possible to compute an expected standard deviation for any given EQR value and by relating this expected standard deviation to the position of the status class boundaries enables the confidence of class to be calculated. The principle is illustrated by Figure 6.6. The risk of face-value misclassification is then computed as the sum of probabilities of membership of all classes except for the observed class. In practice, there will always be a 50% chance of misclassification when the observed EQR is on the border of two status classes but the risk of misclassification will fall towards the middle of the class. Because the confidence of class predictions are dependent upon the standard deviation, which itself depends upon sample size, the risk of misclassification based upon various sampling scenarios can be modelled.

6.3.3 Results

Temporal variability in rivers

Within-site standard deviation for all sites with ≥ 6 samples shows a curvilinear relationship to mean EQR (approximating to a third-order polynomial), although there is a considerable amount of variation for any given EQR value (Figure 6.7). Few sites, however, have standard deviations < 0.05, and these occur only at the extremes (EQR < 0.5 and > 0.95). The maximum variability is encountered at the centre of the gradient (approximately at the position of the good/moderate status boundaries) and here the standard deviation occasionally exceeds 0.2.

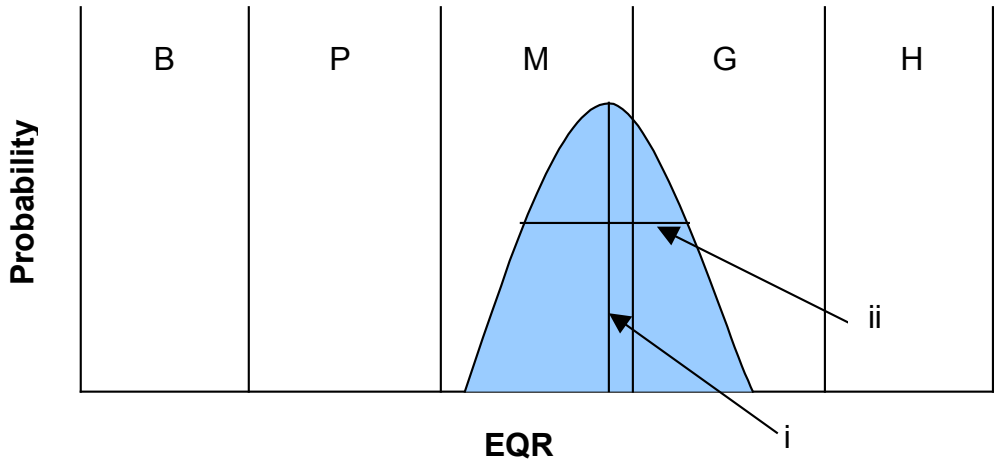


Figure 6.6. Schematic diagram showing the basis for calculation of confidence of class and risk of misclassification. i = observed EQR value; shaded blue area is a probability distribution associated with this EQR value, based on the predicted standard deviation (ii). Vertical lines show boundaries between B (bad), P (poor), M (moderate), G (good) and H (high) status classes. The normal distribution, in this case, straddles moderate and good status classes and, while the observed value suggests moderate status, there is also a possibility that the true condition of the site is 'good status'.

When this information is integrated with the status class boundaries, the confidence with which a sample can be assigned to each class can be represented as a bell-shaped curve with the maximum at the centre of the relevant status class (Figure 6.8). The tails of these curves overlap, so that an observed EQR has the potential for belonging to up to four status classes. If this EQR was 0.6, for example, the probability of the long-term condition of the water body being 'moderate status' is about 70% (based on a single sample per classification period), and there is an approximately 20% likelihood that the true condition is 'poor' and a 10% likelihood that the true condition is 'good'.

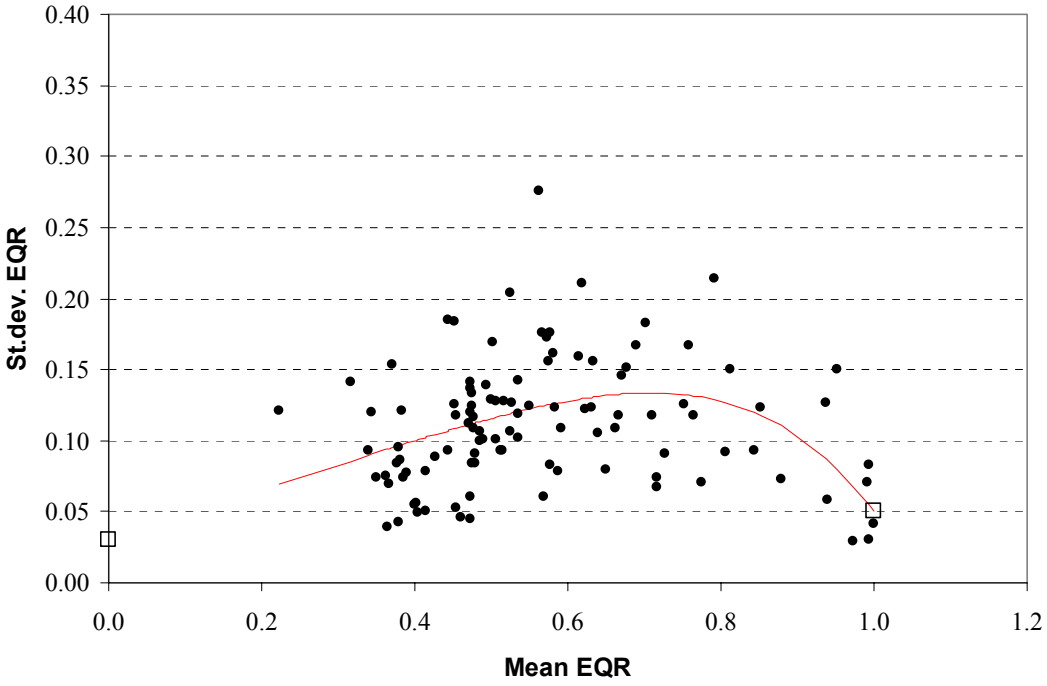


Figure 6.7. Within-site variability for UK rivers with ≥ 6 samples in the DARES database. The red line shows a line fitted to a polynomial function, anchored at EQR = 0 (std dev = 0.03) and EQR = 1 (std dev = 0.05). RMSE = 0.171; R^2 = 0.188.

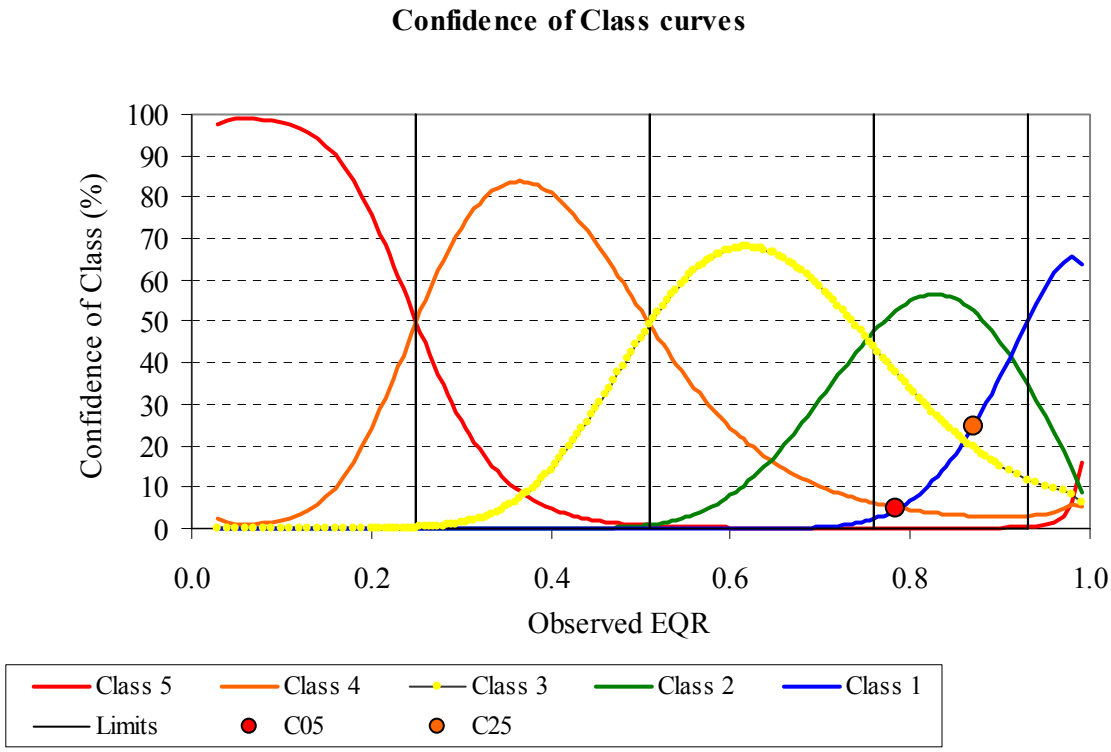


Figure 6.8. Confidence of a correct ecological status class prediction for UK rivers, based on a single sample in a classification period.

The situation changes if there are six samples within a classification period (corresponding to the likely sampling effort of two samples per year for three years), with the maximum for each curve now > 90% at the centre of the relevant status class (Figure 6.9). A site with a mean EQR of 0.6 now has an approximately 90% probability of being 'moderate status' and the probability of the long-term condition being poor is about 10%. The chances of the true condition being 'good' are negligible.

If the 'true' status is 'good status', then the combined risk of placing a site in a status class other than 'good' can be calculated to give an estimate of the risk of face-value misclassification (i.e. of placing a site in any status class other than the correct one). This risk decreases with distance from class boundaries, with the lowest risk of misclassification occurring at the centre of a status class. As for confidence of class, the risk of misclassification varies with the number of replicates (Figure 6.10) but the maximum risk of misclassification is always 50% – the value obtained at the class boundaries themselves, where there is an equal likelihood that a sample will belong to the lower, rather than to the higher, status class. Increasing the number of replicates influences the minimum risk of classification, but this is also affected by the width of an ecological status class, with narrower classes leading to a greater risk of misclassification.

If a site only achieves moderate status or lower, a Programme of Measures is required and the financial implications of this means that there is a particular interest in the ability of a classification tool to predict whether a site is 'moderate status or lower' rather than 'good status or better'. This is shown in Figure 6.11 and indicates that, if a site is classified using six samples within a classification period, then even though the good/moderate boundary was placed at EQR = 0.78, it is only sites with EQR < 0.671 that can be placed in 'moderate status or poorer' with 95% confidence. Conversely, only sites with EQR > 0.88 can be placed in 'good status or better' with a similar level of confidence.

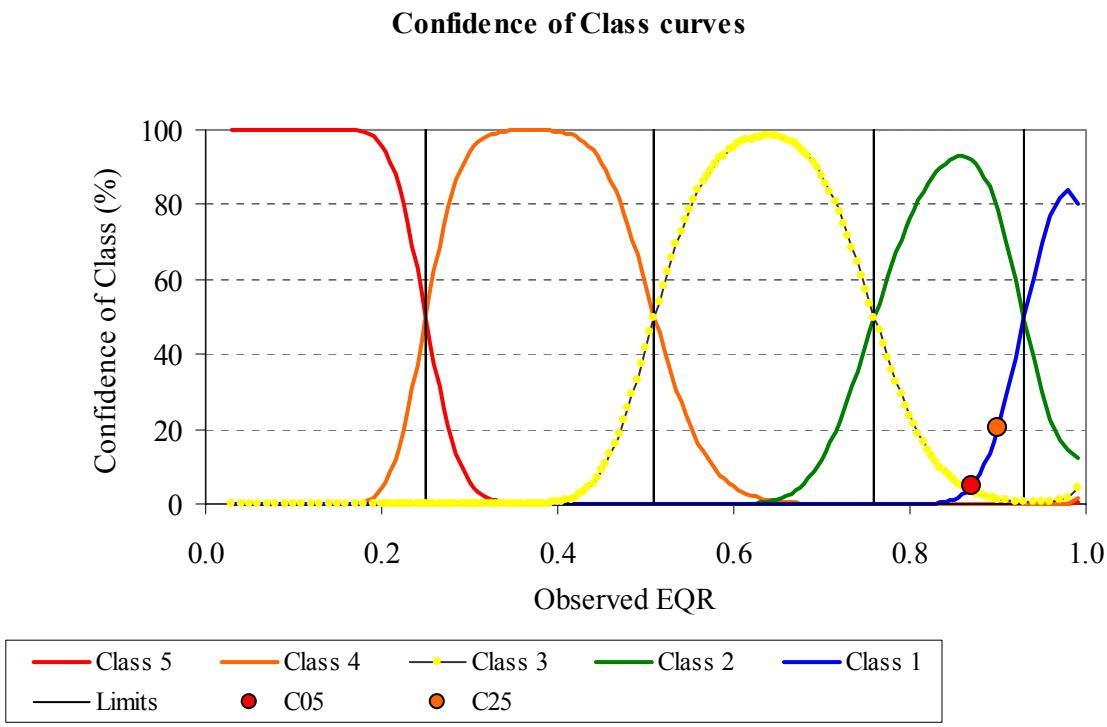


Figure 6.9. Confidence of a correct ecological status class prediction for UK rivers, based on six samples in a classification period.

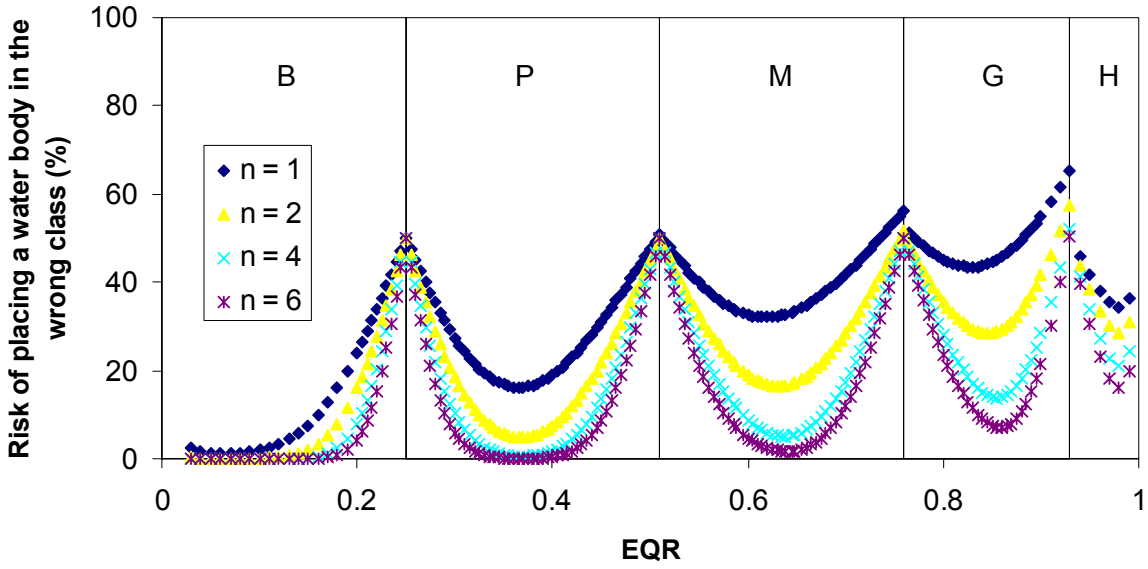


Figure 6.10. Risk of face-value misclassification for UK rivers, based on different numbers of replicates in a classification period.

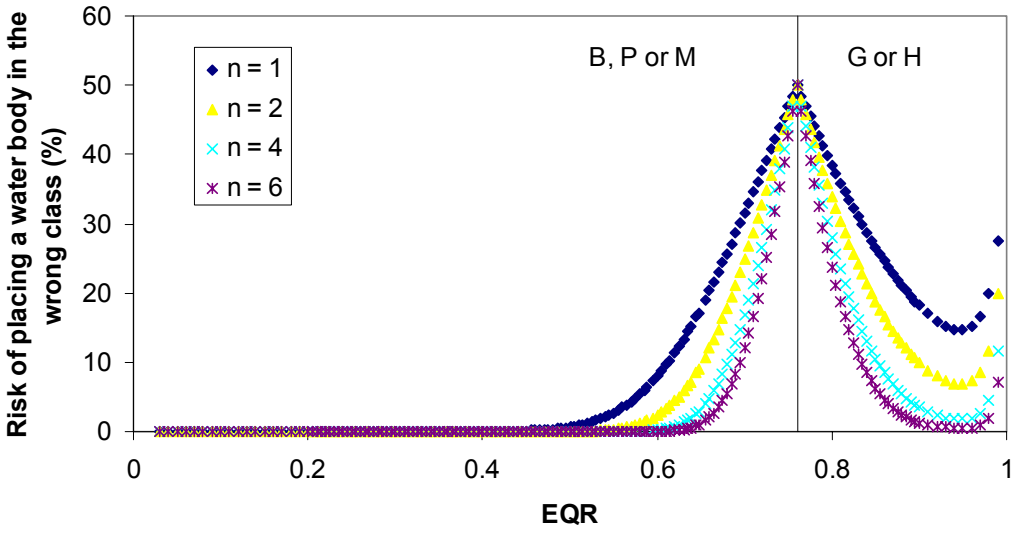


Figure 6.11. Risk of face-value misclassification for UK rivers, focusing only on the risk of misclassifying a site as 'moderate status or lower' versus 'good status or better'.

Spatial variability in rivers

The same approach was adopted to assess spatial variability in rivers. Figure 6.12 shows within-site variability, with one outlier (SD = 0.178) removed, and Figures 6.13 and 6.14 show the risk of face-value misclassification. Levels of uncertainty are much lower than for temporal variability, with two replicates being sufficient to attain 95% confidence mid-class in most cases.

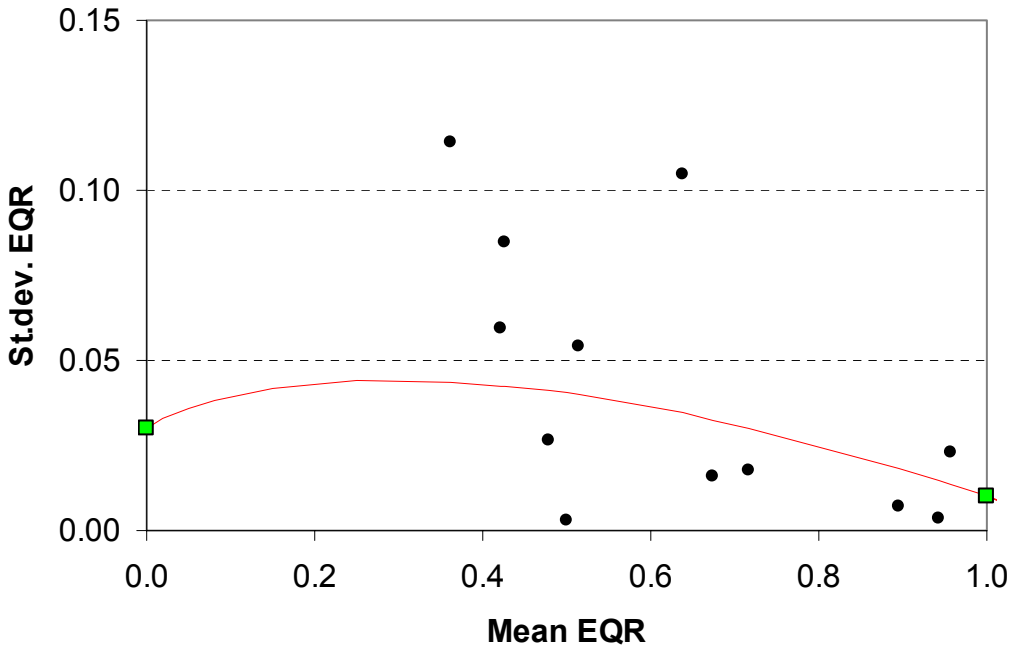


Figure 6.12. Within-site variability for UK rivers, based on three replicate samples collected on the same day. The red line shows a line fitted to a polynomial function, anchored at EQR = 0 (std dev = 0.03) and EQR = 1 (std dev = 0.01). RMSE = 0.025; R^2 = ¯0.095.

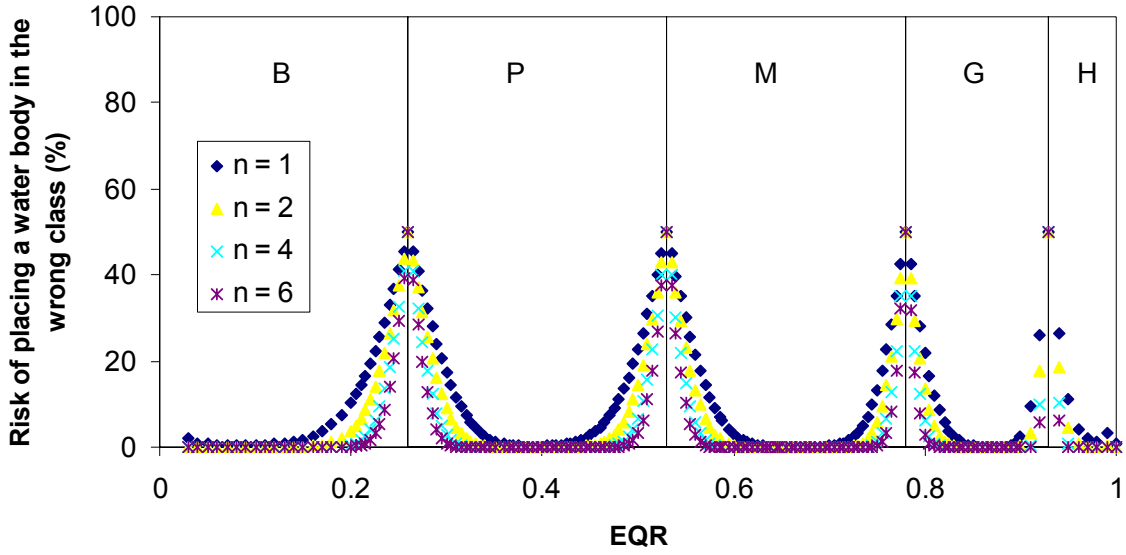


Figure 6.13. Risk of face-value misclassification for UK rivers, based on different numbers of spatial replicates collected on the same day.

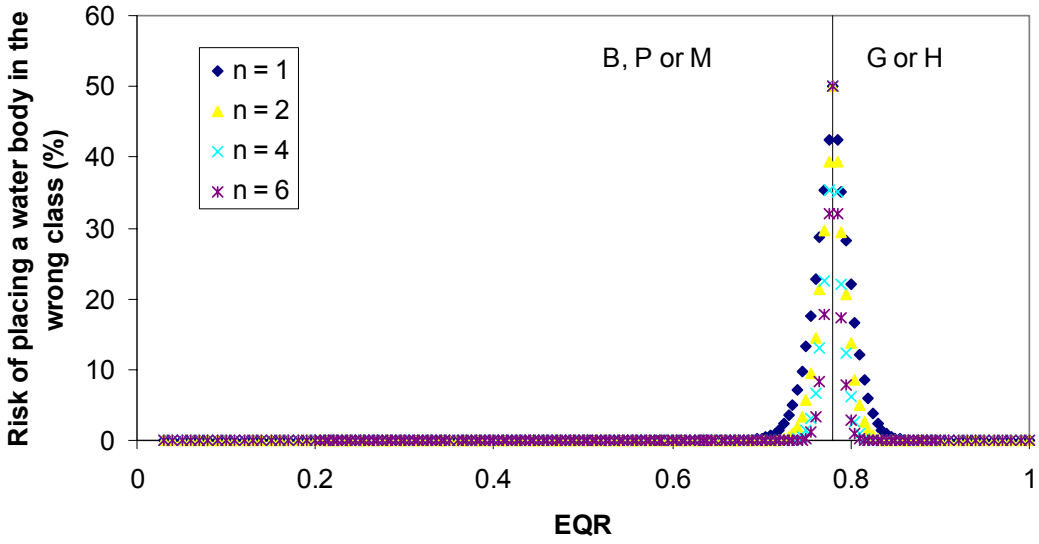


Figure 6.14. Risk of face-value misclassification for UK rivers, based on different numbers of spatial replicates collected on the same day and focusing only on the risk of misclassifying a site as 'moderate status or lower' versus 'good status or better'.

Temporal variability in lakes

Figures 6.15 to 6.17 show a similar sequence of graphs based on lake sites for which at least six temporal replicates were available. The scale of variability is very similar to that observed in Figures 6.10 and 6.11 for rivers, suggesting that at least six replicates are required within a three-year classification period to ensure 95% confidence of class at the middle of status classes.

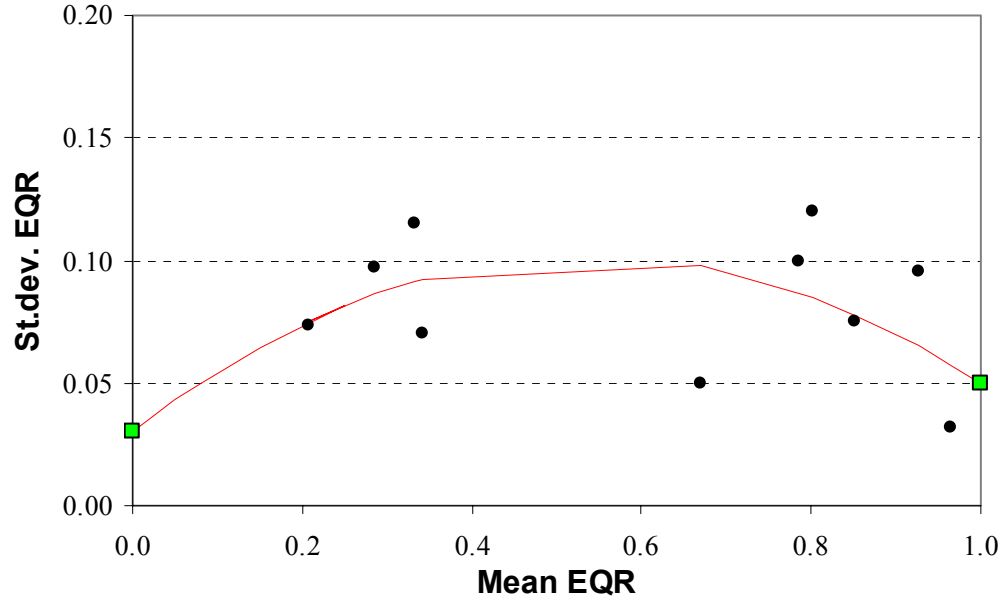


Figure 6.15. Within-site variability for UK lakes with ≥ 6 samples in the DALES database. The red line shows a line fitted to a polynomial function, anchored at EQR = 0 (std dev = 0.03) and EQR = 1 (std dev = 0.05). RMSE = 0.006; R^2 = - 0.088.

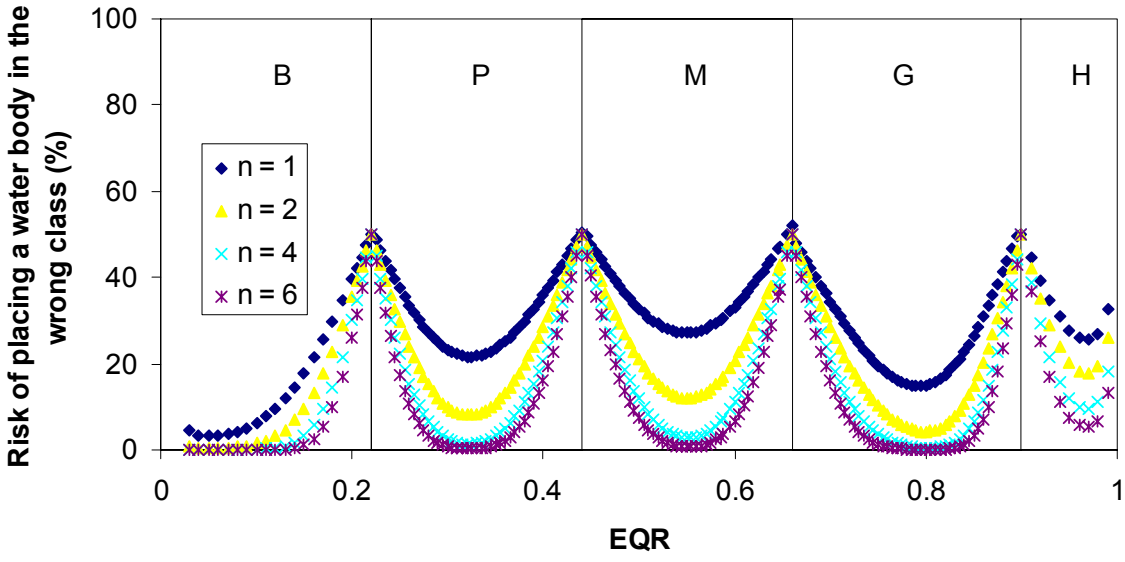


Figure 6.16. Risk of face-value misclassification for UK lakes, based on different numbers of replicates in a classification period.

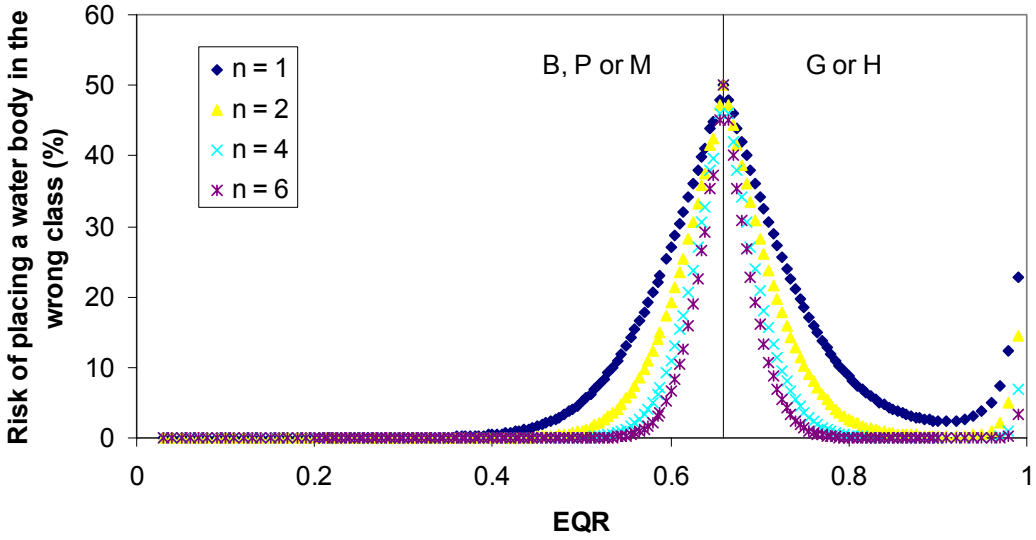


Figure 6.17. Risk of face-value misclassification for UK lakes, focusing only on the risk of misclassifying a site as 'moderate status or lower' versus 'good status or better'.

Spatial variability in lakes

Finally, Figures 6.18 to 6.20 show the spatial variability encountered in lakes and illustrate that this is of a similar magnitude to that observed in rivers. As for rivers, the spatial variability in lakes was considerably lower than the temporal variability.

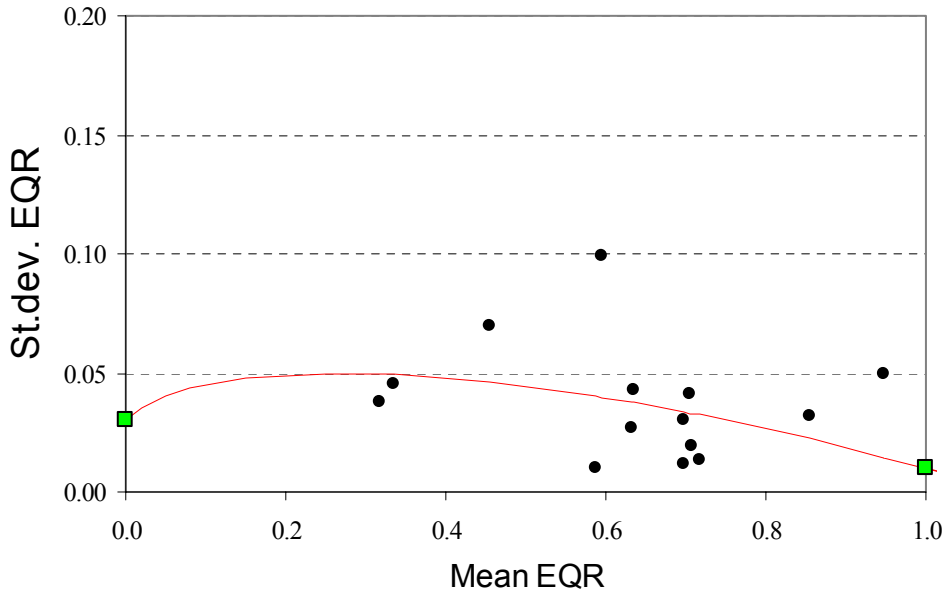


Figure 6.18. Within-site variability for UK lakes, based on three replicate samples collected on the same day. The red line shows a line fitted to a polynomial function, anchored at EQR = 0 (std dev = 0.03) and EQR = 1 (std dev = 0.05).

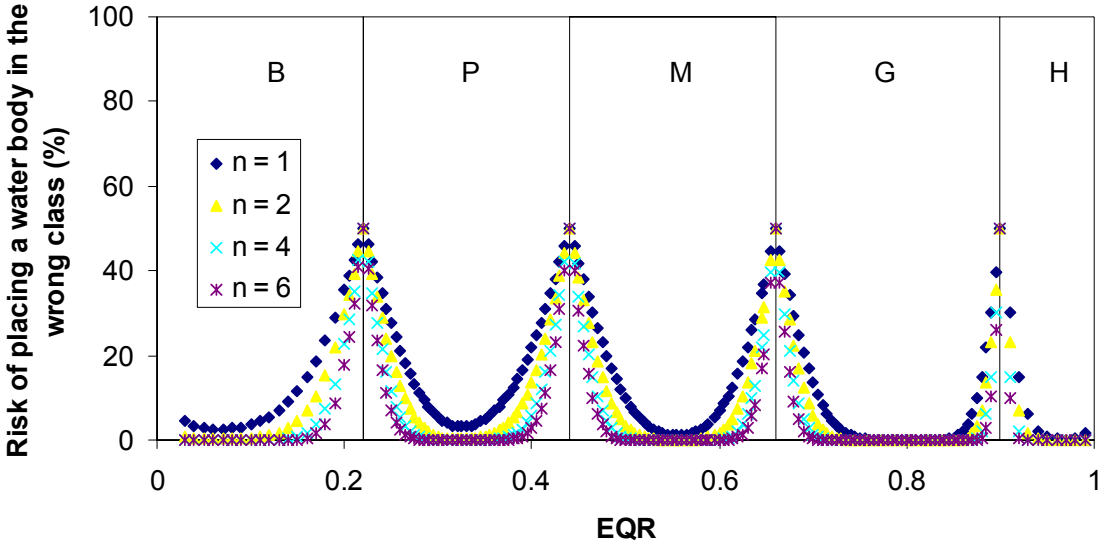


Figure 6.19. Risk of face-value misclassification for UK lakes, based on different numbers of spatial replicates collected on the same day.

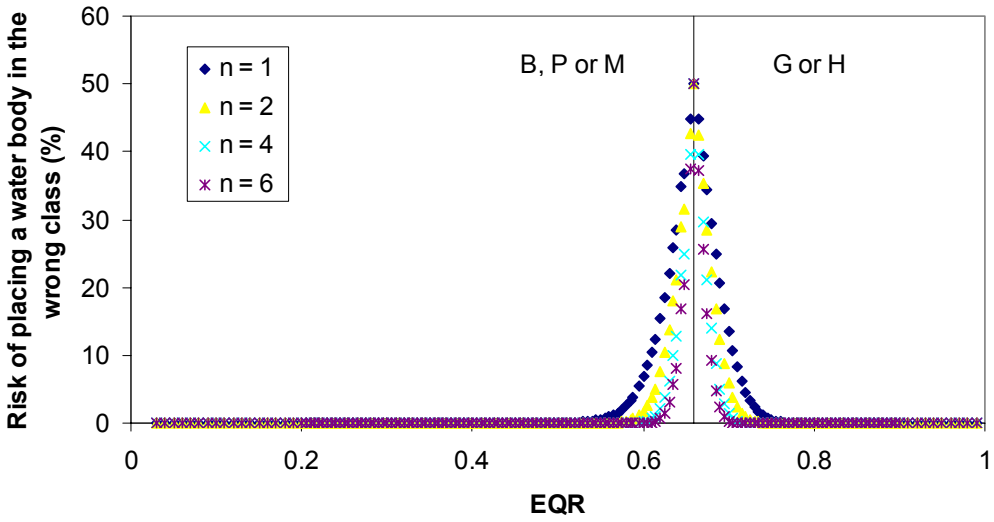


Figure 6.20. Risk of face-value misclassification for UK lakes, based on different numbers of spatial replicates collected on the same day and focusing only on the risk of misclassifying a site as 'moderate status or lower' versus 'good status or better'.

Comparison

Figure 6.21 shows the relationship between risk of misclassification and sampling intensity for a sample with an EQR located in the middle of 'moderate status' for each of the scenarios modelled above (temporal versus spatial variability, lakes versus rivers).

Temporal variation is approximately one order of magnitude greater in both lakes and rivers than spatial variation. The nature of the study design is such that these temporal variation estimates probably represent 'global uncertainty', incorporating all

aspects of sampling uncertainty. The results suggest that reliable indications of status class in both rivers and lakes will need to be based on repeated sampling from the same location. Risk of misclassification due to spatial sampling alone is much lower partly because it fails to capture all the 'uncertainty' present at a site.

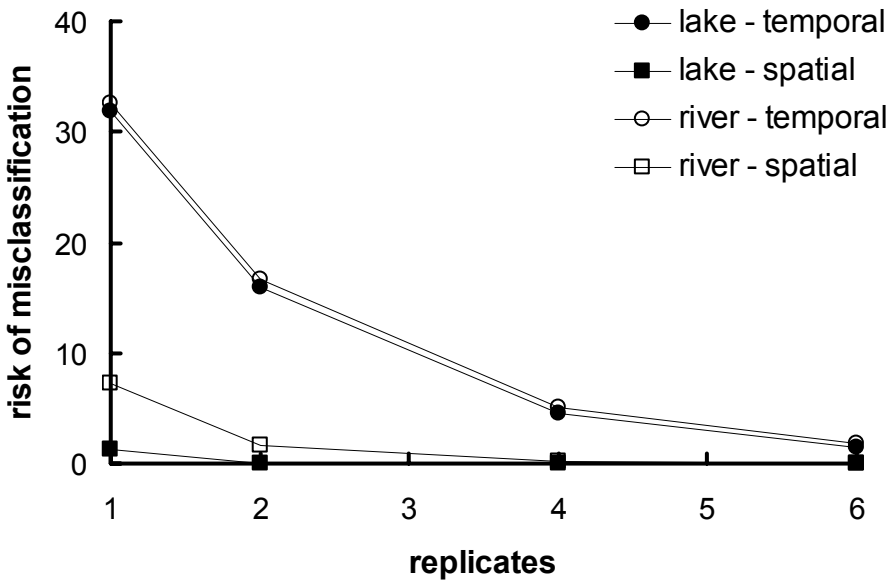


Figure 6.21. Risk of misclassifying a site with a hypothetical EQR at the middle of moderate status (0.635 for rivers; 0.55 for High Alkalinity and Medium Alkalinity lakes): comparison of spatial and temporal uncertainty in lakes and rivers.

6.4 Conclusions

i. Errors associated with making slides are relatively small and differences between lakes and rivers are minor (see section 6.2.2 and Lavoie *et al*., 2005). If analysts adhere to protocols, one slide per sample is sufficient to estimate the taxonomic composition and derived indices from a sample.

ii. The variance between replicate samples taken at one time from one location in rivers was relatively large. It is possible that this variability is partly due to operators, but it is more likely that a large part represents natural spatial heterogeneity. Sample variance within rivers was itself variable; that is, some rivers show more spatial variability than others and preliminary observations (unpublished) suggest that there may be a relationship between variability and ecological status. Although localised spatial heterogeneity has been observed elsewhere (Cazaubon *et al*., 1995; Passy, 2001), the reason why such heterogeneity might vary with ecological status is not clear and further work on this topic is needed.

iii. The model is critically dependent on the form and accuracy of the error distributions. Sampling from a larger pool of samples, covering as wide a range of conditions as we might expect to encounter during sampling, would enable us to examine how these error terms behave. This would enable us to analyse the sensitivity of the results (in particular the

probabilities of misclassification) to variation in the model parameters. An assessment of how variation in the values and distributions of the error terms may influence the results would be useful.

iv. Simulations (section 6.2.2) highlight that our ability to distinguish between the rivers on the basis of their ecological status increases with increasing sample size. The effect of pooling more stones from sites that exhibit higher spatial variability could be examined. However, results in section 6.3 suggest that the benefits of increasing effort for particular samples has to be offset against the amount of variation observed between samples from the same site over a period of time.

v. The variance between replicate samples taken at one time from one location in lakes was smaller than in rivers. It could be concluded that lakes are more homogeneous and predictable in terms of their diatom communities.

vi. Temporal variation is approximately one order of magnitude greater in both lakes and rivers than spatial variation. The results suggest that reliable indications of status class in both rivers and lakes will need to be based on repeated sampling from the same location. Results in section 6.3 suggest that at least six replicates (i.e. two per year for three years or three per year for two years) will be required in order to provide a firm basis for regulation. A sampling intensity greater than this might be at risk of 'pseudo-replication'.

vii. The risk of misclassification depends on the proximity of the mean EQR for a site to the status class boundary. When the EQR value is very close to the boundary, the risk of misclassification will be approximately 50%, regardless of the number of samples available.

7 Reconciling spatial and temporal changes in rivers

7.1 Background and rationale

A prerequisite for distinguishing 'high' ecological water quality status is to be able to define reference conditions (Bailey *et al*., 1998; Allan, 2004), which is the condition of an ecosystem expected under circumstances of no more than 'very minor' human-made alterations to physicochemical, hydromorphological and biological quality elements (Pollard and Huxham, 1998). However, there are few, if any, true reference sites available in Europe for river or lake ecosystems, and it has been recommended to best approach the determination of reference states through a combination of available data, palaeolimnological approaches, hindcasting and expert judgement (Moss *et al.*, 2003). Using palaeolimnological methods, it has been possible in lakes to infer the baseline state of environmental variables such as pH (Flower *et al.*, 1997) and nutrients (Wessels *et al*., 1999; Bennion *et al.*, 2004) using diatoms.

In UK rivers, however, it has been necessary to adopt a 'spatial state' scheme in which the biota at sites without known anthropogenic impacts are considered to be the reference against which other sites are compared, based on a 'space for time' substitution (Pickett, 1988). This approach is clearly limited by the availability of unimpacted sites that are truly comparable to impacted sites, but is the only option where historical diatom data from rivers is not available. In a few studies, it has been possible to examine the historical state of rivers either from old records and samples (Taylor *et al.*, 2005) or from diatoms preserved with herbarium specimens of macrophytes (van Dam and Mertens, 1993; Denys, 2000, 2003; Cocquyt and de Wever, 2002).

We assessed the feasibility of the use of herbarium specimens for UK rivers by comparing diatom community composition between matched recent and historical diatom samples. One criticism that can be levelled at such an approach is that comparisons are being made between diatom floras associated with different substrata. We investigated the feasibility of such an approach by comparing variability between stone (epilithic) and macrophyte (epiphytic) samples to account for differences associated with substratum specificity (Rothfritz *et al.*, 1997; Potapova and Charles, 2005). Occasionally, when field sampling, it is impossible to find suitable stone substrata (chapter 3). This study also addressed questions relating to use of suitable alternative substrata for sampling periphytic diatoms.

7.2 Methods and statistical approach

7.2.1 Substratum specificity

In order to assess the extent to which habitat specificity influenced the use of macrophyte specimens from herbaria, matched contemporary diatom samples were collected in the autumn of 2004 and 2005 from stone and macrophyte (*Ranunculus* spp.) substrata at 16 sites in the northeast and southwest of the UK (Table 7.1). In

the field, diatom samples were collected as a pooled sample from five cobbles/macrophyte fronds, following standard methods (chapter 3).

7.2.2 Herbarium collections

Herbarium samples were obtained from a variety of aquatic plant species and samples prepared as above. Samples were selected for which contemporary matches could be made for the same river (Table 7.2). The age of the herbarium specimens were all pre-1930s, prior to a period of increased intensification of agricultural practices.

7.2.3 Data analysis

In order to assess differences in community patterns between substrata we examined differences in species richness, diversity, TDI and percentage of motile valves (chapter 4) as well as differences in the percentage abundances for selected diatom species. Taxa with sensitivity values of 1 and 2 were pooled to form a category of 'nutrient sensitive' taxa, and taxa with sensitivity values of 4 and 5 were pooled to form a category of 'nutrient tolerant' taxa. Differences between these diatom community parameters were compared between the two habitat categories using ANOVA. Tests were undertaken for homogeneity of variance and normality

Table 7.1. Site details for locations (n = 16) of matched diatom samples from stone and macrophyte (*Ranunculus* spp.) substrata, sampled in the autumn of 2004 and 2005. Abbreviations: U/s = upstream; D/s = downstream.

River	Reach
Bowmont Water	Thornington
River Browney	Langley Park
River Ely	U/s St Fagans
River Glen	Bridge End
River Ithon	U/s Llandrindod Wells
River Ribble	D/s Clitheroe STW
River Tees	Blackwell Bridge
River Usk	Llantrisant
River Wear	Shincliffe
River Wear	U/s Gaunless STW
Wooler Water	D/s Wooler STW
Wooler Water	U/s River Till
Wooler Water	U/s Wooler STW
River Wye	Builth Wells
River Wye	Hafodygarreg
River Wylye	Boyton Manor

Table 7.2. Site details for locations of matched old (herbarium-derived diatoms from preserved macrophytes) and recent (diatoms from stones) for selected rivers.

River	Site	Date	Sample code
R. Avon (new)	Eckington	04.10.2001	101095
R. Avon (old1)	Tewkesbury	1875	LIVP15
R. Loddon (new1)	Twyford	30.07.1999	99354
R. Loddon (old1)	Twyford	1898	LIVP26
R. Ribble (new4)	u/s Clitheroe	01.09.1999	100196
R. Ribble (new5)	d/s Calder	09.09.1999	100200
R. Ribble (new6)	d/s Brockholes	03.09.1999	100201
R. Ribble (new7)	u/s Clitheroe	14.07.2000	101005
R. Ribble (new8)	d/s Calder	19.07.2000	101009
R. Ribble (new9)	d/s Brockholes	09.08.2000	101010
R. Ribble (old1)	Chatburn	1911	LIVP2
R. Ribble (old2)		1911	LIVP4
R. Ribble (old3)	east of Preston	1914	NMGW3
R. Ribble (old4)	nr Preston	1914	LIVP1
R. Ribble (old5)		1916	LIVP3
R. Wye (new1)	Ross-on-Wye	17.07.2002	102296
R. Wye (new2)	Ross-on-Wye	25.09.2002	102297
R. Wye (new3)	Ross-on-Wye	15.07.2003	103301
R. Wye (new4)	Ross-on-Wye	25.09.2003	103402
R. Wye (old1)	Ross-on-Wye	1852	LIVP16
R. Wye (old2)	Foy nr Ross	1883	LIVP13
R. Wye (old3)	Ashe nr Ross	1889	LIVP14
R. Wye (old4)	Carey nr Ross	1893	LIVP18
R. Wye (old5)	Sellack nr Ross	1893	LIVP19
R. Wye (old6)	Carey nr Ross	1894	LIVP21

of residuals and transformed where necessary. *A priori* tests to identify statistical differences were undertaken using Tukey's HSD test. To assess variations in floristic composition, all samples were ordinated using Detrended Correspondence Analysis (DCA, in Canoco 4.5 for Windows). To assess the degree of floristic change in both datasets the squared chord distance coefficient was determined (Overpeck *et al*., 1985). A maximum score of 2 indicates an identical match between two samples whereas a score of 0 indicates a complete dissimilarity. A score of ≤ 0.48, showing insignificant change at the 5th percentile, was used to identify matched river sites where differences in species composition were low (Bennion *et al*., 2004).

7.3 Results

7.3.1 Comparison of contemporary substratum specificity data

Macrophyte samples represented an average proportion of 76.1% of the total species pool at each site, compared to a value of 71.9% for stone samples. There were no statistically significant differences between the proportions of the total species pool represented by each substratum type. Macrophyte-derived samples had a mean TDI value of 56.7 (SD = 15.2; median = 64.6), mean species richness of 34.8 (SD = 9.2; median = 33.0) and species diversity of 1.09 (SD = 0.18; median = 1.06). Matched stone-derived samples mirrored these ranges, with a mean TDI value of 58.6 (SD = 15.9; median = 64.9), mean species richness of 32.3 (SD = 5.8; median = 31.5) and species diversity of 1.08 (SD = 0.18; median = 1.14). Correlation analysis showed that TDI values from macrophyte and stone communities were significantly correlated (Pearson's r = 0.904; $p < 0.001$) (Figure 7.1), indicating that there were no gross variations in community structure.

The most abundant and widespread diatom species in the dataset were *Achnanthidium minutissimum* (Kützing) Czarnecki, *Cocconeis pediculus* Ehrenberg, *Cocconeis placentula* Ehrenberg var. *euglypta* Ehrenberg, *Fragilaria capucina* Desmazières var. *gracilis* (Østrup) Hustedt, *Fragilaria vaucheriae* (Kützing) Petersen, *Navicula capitatoradiata* Germain and *Nitzschia fonticola* Grunow in van Heurck. The relative abundances of these species varied widely between rivers and did not show statistically significant differences between substrata (Table 7.3 and Figure 7.2). Similarly, the occurrence of nutrient sensitive and tolerant diatom taxa was not statistically significantly different (Table 7.3) and did not vary systematically between substrata (Figure 7.3).

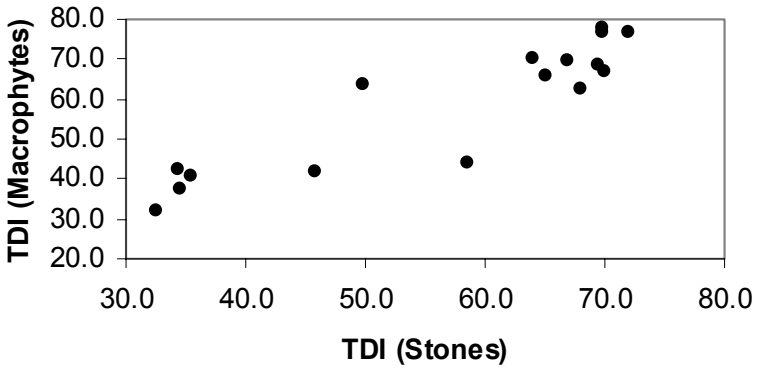


Figure 7.1. Comparison between TDI scores for stone and macrophyte substrata (n = 32).

Table 7.3. Differences in relative abundance values of the most common, nutrient sensitive (s values of 1 and 2) and nutrient tolerant (s values of 4 and 5) diatom species in the dataset from matched macrophyte and stone samples collected at 16 locations in the northeast and southwest of the UK in the autumn of 2004 and 2005 as calculated by ANOVA. Abbreviations: n.s. = not significant.

Taxon	Mean (SD)		$F_{1,31}$	p
	Macrophytes	Stones		
Achnanthidium minutissimum (AMIN)	8.2 (12.6)	14.8 (17.0)	1.29	n.s
Cocconeis pediculus (CPED)	3.0 (4.8)	4.6 (5.5)	0.49	n.s
Cocconeis placentula var. *euglypta* (CPLE)	11.9 (17.7)	10.9 (12.7)	0.11	n.s
Fragilaria capucina var. *gracilis* (FCGR)	7.2 (9.5)	3.1 (3.6)	1.31	n.s
Fragilaria vaucheriae (FVAU)	2.8 (3.5)	3.4 (6.6)	0.11	n.s
Navicula capitatoradiata (NCPR)	6.2 (11.9)	2.3 (5.0)	1.14	n.s
Nitzschia fonticola (NFON)	4.2 (6.5)	7.1 (13.7)	0.77	n.s
Nutrient sensitive species (s value 1 or 2)	24.5 (18.7)	26.9 (23.8)	0.10	n.s
Nutrient tolerant species (s value 4 or 5)	50.5 (25.1)	54.9 (26.7)	0.22	n.s

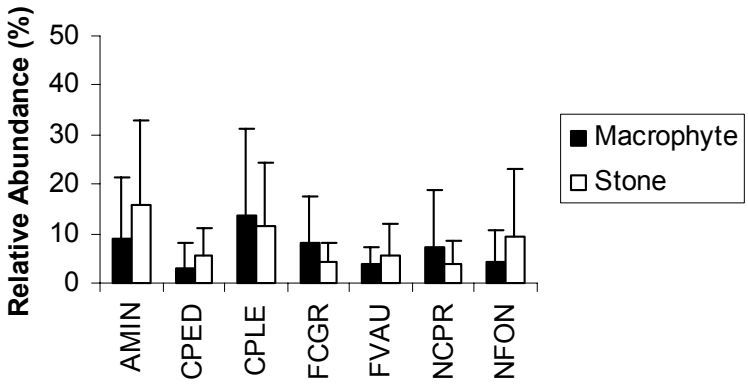


Figure 7.2. Comparison of the mean relative abundance values (including SD) of the most common species in the dataset between matched macrophyte and stone samples. Abbreviations: AMIN, *Achnanthidium minutissimum*; CPED, *Cocconeis pediculus*; CPLE, *Cocconeis placentula* var. *euglypta*; FCGR, *Fragilaria capucina* var. *gracilis*; FVAU, *Fragilaria vaucheriae*; NCPR, *Navicula capitatoradiata*; NFON, *Nitzschia fonticola*.

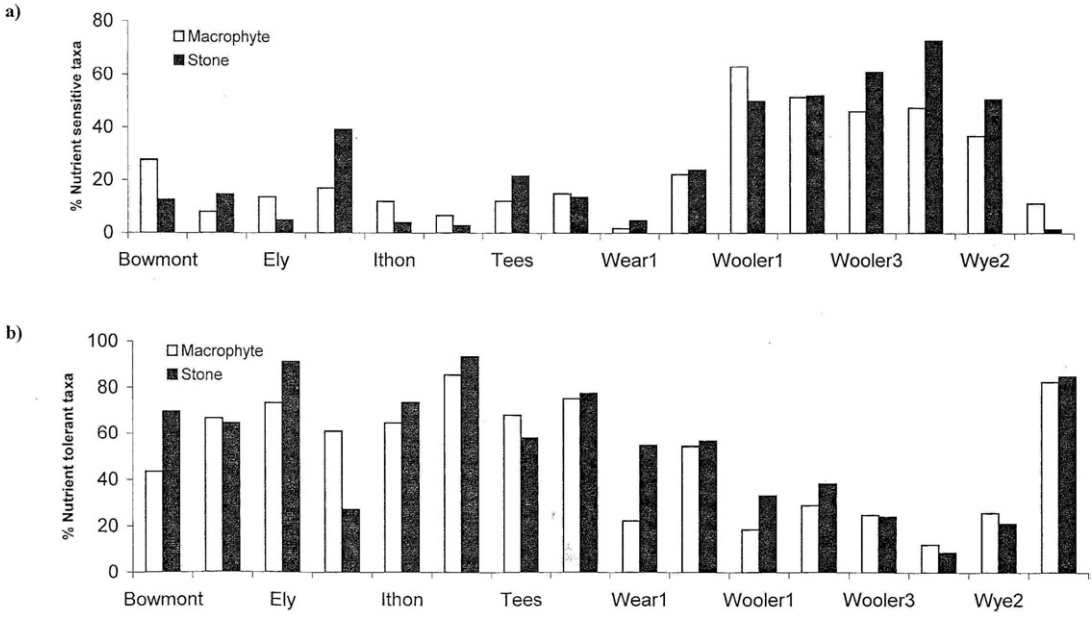


Figure 7.3. Site comparisons of mean relative abundance values of (a) nutrient sensitive and (b) nutrient tolerant diatom taxa in the dataset between matched macrophyte and stone samples. Nutrient sensitive taxa have sensitivity values of 1 and 2, whereas nutrient tolerant taxa have sensitivity values of 4 and 5.

Patterns in community composition were confirmed by DCA ordination analysis on the matched samples from 16 rivers, in which the eigenvalues of the first two DCA ordination axes (λ_1 = 0.28, λ_2 = 0.11) accounted for 26.8% of the cumulative variance in the species data. Along the first ordination axis, diatom communities were ordinated in response to nutrient status. Samples separated into two groups, which differed statistically in their sample scores along the first ordination axis ($F_{1,31}$ = 56.4; p = 0.000): group 1 (mean DCA score = 1.81; n = 13) covered a range of TDI values from 30.0 to 60.0 whereas group 2 (mean DCA score = 0.78; n = 19) covered a range from 60.1 to 80.0 (Figure 7.4). Accordingly, diatom species which occurred at the low end of the first ordination axis are ones indicative of high nutrient status, such as *Psammothidium lauenburgianum*, *Staurosirella pinnata* and *Navicula menisculus*, with sensitivity values of 4 and 5, while diatom species which occurred at the high end of the first ordination axis had generally lower sensitivity values (Figure 7.5). Some species separated strongly along the second ordination axis with *Nitzschia lacuum*, *Ctenophora pulchella* and *Fragilaria bidens*, at one end and *Pseudostaurosira brevistriata*, *Staurosira construens* var. *binodis* and *Synedra arcus* (Krammer and Lange-Bertalot, 1986–2004).

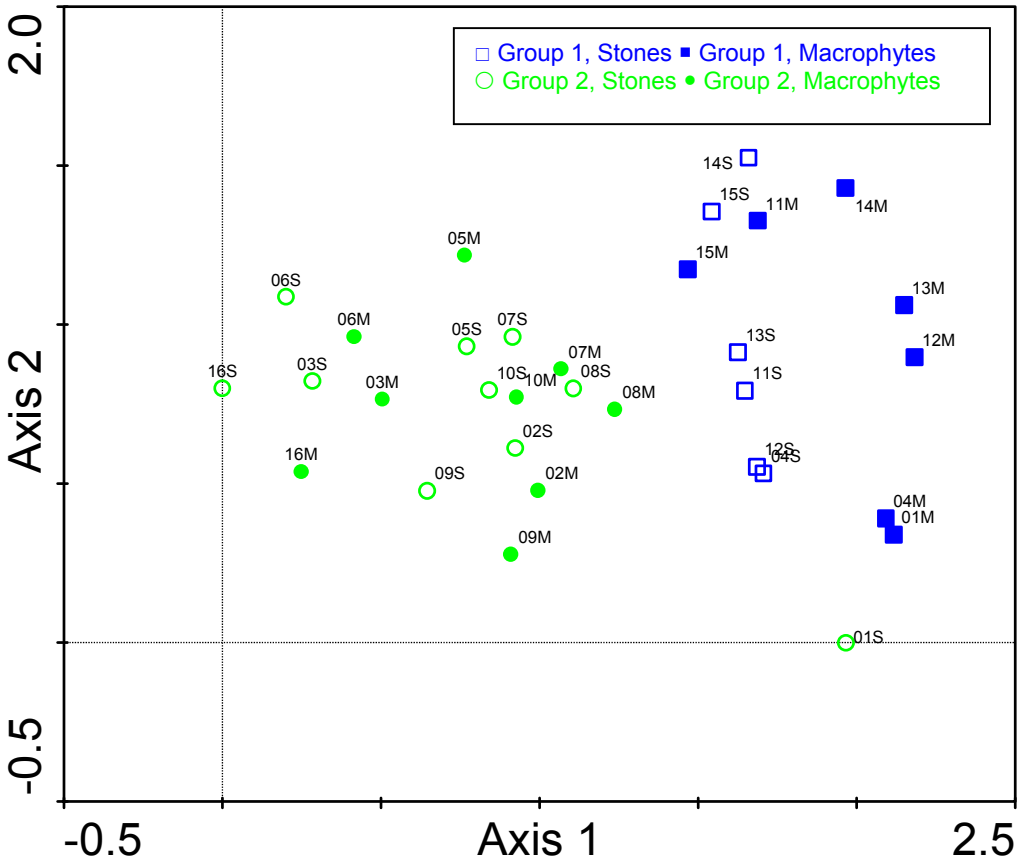


Figure 7.4. DCA ordination diagram of 32 samples from matched macrophyte and stone substrata at 16 sites in the northeast and southwest of the UK. Samples with TDI values ranging from 30.0 to 60.0 are group 1, n = 13; samples with TDI values ranging from 60.1 to 80.0 are group 2, n = 19.

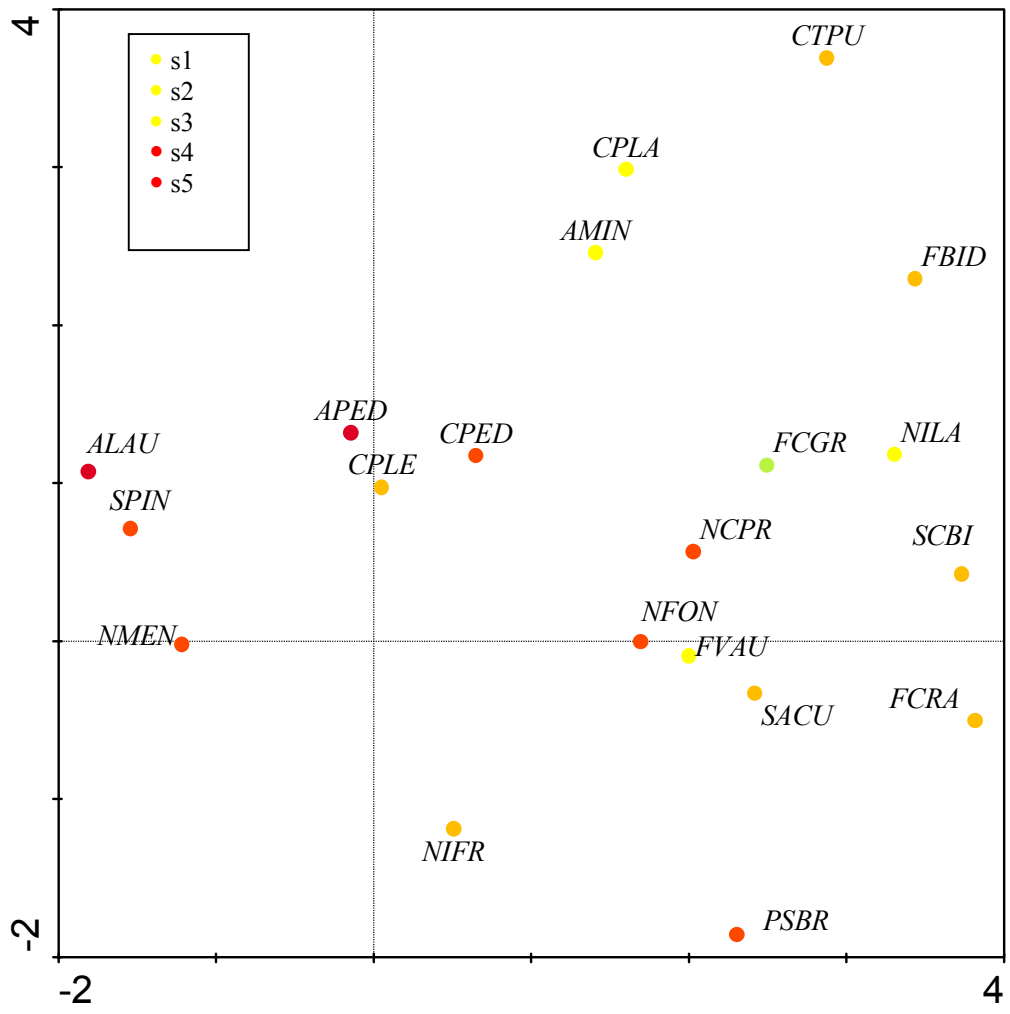


Figure 7.5. DCA ordination diagram of 19 selected diatom species from matched macrophyte and stone substrata at 16 sites in the northeast and southwest of the UK. Abbreviations: ALAU, *Psammothidium lauenburgianum*; AMIN, *Achnanthidium minutissimum*; CPED, *Cocconeis pediculus*; CPLA, *Cocconeis placentula* var. *placentula*; CPLE, *Cocconeis placentula* var. *euglypta*; CTPU, *Ctenophora pulchella*; FBID, *Fragilaria bidens*; FCGR, *Fragilaria capucina* var. *gracilis*; FCRA, *Fragilaria capucina* var. *radians*; FVAU, *Fragilaria vaucheriae*; NCPR, *Navicula capitatoradiata*; NFON, *Nitzschia fonticola*; NIFR, *Nitzschia frustulum*; NILA, *Nitzschia lacuum*; NMEN, *Navicula menisculus*; PSBR, *Pseudostaurosira brevistriata*; SACU, *Synedra arcus*; SCBI, *Staurosira construens* var. *binodis*; SPIN, *Staurosirella pinnata*.

7.3.2 Comparison of herbarium and contemporary matched samples

Having established that differences due to substrata are small relative to differences between sites, we went on to compare diatoms removed from herbarium sheets with those from contemporary epilithon samples. Structural parameters of the diatoms from herbarium and contemporary samples showed significantly different patterns. The TDI for herbarium samples had a mean of 40.1 (SD = 11.7; median 43.0), mean species richness of 18.8 (SD = 5.2; median =17.0); and species diversity of 0.75 (SD = 0.16; median = 0.7). The mean TDI for recent stone samples was significantly higher (ANOVA; $F_{1,24} = 71.29$; $p < 0.001$) at 71.3 (SD = 6.6; median = 72.9); mean species richness was significantly higher (ANOVA; $F_{1,24} = 21.40$; $p < 0.001$) at 33.0 (SD = 9.9; median = 31.5); and mean diversity significantly higher (ANOVA; $F_{1,24} = 21.40$; $p < 0.001$) at 1.14 (SD = 0.167; median =1.10). The percentage of motile cells was significantly greater in the recent samples (ANOVA, $F_{1,24}= 135.58$; $p < 0.001$) at 46.40 (SD = 17.34, median = 17.34) compared with 4.47 (SD 3.72, median = 2.83) for the historical macrophyte-derived samples.

The most common and widespread species in this dataset were *Achnanthidium minutissimum*, *Cocconeis pediculus* and *Cocconeis placentula* Ehrenberg var. *euglypta* Ehrenberg. The relative abundance of *Achnanthidium minutissimum* was significantly greater ($p < 0.001$) in the samples taken on material from herbarium samples than on the stones collected recently (Table 7.4) but there were no significant differences between the relative abundance of the other two most common species. Some species, including *Navicula subminuscula, Nitzschia palea* and *Planothidium lanceolatum* were exclusively found in the new samples but with low mean relative abundance (~ 2%). Some species occurred rarely in the herbarium samples yet were common representatives of the newer samples. In particular, the mean relative abundance of *Amphora pediculus* was 8.68% ± 5.96% in the new samples and 0.47% ± 0.26% in the historical samples. *Navicula tripunctata* had a mean relative abundance of 5.17% ± 5.94% in the new samples and was only found in one historical sample with a relative abundance of < 1.0%. *Nitzschia fonticola* was common in new samples (mean relative abundnace 11.78% ± 10.57%) and rare in old material (1.17 ± 0.40). Conversely, some species were more common on the historical samples. *Gomphonema parvulum* var. *exilissimum* was recorded in half of the historical samples with a mean relative abundance of 3.03 ± 3.90% but was not recorded on any recent samples. *Cocconeis placentula* var. *lineata* was common in old samples (mean relative abundnace 25.36% ± 19.38%) but only found in one site from the new samples (relative abundnace < 1.0%). *Encyonema minutum* was relatively more common in samples from historical sites (mean relative abundnace 5.25% ± 6.91%) and rare in new sites (mean relative abundnace < 1.0%). The mean percentage of sensitive valves was significantly greater in the historical samples (51.50 ± 26.23) compared to 5.72 ± 3.05 in the recent samples. The mean percentage of tolerant valves was significantly higher in the recent samples (73.88 ± 11.09) compared with 22.15 ± 20.12 for the recent samples (Table 7.4).

Chord distances between historical and recent samples were all > 1 (mean: 1.436) suggesting a significant change in assemblage (Table 7.5). By comparison, the mean chord distance for the stone–macrophyte pairs was 0.453 and none of the pairs had a chord distance > 1.

A detrended correspondence analysis was carried out using the entire dataset of contemporary stone–macrophyte matched pairs and all historical and modern diatom samples for 16 rivers (Figure 7.6). There was a clear separation of the historical samples (situated to the right of the ordination on axis 1) and their modern counterparts (situated to the left of the ordination) with the matched pairs of stone–

macrophyte samples overlapping to some extent with the recent stone samples but distant from old macrophyte samples.

Table 7.4. Differences in relative abundance values of the most common, nutrient sensitive (s values of 1 and 2) and nutrient tolerant (s values of 4 and 5) diatom species in the dataset from old (herbarium) and recent diatom samples from matched rivers in the northeast and southwest of the UK as calculated by ANOVA. Abbreviations: n.s. = not significant.

Diatom species	Mean (SD)		$F_{1,25}$	p
	Macrophytes	Stones		
Achnanthidium minutissimum (AMIN)	31.96 (19.95)	2.43 (2.29)	66.24	< 0.001
Cocconeis pediculus (CPED)	2.17 (4.80)	3.70 (3.87)	1.83	n.s.
Cocconeis placentula var. *euglypta* (CPLE)	14.35 (19.09)	18.85 (16.5)	1.79	n.s.
Nutrient sensitive species (s value 1 or 2)	52.9 (27.2)	7.9 (8.3)	25.87	< 0.001
Nutrient tolerant species (s value 4 or 5)	19.5 (18.1)	72.6 (11.6)	77.16	< 0.001

A DCA for the River Ribble sites only is shown in Figure 7.7. The sites are divided into two distinct groups with all of the historical samples on the right of the ordination and the matched stone–macrophyte pair and recent stone samples clumped to the left of the ordination. The recent stone–macrophyte samples are spread out along axis 2 indicating another underlying environmental pressure, leading to differences in species composition at these sites.

A DCA for the River Wye sites only (Figure 7.8) shows a clear separation of recent and historical samples in ordinal space. The matched stone–macrophyte samples occupy an intermediate position along axis 1.

7.4 Discussion

7.4.1 Substratum specificity

Both macrophytes and stones supported diverse communities of diatoms. That no significant differences were observed in the relative abundance of the seven most common diatom species, including *Achnanthidium minutissimum*, *Cocconeis placentula var euglypta*, *Cocconeis pediculus, Fragilaria capucina* var. *gracilis, Fragilaria vaucheriae, Navicula capitoradiata* and *Nitzschia fonticola* indicated no major substratum preference. These observations were supported by comparisons between the mean species richness, diversity, and the percentage of both tolerant and sensitive valves where no significant differences were observed. The TDI values for the macrophyte and stone-derived samples covered a similar range and no significant differences were observed between them. Michelutti *et al.* (2003) noted that several taxa exhibited strong habitat preferences in ultra-oligotrophic ponds.

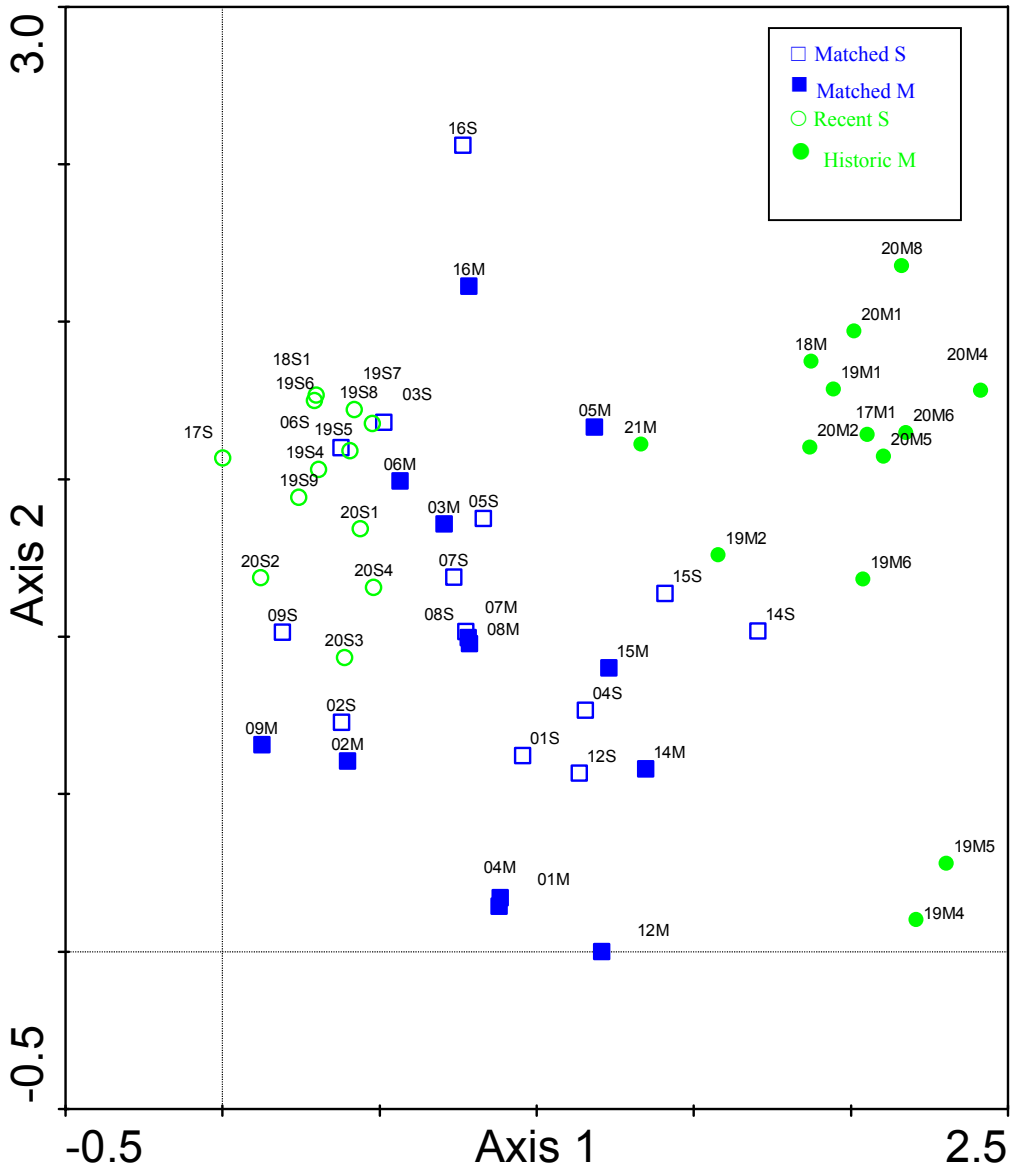


Figure 7.6. DCA ordination diagram of matched recent stone–macrophyte pairs and recent and historical river matches.

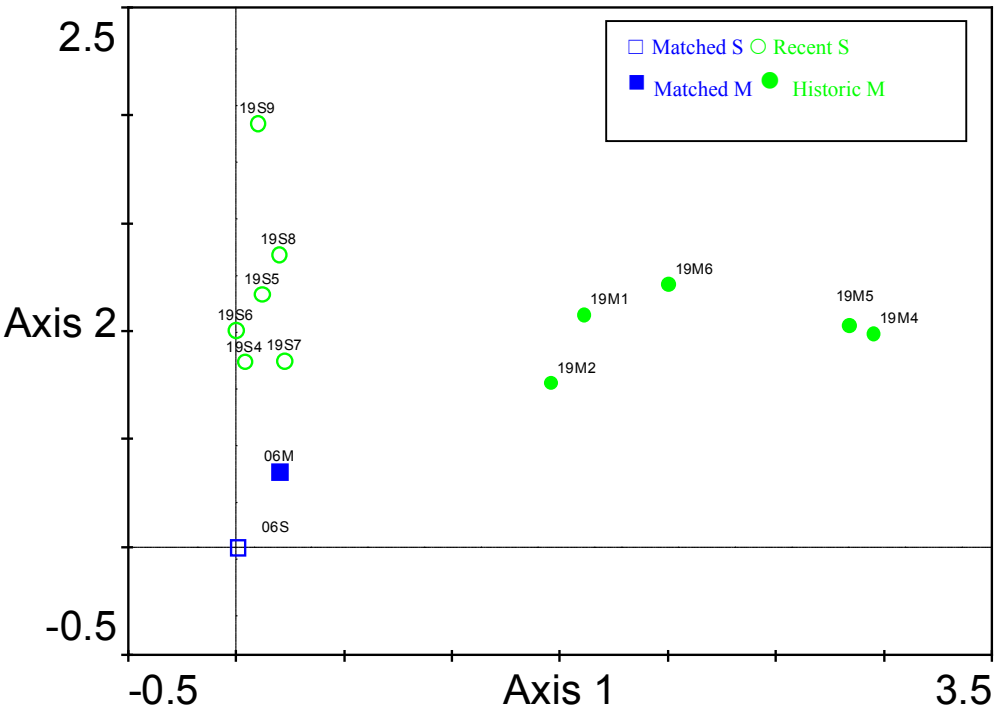


Figure 7.7. DCA ordination diagram for River Ribble sites (n = 13)

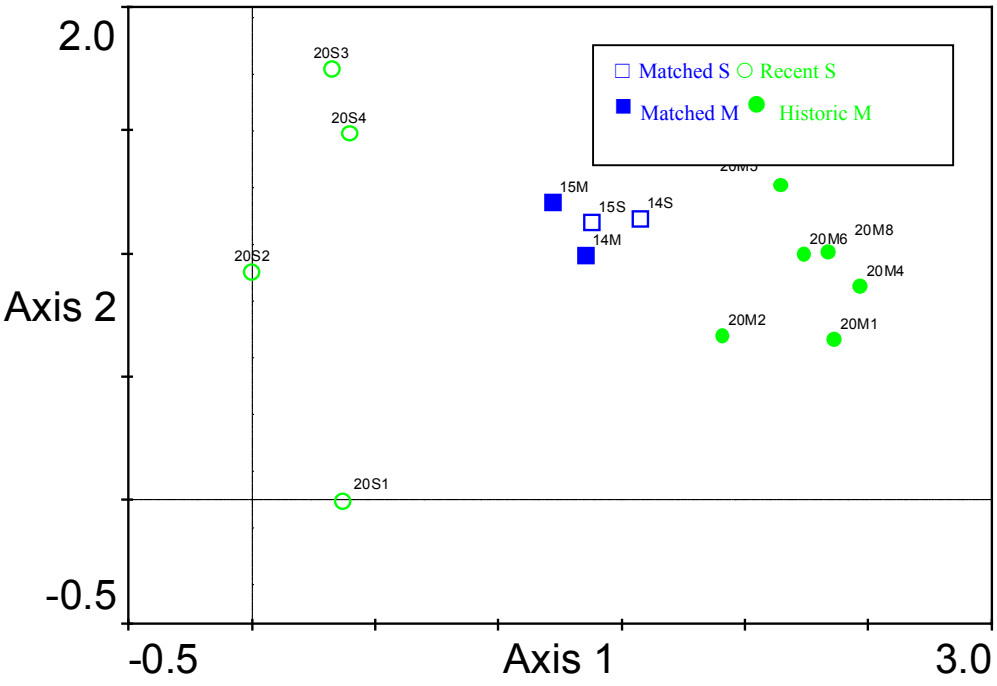


Figure 7.8. DCA ordination diagram for River Wye sites (n = 16)

Table 7.5. Squared chord dissimilarity scores for the matched stone–macrophyte pairs (S–M) and herbarium-stone pairs (H–S). Pairs with scores with values ≤ 0.39 show insignificant changes and those > 1.0 (asterisked) have marked dissimilarity in diatom composition.

Site	Squared chord distance dissimilarity score
Bowmont Water (S–M)	0.893
Browney (S–M)	0.402
Ely (S–M)	0.3
Glen (S–M)	0.762
Ithon (S–M)	0.299
Ribble (S–M)	0.178
Tees (S–M)	0.319
Usk (S–M)	0.426
Wear (S–M)	0.358
Wooler (S–M)	0.445
Wye (S–M)	0.812
Wye (S–M)	0.25
Wylye (S–M)	0.446
Avon (H–R)	1.541*
Loddon (H–R)	1.038*
Ribble (H–R)	1.612*
Ribble (H–R)	1.659*
Ribble (H–R)	1.504*
Wye (H–R)	1.232*
Wye (H–R)	1.405*
Wye (H–R)	1.497*

Other research contradicts these findings and suggests that these taxa do not exhibit strong habitat specificity (Lim *et al.,* 2001). Stones are regarded as inert substances (Burkholder, 1996) while macrophytes may leach nutrients that could be taken up by attached diatoms. However, Wetzel (1969) concluded that host specificity appeared to be less pronounced if the overlying water was eutrophic. No significant differences were found in the relative abundance of the common diatoms between the two substrata or between the TDI, hence the analysis did not lead to different indications of trophic status. Poulíčková *et al*. (2004) found differences in diatom-derived indices of trophic state between substrata from seven perialpine lakes. Many of the studies addressing questions about substratum specificity have been conducted on a relatively small sample size.

Sixteen rivers were sampled for the present analysis and they spanned a wide range of trophic conditions. Ordination of the samples from matched substrata resulted in the separation of the sites into two groups. Group 1 comprised sites with relatively low TDI values ranging from 30 to 60 and were separated from group 2 sites covering the higher TDI scores of 60 to 80. However, the matched macrophyte–stone site pairs were located in close proximity in each case. This indicates that over a wide range of ecological status, the diatom composition on the stones or macrophytes taken from one site are comparable in terms of their potential to describe ecological status. The strong separation of certain species along axis 2 with *Nitzschia lacuum, Cocconeis pulchella and Fragilaria bidens* positioned at one end along axis 2 and *Pseudostaurosira brevistriata, Staurosira construens* var. *binodis and Synedra arcus* may reflect differences in the hydromorphological regime of the sites or other pressure gradients, though again there were no marked differences in samples from two substrata from the same site.

Compositional changes that were observed between rivers or different sites within a river on either of the substrata could be attributed to other localised pressures, for instance differences in hydromorphology (Stevenson, 1996), microtopography, differential grazing pressures (Steinman, 1996) or different physical conditions influencing the biofilms. Evidence presented here would indicate that while there may be some taxonomic differences between the diatom flora on different substrata from the same water body, overall, these differences do not lead to significant differences in any of the metrics or indices derived from the diatom compositional data used to assess the ecological status of the water bodies.

7.4.2 Comparison of historical and recent diatom samples

In contrast to the matched stone–macrophyte samples there were marked differences in a number of the structural parameters used to measure diatom composition in the historical–recent dataset. Only three species were common in both recent and historical samples (*Achnanthidium minutissimum*, *Cocconeis pediculus* and *Cocconeis placentula* var. *euglypta).* These species were also the three most common species found in the sites selected for the matched stone–macrophyte comparison. However, in contrast, *Achnanthidium minutissimum* was significantly more abundant in the herbarium samples than in the recent samples. *Achnanthidium minutissimum* is a group 2 species, and is relatively intolerant of eutrophication. The low percentage abundance of this species in the newer samples suggests a marked change in these sites in terms of their ecological status over the past 100 or so years. *Cocconeis placentula* var. *euglypta* is classed as a group 3 species and was common in both old and new samples. *Cocconeis pediculus* as a group 4 species is tolerant to eutrophication though did not form significant populations in either of the groups.

The percentage of nutrient sensitive valves was significantly greater in herbarium samples, and the percentage of nutrient tolerant valves significantly lower in the herbarium samples, again suggesting a marked difference in these rivers through time. It is not likely that these differences could be due to the different substrata being compared within a water body as no significant differences were observed in these variables in the matched stone–macrophyte pairs.

Species richness was significantly lower in the herbarium samples compared with recent samples. No differences were observed in species richness between the matched stone–macrophyte samples. Some species including *Navicula subminiscula, Nitzschia palea* and *Planothidium lanceolatum* were exclusively associated with recent samples while others such as *Amphora pediculus, Navicula*

tripunctata and *Nitzschia fonticola* were far more commonly occurring species in recent samples. The significant increase in percentage of motile species in the recent samples may largely account for the overall increase in species richness. Many of these motile species have a higher tolerance to eutrophication. The TDI was significantly lower in the historical samples. Biofilms from unenriched streams tend to support a lower biomass (Biggs *et al.,* 1998) and nutrient limitation can occur earlier on in the development of the biofilm leading to degradation or removal of the accruing biofilm. As a result, the communities sampled in unenriched waters may not support as many species as the denser, mature biofilms found in enriched water bodies. Many more loosely attached or motile species may become established in the latter stages of biofilm succession (Yallop and Kelly, 2006).

The partitioning of the historical samples in a relatively discrete group in ordinal space provides further supporting evidence that these samples were obtained from a time when the ecological status of these waters was superior to their present state. Separation of old and new samples sites from the Ribble and Wye suggest a particularly marked decline.

The squared chord dissimilarity scores for all the matched recent–historical sites were > 1, indicating marked changes in community composition. For most of the contemporary stone–macrophyte comparisons the dissimilarity values were < 0.48, which indicates little floristic change.

7.4.3 Conclusions

i. Diatom samples obtained from macrophytes are reliable indicators of ecological status in rivers.

ii. No significant differences were found between the species composition of diatoms obtained from macrophytes or stones in the same water body.

iii. Given that no significant differences were found we conclude that diatoms collected from herbarium samples can be used to infer ecological status of a given water body at the time of sampling.

iv. Diatoms obtained from herbarium samples can therefore be used to assess the degree of florisitic change over time-scales > 100 years.

v. Herbarium samples could be used to 'fill in gaps' for water bodies where there is a limited availability of suitable 'reference sites', for instance types 3 and 4 in the DARES database.

8 Application of the models

8.1 River Wye

8.1.1 Introduction

Compared with much of the UK, the Wye catchment is relatively rural with few large towns and, as a result the Wye supports high biodiversity (Edwards and Brooker, 1984). Indeed the river, along with several of its tributaries, is designated as a Site of Special Scientific Interest under UK legislation, and a Special Area of Conservation under the terms of the Habitats Directive (European Community, 1992). Among the protected species listed in the Directive that are found in the Wye are Eurasian otter (*Lutra lutra*), white-clawed crayfish (*Astacus pallipes*), freshwater pearl mussel (*Margaritifera margaritifera*) and fish including Atlantic salmon (*Salmo salar*), bullhead (*Cottus gobio*) and twaite shad (*Alosa fallax*). The Habitats Directive places a legal responsibility on the UK Environment Agency and others to ensure that the river remains in a state that can support these organisms.

The lower part of the river is also designated as a 'sensitive area', under the terms of the Urban Wastewater Treatment Directive (European Community, 1991), which requires large sewage works within the sensitive area to be equipped with nutrient stripping facilities. To date, three major sewage works on the Wye have been set up to strip nutrients, along with one sewage works and one industrial effluent on the River Lugg, a major tributary which joins the Wye just downstream of Hereford .

8.1.2 Dataset

Two samples per year (summer and autumn) were collected from natural substrata at 15 sites on the River Wye between 2002 and 2005. Total alkalinity values used to compute 'expected' TDI values are based on mean values of data collected between approximately January 1996 and December 2004 (the precise dates vary between sites). In many cases, the Environment Agency has ceased measuring total alkalinity due to budget cuts; however, these long-term averages are correlated closely with annual means (e.g. 2003 versus long-term average: r = 0.995).

8.1.3 Results

The uppermost sampling point in this study is 28 km from the source and has a diatom flora typical of a circumneutral stream with low nutrient concentrations. Samples in this area are dominated by *Achnanthidium* spp. (principally *Achnanthidium minutissimum*) but with several other nutrient sensitive taxa present (e.g. *Achnanthes oblongella*, *Fragilaria capucina* and varieties, *Meridion circulare*). By km 37, the cosmopolitan species, *Cocconeis placentula* becomes established, although numbers of nutrient tolerant taxa remain low. Between km 37 and km 140 there is a gradual increase in the proportions of motile taxa recorded, with *Navicula capitatoradiata* becoming particularly common. Accordingly, the TDI increased from values at or close to reference at km 28 to those suggesting moderate nutrient enrichment between km 37 and km 140 (Figure 8.1).

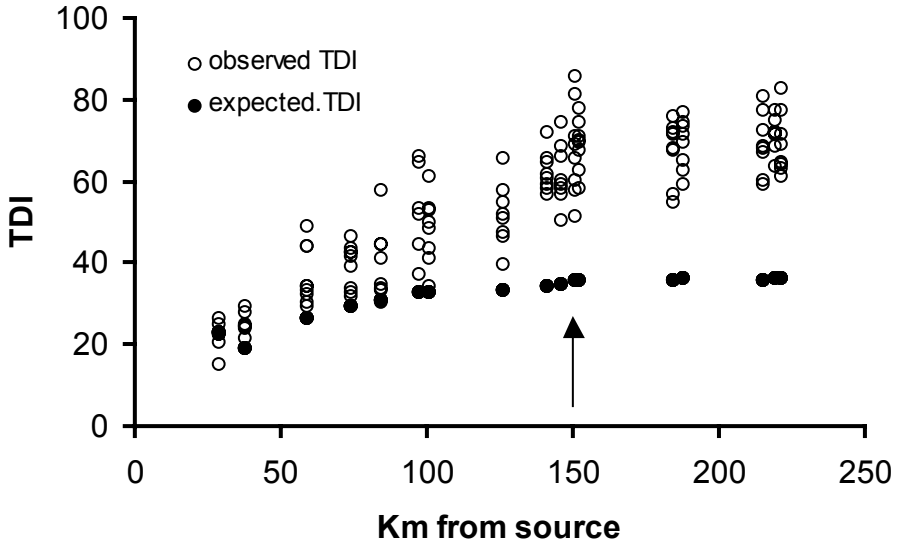


Figure 8.1. Observed and expected values of the TDI in the River Wye. Arrow indicates the confluence with the River Lugg.

Effluents from Hereford enter the river at km 142, and the confluence with the River Lugg is at km 149. From this point to the tidal limit at Bigsweir (215 km) the diatom flora is dominated by taxa typical of eutrophic conditions (*Diatoma vulgare*, *Rhoicosphenia abbreviata*, *Nitzschia palea*) and values of the TDI remain high, with little longitudinal change. Nutrient sensitive taxa are correspondingly less important in these lower reaches.

Only the uppermost sites are unambiguously high or good status, with all other sites showing at least occasional lapses into moderate or lower status classes (Figure 8.2). By Bridge Sollers (126 km), samples at moderate status predominate, although the mean value has not fallen below the critical threshold of 0.68 – when the risk of misclassification is < 5%. However, from upstream Eign STW onwards (i.e. Hereford and downstream), the diatom flora is unambiguously below good status, with the lower five sites indicating poor status.

These results need to be set in context: annual mean SRP concentrations are always < 0.04 mg l^{-1} in the River Wye upstream of Hereford (Figure 8.3) and total oxidised nitrogen concentrations are also low (Figure 8.4). Downstream of Hereford and the confluence with the River Lugg there is a marked increase in SRP even in samples collected after the onset of nutrient stripping, although measured concentrations are still low by the standards of many UK rivers. After the onset of nutrient stripping, mean summer concentrations are always < 0.1 mg l^{-1}, and have often been less than half of this. In other words, nutrient concentrations are well below the regulatory standards set for UK rivers and major point sources of nutrients have already been controlled but the diatom flora is still showing signs of enrichment.

The reasons for these discrepancies need further investigation but the following points may provide partial explanations:

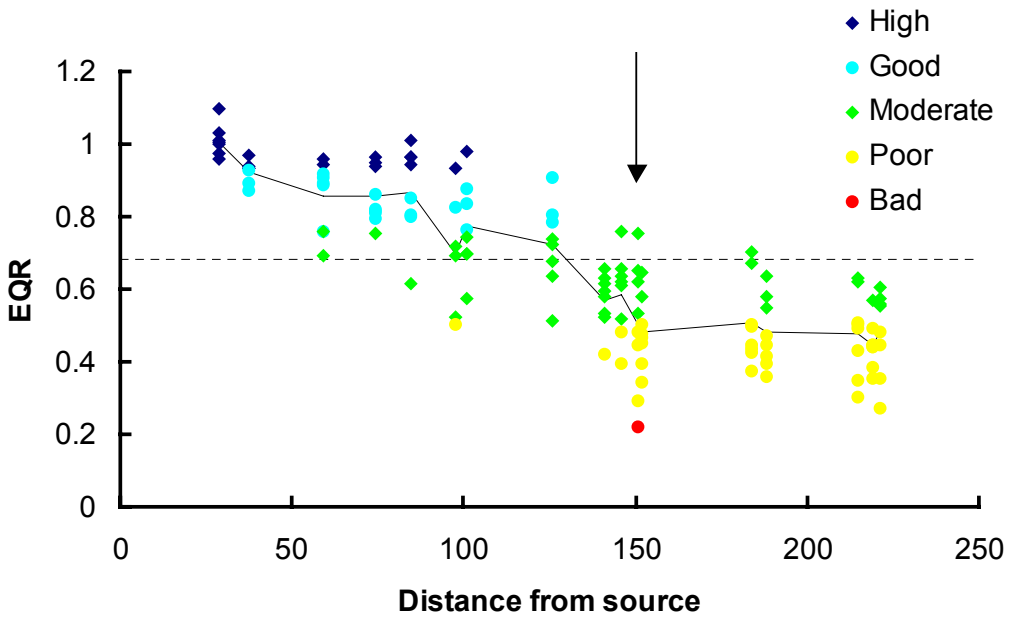


Figure 8.2. EQR values in the River Wye, along with predicted status classes. Arrow indicates the confluence with the River Lugg. Solid line indicates mean value at each site and dashed line is EQR = 0.68 (the point on the EQR scale when there is > 95% confidence that a site is not at good status – see chapter 6 for more details).

- When nutrient loads are naturally low it is likely that algae are utilising forms of P other thanSRP. Additionally, many algae practise 'luxury consumption' of nutrients and are able to utilise short pulses of high nutrient concentrations (particularly in the spring), and annual mean concentrations in an unregulated river such as the Wye may not give an accurate picture of the nutrient concentrations during the periods when algae are most prolific. The practice of monthly sampling of a single P determinand is likely to underestimate the true phosphorus load in a river and the use of 90th percentiles to derive regulatory standards may not be sufficiently protective.

- While the impact of eutrophic tributaries of the Wye (e.g. Llynfi and Lugg) may not be great in terms of their contribution to the total nutrient load, it is possible that the increased algal productivity in these streams may contribute inocula to the main river that decouples the link between algae and water chemistry. There is some evidence for this from the Wye (Roe, Kelly and Sayer, unpublished data). As ecological status will be the criterion by which a river is judged in the future, improvements in ecological status in the lower river may only be possible if high nutrient concentrations in tributaries upstream are first dealt with as it is only by reducing the quantity of these inocula that ecological status lower down in the catchment can be improved. The mechanism by which such processes operate will, however, require more work.

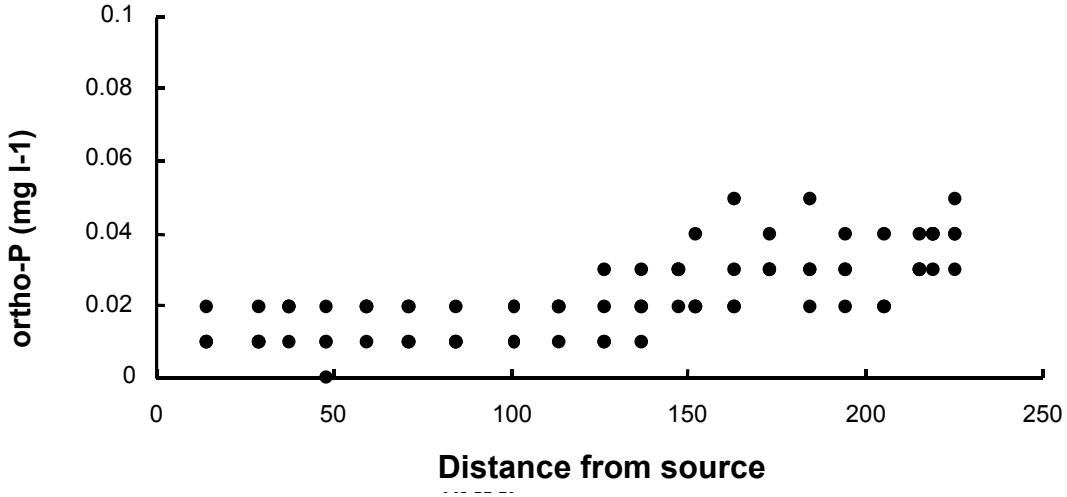


Figure 8.3. Longitudinal changes in annual mean SRP concentrations in the River Wye, 2002–2005.

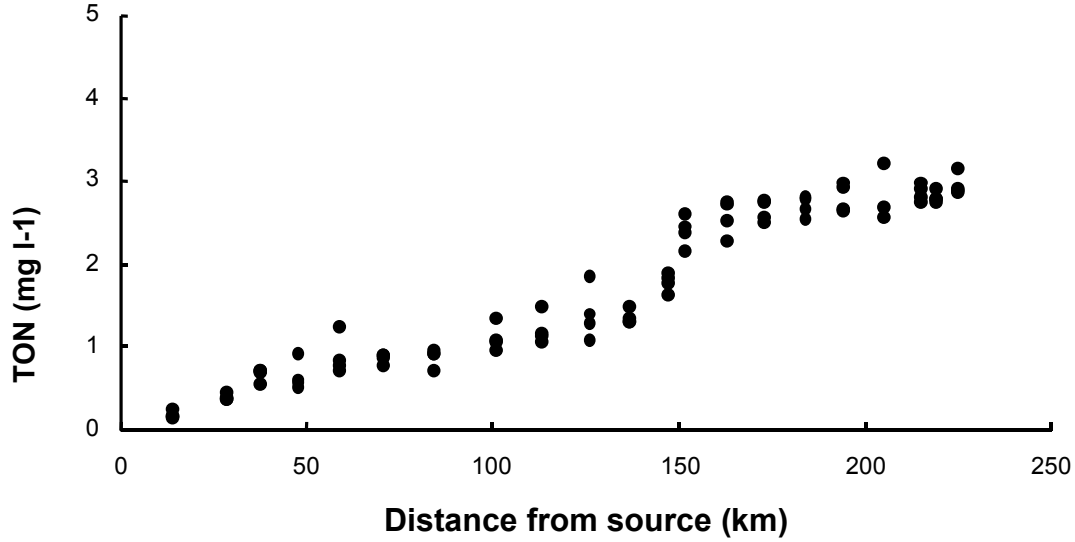


Figure 8.4. Longitudinal change in total oxidised nitrogen (TON) in the River Wye, 2002–2005.

- Diatom assemblages are naturally variable and this translates into variations in EQR values for individual samples. The lowest values may be associated with long periods of low flow (i.e. when nutrient concentrations may be naturally elevated) and it may be possible to account for this in future versions of the model.

Figure 8.5 shows the relationship between TDI and Hill's N_2 diversity for samples from the River Wye. In section 4.5 the possibility of using the maturity of the biofilm to

predict 'expected' TDI was raised. A mature biofilm is likely to have a more diverse flora and Figure 8.5 shows that a strong linear relationship does exist between diversity and TDI when the TDI is low but that the variability in diversity increases at higher TDI values, reducing the predictive value of this relationship. Moreover, high status, in particular, is associated with relatively low diversity and values of Hill's N_2 diversity > 10 are unusual at high and good status. There are several possible explanations for this but it is important to remember that a diversity measure applied to diatoms encapsulates just part of the total diversity of the biofilm and also that the sampling method will integrate within-site spatial heterogeneity (which also increases along the EQR gradient – see chapter 6). A further possibility is that low levels of pressure in the Wye (and elsewhere in the UK) are associated with low order, often fast-flowing streams and that the low levels of diversity may reflect physical conditions that are not replicated further downstream. Nonetheless, this plot does suggest that there is scope for refining the predictive equation if a property that was not dependent upon status could be developed.

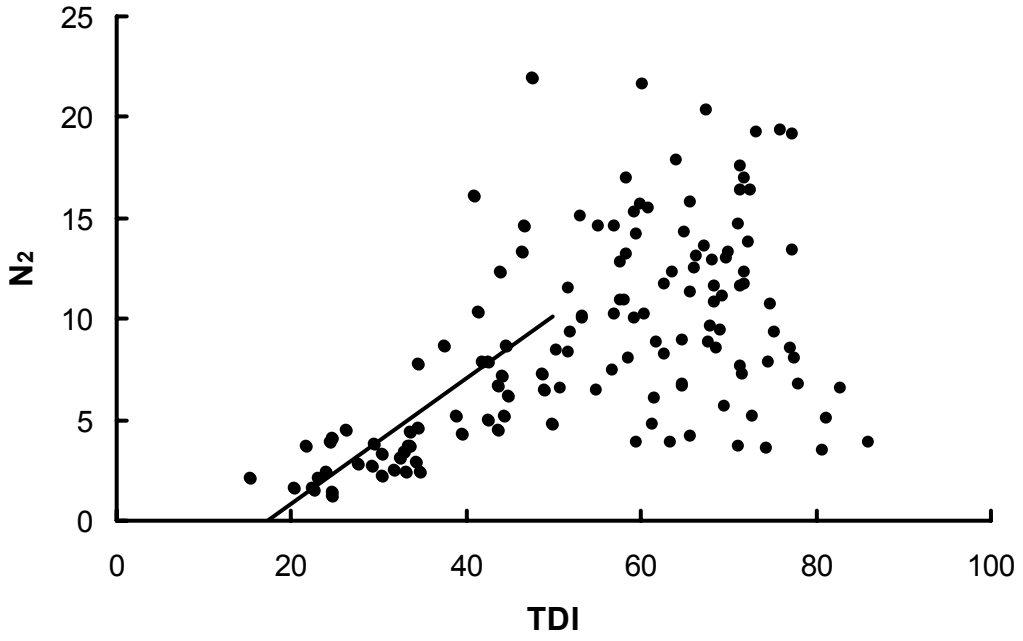


Figure 8.5. Relationship between TDI and Hill's N_2 diversity (Hill, 1973) for samples from the River Wye. The straight line is fitted to points with TDI ≤ 50 (R^2 = 0.439).

8.2 River Axe

8.2.1 Introduction

The River Axe is a very different river to the River Wye, rising in the rich agricultural land of south Devon although the section between Wadbrook and Colyford has, like the Wye, been proposed as a candidate Special Area of Conservation, under the terms of the Habitats Directive (European Community, 1992), due to the presence of interesting plant communities (including nationally scarce short-leaved water-starwort) and three fish species of European importance (bullhead, brook lamprey and sea lamprey). Background information on the catchment is given in Environment Agency (2001) and ENTEC (2003).

8.2.2 Dataset

Three samples per year (spring, summer, autumn) were collected between 2002 and 2003. In addition, summer and autumn samples were collected in 2001 and some sampling also took place in 1998. Annual mean total alkalinity data are available only for 2003 and not for all sites. Values for sites that lacked measurements are the means of the upstream and downstream values. As total alkalinity levels are high in the Axe, the effect of these interpolations is insignificant.

8.2.3 Results

Figures 8.6 and 8.7 show longitudinal changes in phosphorus and nitrogen in the River Axe. Phosphorus concentrations, in particular, are elevated with annual mean concentrations even at the headwaters close to or exceeding the proposed regulatory standard. Concentrations increase at km 22, where there is an input from a creamery, and remain elevated until the tidal limit.

Achnanthidium minutissimum was abundant in samples from the first 10 km of the River Axe, particularly in spring samples. At other times of the year and at all sites downstream from here, however, samples were dominated by taxa more indicative of moderate or high nutrient concentrations. The spring samples at these lower sites were dominated by *Navicula lanceolata* and *Navicula gregaria*, while autumn samples were dominated by *Amphora pediculus*. These seasonal changes are fairly characteristic of lowland rivers, although the underlying reason is not known.

TDI values in the River Axe showed relatively little longitudinal variation, except at the uppermost sites (Figure 8.8). The mean EQR value for all sites fell below the threshold EQR value of 0.68, and only two samples from Cheddington, the uppermost site, fall into high or good status (Figure 8.9). The first three sites on the river (Cheddington, upstream and downstream Mosterton) all had three-year mean values that indicated moderate status but all other sites would be classified at poor status on the basis of these results.

Despite the designation of the River Axe as a candidate Special Area of Conservation, the problems in the River Axe are recognised and steps are already

being taken to address these. However, the water quality problems here extend the whole length of the river and both point and diffuse sources contribute nutrients. Improving ecological status in a rich agricultural catchment such as this will be challenging, particularly when the Axe is viewed alongside the River Wye, where nutrient concentrations are much lower but several sites are still failing to achieve good status.

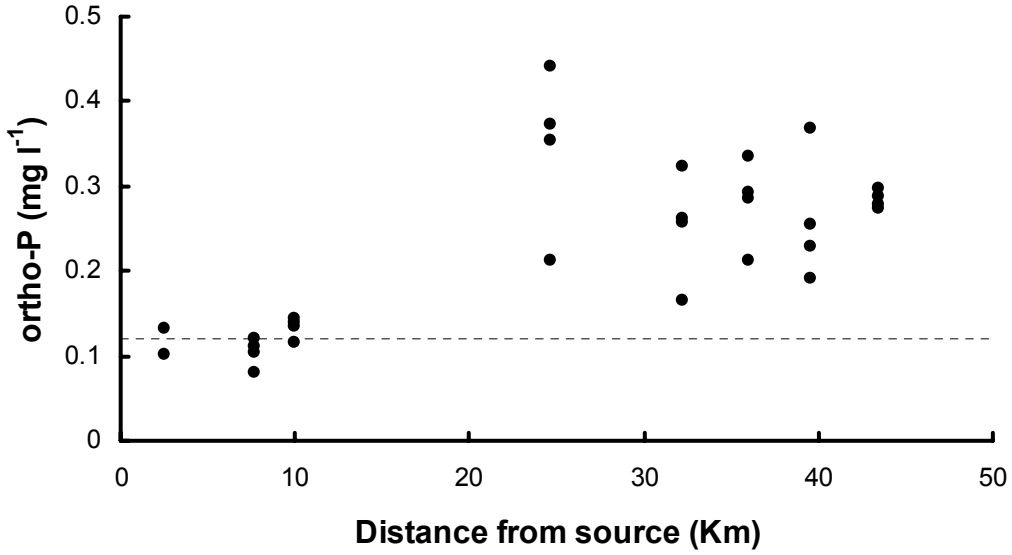


Figure 8.6. Longitudinal change in annual mean concentrations of SRP in the River Axe between 2000 and 2003. Dotted line indicates position of proposed regulatory standard for P in UK rivers.

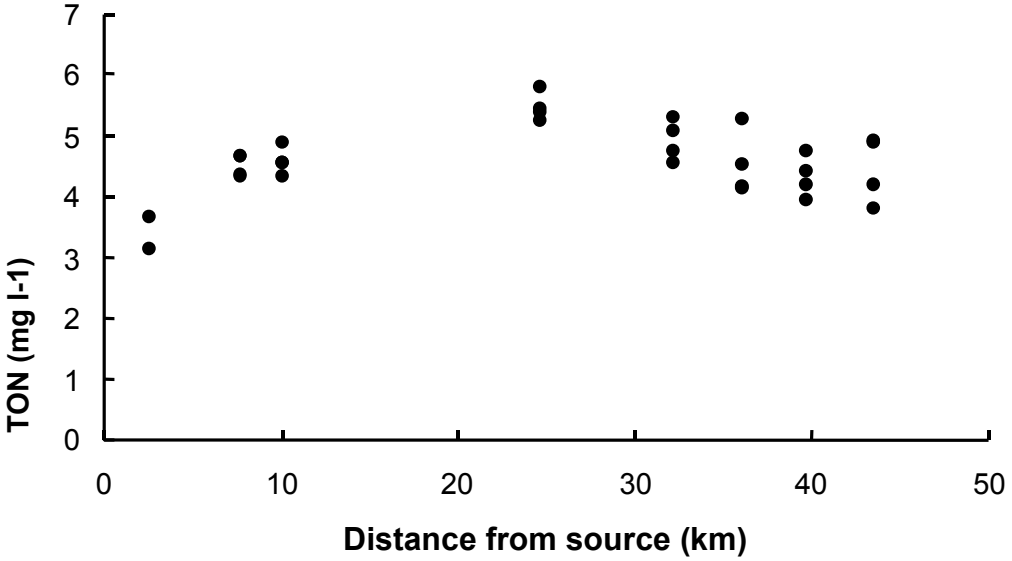


Figure 8.7. Longitudinal change in annual mean concentrations of total oxidised nitrogen (TON) in the River Axe between 2000 and 2003.

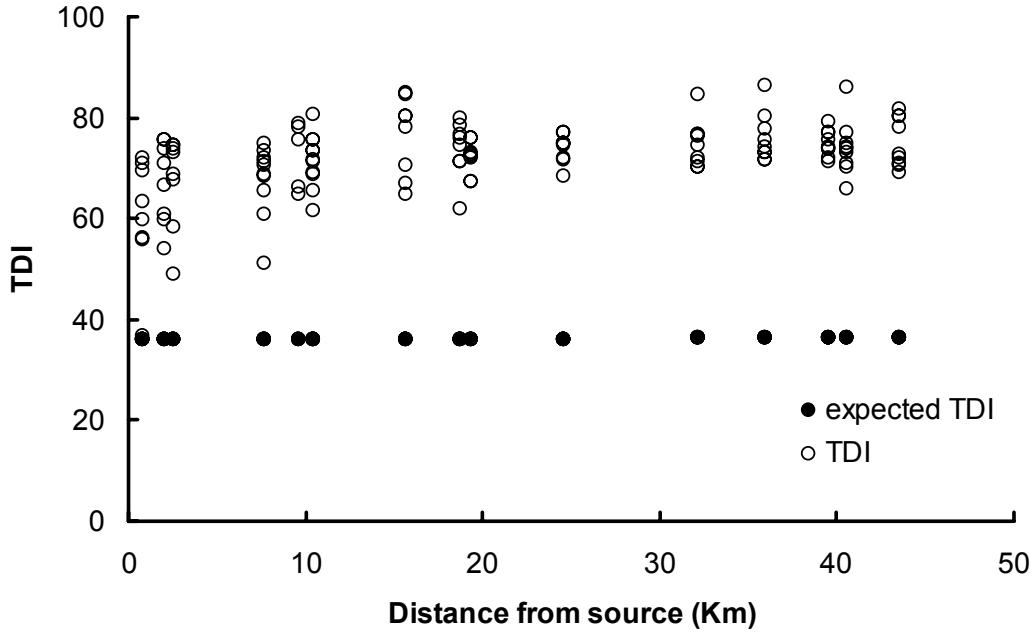


Figure 8.8. Observed and expected values of the TDI in the River Axe.

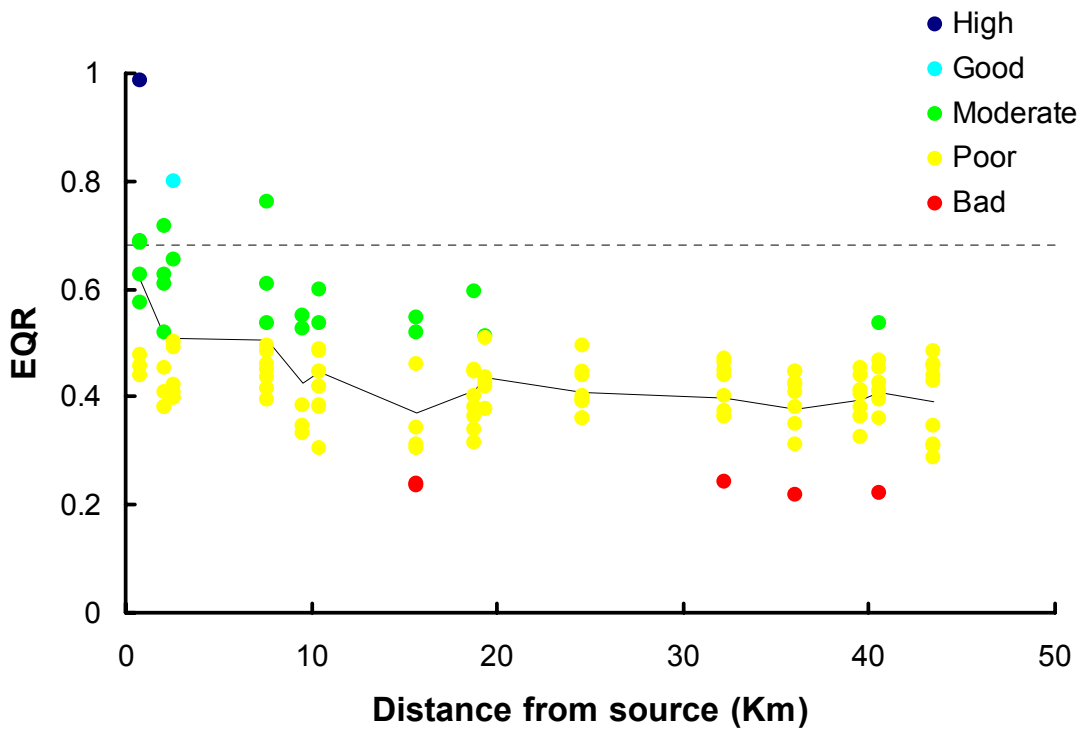


Figure 8.9. EQR values in the River Axe, along with predicted status classes, based on data collected between 1998 and 2003. Solid line indicates mean value at each site and dashed line is EQR = 0.68 (the point on the EQR scale when there is > 95% confidence that a site is not at good status – see chapter 6 for more details).

8.3 Lake District lakes

King *et al*. (2000) explored the relationship between epilithic algae and environmental variables from 17 lakes in the English Lake District along a trophic gradient. Each lake was visited three times (June 1997, September 1997 and September 1998) resulting in a total of 51 epilithic diatom samples. These data have been harmonised with the DALES dataset and the model has been applied.

This is a useful set of lakes with which to assess the performance of the DALES classification tool as these are well-studied sites for which a range of biological and chemical data are available. This includes EQRs based on total P, chlorophyll a and oxygen concentrations, and status classes based on macrophyte communities, the chironomid pupal exuvial technique (CPET) method and the chord distance from palaeoecological studies. A descriptive summary class for each lake has been derived on the basis of these various outputs in order to aid comparison with the DALES classification.

The results show that the DALES tool is good at correctly predicting lakes at high and good status but is poor at predicting lakes at 'less than good' status. The LTDIs increase and thereby the EQRs decrease for those sites classed by other methods as 'less than good' status but the current position of the class boundaries proposed in section 5.4.2 means that these sites are still classed as good. There are two possible reasons for these apparently low thresholds. The first is that all of the lakes in this test set are of Low and Medium Alkalinity types and given the limitations of the DALES dataset for these types (particularly the Low Alkalinity lakes) it is not surprising that the tool fails to detect any nutrient impact.

However, there is an alternative explanation. In section 5.3.1 we discussed the extent to which the reference community in Low Alkalinity lakes might be 'buffered' against change as the dominant taxa in the biofilms (e.g. *Achnanthidium minutissimum*) were relatively tolerant to mild enrichment. We also indicated that, in palaeoecological studies in Low Alkalinity lakes, it was often the planktonic taxa that were responsible for changes observed in the sediment record. One implication of this is that the littoral biofilm can be said to be at 'good status' even though other components of the lake ecosystem are showing signs of enrichment. Following this reasoning, the boundaries proposed in chapter 5 give a correct indication of the state of the littoral biofilm but need to be recalibrated in order to give a true indication of the state of the lake as a whole. The dataset used in this study is too small to attempt this but it may be a useful exercise in the future.

Table 8.1. Comparison of DALES classification with other status assessments for lakes in the English Lake District.

WBID	NAME	Type	Grid reference		Max. depth (m)	Mean depth (m)	Alk	MEI	Mean TP 2000 (ug/l)	Mean Chl 2000 (ug/l)	Ref TP (ug/l)	Ref Chl (ug/l)	P risk	Status based on TP EQR	Status based on oxygen	Status based on Chla EQR	Status based on plants	Status based on CPET	Squared chord distance from palaeo	Summary status	DALES class
29183	Wastwater	LAD	NY14973		76	39.73	60	0.002	1	1.19	4	1.5	No					15.1	0.44	H	H
29052	Buttermere	LAD	NY19161	15423	28.6	16.60	64	0.004	1.4	1.59	5	1.9	No					14.1	0.48	H	H
29062	Ennerdale Water	LAD	NY11232	14977	42	17.76	62	0.003	1.9	1.26	5	1.8	Low					28.9	0.68	H–G	H
29116	Brothers Water	MAS	NY40215	13003	15	6.20	202	0.033	6.6	2.18	8	3.3								H	H
29000	Crummock Water	LAD	NY16276	18305	43.9	26.70	50	0.002	3.2	2.95	4	1.5	No					24.9	0.16	H–G	H
28965	Derwent Water	LAS	NY26460	22727	22	5.50	127	0.023	7.5	4.73	8	3.0	No					21.6	0.43	H–M	G
29321	Coniston Water	MAD	SD30971	95228	56.1	24.10	222	0.009	7.5	5.68	6	2.3	Low					37.6	0.73	H–M	H–G
28955	Ullswater	MAD	NY38736	18849	62.5	25.30	248	0.010	9.9	4.85	6	2.4	No					26.5	0.28	H–M	H–G
29233	Windermere N Basin	MAD	SD38929	96301	64	25.10	250	0.010	12.3	4.35	6	2.4	High					35.3		G–M	H–M
29233	Windermere S Basin	MAD	SD37979	87002	64	16.8	250	0.015	13.9	6.42	7	2.7						55.4	1.17	G–M	G
28986	Loweswater	LAS	NY12682	21216	16	8.37	185	0.022	16.4	9.62	8	2.9	Mod					47.2	0.54	G–M	H–G
29197	Rydal Water	LAS	NY35616	6343	< 10	5.30	160	0.030	18.1	6.53	8	3.2								M–P	G
28847	Bassenthwaite Lake	LAS	NY22196	27349	19	5.30	180	0.034	20.85	14.53	9	3.3	Mod					40.3	0.98	M–P	G
29328	Esthwaite Water	MAS	SD36341	96475	15.5	6.40	459	0.072	30.26	21.02	10	4.0	High						1.34	M–P	H–G
29270	Blelham Tarn	MAS	NY36803	628	14.5	6.80	558	0.082	31.7	14.94	11	4.1							0.33	M–P	H–G
29184	Grasmere	LAS	NY34273	6081	21.5	7.74	168	0.022	23.6	12.34	8	2.9	High						0.65	M–P	H
29222	Elterwater	LAS	NY33769	3944	7.4	2.90	135	0.047	50.7	30.32	9	3.6							0.65	P–B	H–G

9 Discussion

9.1 General comments

The issues that have emerged from each chapter have been discussed in detail within each chapter and this final discussion is intended just to pull the various strands together and to identify areas where additional work will be needed in the future. Issues associated with the normative definitions themselves are not covered here, but the comments in chapter 1 are expanded upon in Kelly *et al*. (2006) and will also be discussed in the forthcoming report on the Central/Baltic Geographical Intercalibration Group (GIG) phytobenthos intercalibration exercise.

9.2 Definition of reference sites and 'high status'

Reference conditions were, of necessity, defined differently for lakes and rivers although, in both cases, the intention was the same: to find contemporary sites that reflected a pre-industrial condition. In the case of standing waters (chapter 5), palaeoecological data were available and provide a robust baseline (limitations are discussed in section 5.2 and Burgess *et al*., 2005). Moreover, the pool of potential reference sites has, now, been fairly thoroughly explored. The situation in rivers is, however, different. Reference sites were based on a comparison of contemporary nutrient concentrations with those expected in the absence of pressures (see chapter 4). However, the basis for these estimates was a short unreferenced document (Pitt *et al*., 2002) designed to set regulatory standards for the Habitats Directive. This considered phosphorus but not nitrogen and also considered only a single phosphorus determinand (SRP). At the time the project started, a more sophisticated approach based on catchment land use (e.g. Johnes and Heathwaite, 1997) was not possible for all the potential sites. Although the DARES team believe that the diatom flora of riverine reference sites, as defined here, meets expectations for an 'undisturbed' biota, and mean values of metrics from these sites are similar to those obtained from other Member States in Central/Baltic GIG, it would be useful to apply export coefficient models to all sites at high status in order to refine this selection.

All phosphorus analyses reported here are based on annual mean concentrations derived from a relatively low intensity sampling strategy (typically monthly) and a single P determinand ('soluble reactive P', SRP) in order to provide the wide geographical coverage necessary for these analyses. As a result, some of the nuances of nutrient interactions with algae may be missed (e.g. Livingstone and Whitton, 1984; Hantke and Melzer, 1993; Tuchman 1996). Further advantages of determining reference conditions from land use rather than mean annual SRP are discussed, briefly, in section 4.7.

All the river classification tool projects in the UK were expected to derive their own concepts of reference and, while the DARES project considered the status of the invertebrate assemblage in setting its own reference conditions, the status of other biological elements was not considered. Thus, it is possible that a reference site as defined by diatoms is not classified as high status by macrophytes. This was a pragmatic step but is not wholly in accord with the WFD, which defines reference in a more holistic sense, suggesting that if any biological element showed signs of distortion then a site would not qualify as a reference site. Now that most classification tools are at or close to delivery it would be useful to screen all DARES and DALES reference sites against those proposed by other projects and, again, to refine the selections into a list of common reference sites.

9.3 Measuring deviations from reference conditions

For both rivers and lakes a modification of the TDI (Kelly and Whitton, 1995) has been used. Other options (e.g. Bayesian Belief Networks – Adriaenssens *et al*., 2004; Trigg *et al*., 2000) were explored, but a weighted-average metric such as the TDI had the advantage of easy conversion to an EQR. Paradoxically, a Bayesian Belief Network may well have allowed more precise estimation of status classes and associated risk of misclassification, but such an approach would have been difficult to convert to an EQR. This approach does, however, have potential for the future.

A limitation of the approach described here is that only a single pressure gradient has been considered. While the concept of reference conditions is more-or-less universal, only deviations from reference that are caused by nutrients and organic pollution will be assessed by the present EQR. This reflects the primary concerns of the UK regulatory agencies and the underlying principles used to develop the present models provide a framework from which separate metrics for assessing other pressures (acidity, salinity etc) could be developed. Indeed, some work on a metric for assessing pH and acid neutralising capacity in streams has already been done (Juggins and Kelly, unpublished) and the tool itself does include some additional diagnostics to ensure that other pressures are not missed altogether.

Neither the lakes nor the river tool has been tested in situations affected by toxic pollution and it is unlikely that either will be sensitive to these unless further diagnostics are added.

9.4 Definition of 'good status' and the good/moderate boundary

Defining good status is, in many ways, more problematic than defining high status largely because the normative definitions for good and moderate status allow a wide scope for interpretation. 'Good status' allows *slight* changes in the composition and abundance of macrophytic and phytobenthic taxa compared to type-specific communities while communities at moderate status differ *moderately*. The point at which the community ceases to be *slightly* changed and becomes *moderately* changed is the critical point beyond which a water body needs remedial measures in order to achieve good ecological status. The vague wording of the WFD therefore needs to be translated into objective and defensible concepts.

There is no absolute justification for placement of the good/moderate boundary at any point on the ecological status gradient; however, we believe that placing this boundary at the 'crossover' can be justified in two ways. In terms of the taxonomic composition, this is the point at which the numbers of taxa that are tolerant to nutrients (and which are, consequently, scarce in pristine environments) become relatively more abundant than the numbers of those that are sensitive to nutrients and which tend to be most common in pristine environments. This, in turn, reflects structural and ecophysiological changes in the phytobenthos (insofar as this can be inferred from the taxonomic composition of benthic diatoms).

The group of 'sensitive' taxa in both lakes and rivers is dominated largely by *Achnanthidium minutissimum* and *Fragilaria capucina*, while the tolerant category is dominated by *Amphora pediculus, Navicula* and *Nitzschia.* At the lower end of 'good status', therefore, a number of pollution-tolerant taxa are found. Such taxa are, in low numbers, a natural part of the biota and we argue that a concept of 'good status' should embrace the possibility of short-term/chronic pollution events that affect the flora but from which recovery is possible. Lower nutrient resources, where one or more key

nutrients (N, P, Si) may be limiting at any time, will favour 'nutrient generalists' (Carrick *et al*., 1988) with the co-occurrence, in relatively low abundance, of many 'broad-niched' species. A switch to a biofilm dominated by more 'nutrient specialist' species will occur with increasing enrichment of key nutrients and only those species with specialised mechanisms to exploit such conditions will proliferate, outcompeting the broad-niched species. In thicker biofilms, therefore, species diversity will probably be reduced (Tilman, 1982; Fairchild and Lowe, 1984). The marked increase in motile species (e.g. *Navicula gregaria* and *Nitzschia dissipata*) at the good/moderate interface may be explained by their ability to exploit resources unavailable to those occupying a fixed position within the thicker biofilm. In thicker biofilms, the success of adnate species, such as *Achnanthidium minutisimum* and *Cocconeis placentula*, may be compromised as they experience light and nutrient limitation. However, these species can grow as epiphytes on filamentous algae such as *Cladophora*. This alternative strategy removes them from the constraints that develop within thicker biofilms. Under enriched conditions, the most prolific of the sessile diatoms are often those commonly assumed to be 'epiphytes' (e.g. *Cocconeis pediculus, Rhoicosphenia abbreviata*).

9.5 Type-specific versus site-specific predictions

The WFD asks Member States to establish type-specific biological reference conditions against which the observed biota is compared in order to generate an EQR (Annex II, 1.3). While this allows finer discrimination of EQRs than would be possible if a single reference value were chosen, it is still artificial insofar as it imposes a categorical scheme onto systems that show continuous variation. Several Member States have adopted this approach for phytobenthos (Hendrickx and Denys, 2005; Pfister and Pipp, 2005) but there are limitations, one of which is that alternative approaches require large datasets of reference sites and the absence of such a dataset for lakes meant that a type-specific approach was most appropriate (see chapter 5). In the case of rivers, however, there were sufficient data to permit alternative approaches to be explored (section 4.5).

Type-specific predictions yielded four types for UK rivers, separated first by alkalinity and then by altitude. The latter distinction probably separates samples in which taxa associated with cooler water (e.g. *Hannaea arcus, Diatoma mesodon*) are abundant from those without these. These yielded 'expected' TDI values of 29.0 and 25.7 for the two Low Alkalinity types but 37.4 and 35.6 for the Medium/High Alkalinity types. Adding more types theoretically allows finer distinctions between classes but, in practice, these models have a tendency to overfit and the apparent increase in predictive ability is not supported by cross-validation. However, reliance on such a simple typology means that there are step-changes in the 'expected' TDI along a river. A shift in total alkalinity from 49 to 51 mg l^{-1} $CaCO_3$ may lead to an increase in the expected value by eight TDI units using this 'type-specific approach, with consequent implications for interpretation of EQRs.

The reality is, of course, that the 'expected' TDI shows continuous variation along a river and that the type-specific predictions, while providing a neat solution to the problem, introduce errors of their own, particularly when the values of predictor variables are close to thresholds. For this reason, the option of site-specific predictions was also explored. Although these approaches have been used successfully elsewhere (e.g. RIVPACS: Wright *et al*., 1989; LEAFPACS: Wilby *et al.*, 2006), they have had only limited success with diatoms previously (Chessman *et al.*, 1999). In this study, a regression based on environmental variables alone explained 33% of the variance in TDI at reference compared with just 19% when a type-specific prediction was used (section 4.5) and we recommend this approach for rivers. There is, however, considerable opportunity for refining these predictions in the future. In chapter 2 we

described the dynamic nature of the phytobenthos and this suggests that a considerable amount of variation in the diatom assemblage at reference is due to biological processes within the biofilm rather than to easily measured environmental variables. In chapter 8, we noted that there was a significant relationship between TDI and Hill's N_2 diversity measure when the biofilm was at good status or better (Figure 8.5) and preliminary experiments (unpublished) showed that incorporation of N_2 into the equation helped to explain some of the remaining variance. It was, however, not possible to use this for predicting expected values as diversity itself changed along the EQR gradient (Figure 8.5), but this does suggest some routes for future development and the challenge will be to find measures that can act as proxies for biofilm development that are both easily measured and which are unaffected by the EQR gradient.

9.6 Uncertainty in diatom-based estimates of ecological status

The fast growth rate of diatoms coupled with sensitivity to their environment means that diatom communities are naturally variable and this has consequences for the application of the DARES and DALES models. The situation is more acute for rivers, as the river environment is, itself, more dynamic, but the same principles apply to standing waters as well.

The situation is well-illustrated for the River Wye (Figure 8.1). As the distance from the source increases, so the TDI increases (reflecting an increase in 'eutrophication') but so also does the variability within TDI values recorded from a site over a four-year period. At the lowermost sites (downstream of Monmouth), within-site variation of the TDI is approximately 20 units.

Part of this variation will be due to changes in nutrient concentrations in the Wye, reflecting changes in discharge. However, discharge will also have an effect on the maturity of the biofilm as periodic scouring spates will remove much of the biomass and reset the phytobenthos assemblage back to a 'pioneer' state (see chapter 2). Such factors contribute to a temporal variation in the diatom assemblage which combine with the spatial variation and sampling uncertainty described in chapter 6 to give the total uncertainty associated with a single sample. Diatom communities may well be more dynamic than invertebrates or fish, as their life-cycles are shorter. There is, in short, a trade-off between 'sensitivity' and 'uncertainty' which is particularly acute for diatoms as they are being used to assess a pressure (nutrients) that is itself highly variable.

This variation clearly has implications for how diatoms can contribute to the decision-making process. While this means that there are limitations on the interpretation that can be ascribed to a single sample, it also emphasises that ecological status is itself a dynamic concept. A river that achieves 'good status' based on the mean EQR over a three-year classification period may still be expected to show occasional dips into moderate status.

The rationale for uncertainty estimations is outlined in chapter 6 and the recommendation in this chapter is that six samples will be necessary to ensure a robust classification in rivers. There are insufficient data available at present for lakes but the number for them is likely to be lower as first indications are that variability is, generally, lower in lakes. Although we recommend six samples, the difference between four and six samples is slight if the primary purpose is to distinguish between 'good or better' and 'moderate or worse'. If classification into all five ecological status classes is required, then six replicates is preferable. In particular, note that it is not possible to distinguish between 'good' and 'high' status with 95% confidence without six replicates.

We therefore recommend the following for rivers:

- For **surveillance monitoring**, planners should assume that six samples are required for classification, with either two or three samples per year collected in spring, summer or autumn, collected at intervals of not less than two months.
- Although a reliable (i.e. > 95% confidence) classification may be obtained in some cases with four samples, six samples offers benefits when distinguishing between status classes and, in the case of sites that are at moderate status or less, provides a stronger baseline against which changes can be measured.
- For **operational** and **investigative monitoring**, the above guidelines should be followed. The ability to detect the influence of particular inputs will depend upon local circumstances. Guidelines in chapter 7 of the *TDI User's Manual* (Revised Edition – Kelly *et al*., 2001) should be followed when designing sampling programmes for these purposes.

It is important to emphasise that the classifications described above are based on six well-spaced replicates and not necessarily on three years' data. It should be possible to classify a site based on three samples per year for two years although we do not recommend samples more closely spaced than once every two months in order to minimise the risk of pseudoreplication and also we have not tested the reliability of winter samples for obtaining a classification.

9.7 Conclusions

This work is the first attempt to define the benthic diatom flora of UK freshwaters that is expected in the absence of significant anthropogenic activity. It provides a foundation upon which the statutory agencies can start monitoring to assess the status of UK freshwaters, and a statistically sound basis for determining the need or otherwise of Programmes of Measures.

The study has, however, highlighted a number of areas where this work could be developed. Reference concepts and the model itself can both be refined and improved and the way in which the tools are implemented and involved in decision-making will also need further consideration.

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Abbreviations and acronyms

ANOVA	one-way analysis of variance
BES	bad ecological status
CCA	canonical correspondence analysis
CCW	Countryside Council for Wales
CEH	Centre for Ecology and Hydrology
CEN	Comité European de Normalisation
CI	confidence interval
DALES	Diatoms for Assessing Lake Ecological Status
DARES	Diatoms for Assessing River Ecological Status
DCA	detrended correspondence analysis
ECOSTAT	See glossary
ECRC	Environmental Change Research Centre
EHS	Environment and Heritage Service
EN	European Norm
EQR	Ecological Quality Ratio
EU	European Union
GES	good ecological status
GIG	Geographical Intercalibration Group
HES	high ecological status
HSD	Honestly Significant Difference
IPS	Indice de Polluosensibilité
LTDI	Lake Trophic Diatom Index
MEI	morphoedaphic index model
MES	moderate ecological status
MRT	multivariate regression tree
MTR	Mean Trophic Rank
PES	poor ecological status
REFCOND	See glossary
RIVPACS	River Invertebrate Prediction and Classification System
RMSE	root mean squared error
SEPA	Scottish Environment Protection Agency

SRP	soluble reactive phosphorus (≈ ortho-phosphorus, dissolved phosphorus, filterable reactive phosphorus)
TDI	Trophic Diatom Index
TON	total oxidised nitrogen
TN	total nitrogen
TP	total phosphorus
UK TAG	UK Technical Advisory Group (on WFD implementation)
WFD	Water Framework Directive

Glossary

Association	A term used in phytosociology to describe the plant community associated with a particular type of habitat. Each association will have a characteristic assemblage of taxa and a relatively uniform physiognomy.
Biofilm	An aggregation of auto- and heterotrophic micro-organisms on submerged surfaces, along with an associated matrix of extracellular polymeric substances.
Ecological status	An expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters, classified in accordance with Annex V of the WFD.
ECOSTAT	Working group established by the European Commission to produce guidance on the assessment and intercalibration of the ecological status and classification of surface water body types.
Macroalga(e)	Those algae that can be recognised and at least partially identified with the naked eye.
Macrophyte	Larger plants of freshwater which are easily seen with the naked eye, including all aquatic vascular plants, bryophytes, stoneworts (Characeae) and macro-algal growths (CEN, proposed).
Normative definition	Properties of ecological status classes, as described in Annex V of the WFD.
Phytobenthos	All phototrophic algae and cyanobacteria that live on or attached to substrata or other organisms, rather than suspended in the water column (CEN, proposed).
Precautionary principle	Acting now to prevent problems in the future even if there is still scientific doubt about the likelihood or severity of the problem (Environment Agency).
Programme of Measures	Actions taken by a Member State to restore a water body that does not achieve at least good ecological status.
REFCOND	Working group established by European Commission to oversee activities on intercalibration, monitoring, reference conditions and classification of inland waters.
Uncertainty	Parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the quantity subject to measurement (CEN).

Appendicies

Appendix 1: Site details and typologies of lakes in the DALES dataset. Sites are ordered by water body identification number (WBID)

WBID	UK area	Grid reference	Lake name	Site code	GB Lakes Typology					*A priori* status	EQR	Predicted status
					Alk	Depth	Size	Peat	Marl			
2088	S	ND271736	Loch of Mey	MEY	H	V	S	0	0	N/A	0.57	Moderate
2144	S	NC390679	Loch Croispol	CROI	H	D	S	0	1	Good	0.94	High
2161	S	NC381668	Loch Borralie	BORR	H	D	S	0	1	Good	1.06	High
2358	S	ND072602	Loch Calder	CALR	H	S	L	0	0	Good	1.10	High
2490	S	NC463548	Loch Hope	HOPL	L	D	L	0	0	High	1.06	High
2499	S	ND189596	Loch Scarmclate	SCAM	H	V	S	0	0	High	0.79	Good
3904	S	NC621475	Loch Loyal	LOYA	L	D	L	0	0	High	0.96	High
4204	S	ND090482	Loch Meadie	MEAD	L	V	S	0	0	High	0.90	Good
4974	S	NC661448	Loch Syre	SYRE	L	V	S	0	0	N/A	1.09	High
5222	S	NC502410	Loch Meadie	MEAH	L	S	L	0	0	High	1.05	High
5307	S	NC580435	Loch Coulside	COUL	L	V	S	0	0	N/A	1.09	High
5350	S	NC288424	Loch Stack	STAK	L	S	L	0	0	High	1.05	High
5714	S	ND177415	Loch Rangag	RANG	M	S	S	0	0	High	1.09	High
6234	S	NC863390	Loch Culaidh	CULH	L	D	S	0	0	N/A	0.87	Good
6405	S	NC614364	Loch Naver	NAVE	L	S	L	1	0	High	1.09	High
8751	S	NC210245	Loch Assynt	ASSY	M	D	L	0	0	High	1.09	High
8945	S	NC849255	Loch Ascaig	ASCA	L	D	S	1	0	High	0.90	High
9669	S	NC097216	Loch Culag	CULA	L	V	S	0	0	N/A	1.01	High

WBID	UK area	Grid reference	Lake name	Site code	GB Lakes Typology					*A priori* status	EQR	Predicted status
					Alk	Depth	Size	Peat	Marl			
10786	S	NC114139	Loch Sionascaig	SION	L	D	L	0	0	N/A	1.07	High
10934	S	NC213134	Cam Loch	CAM	L	S	L	0	0	High	0.85	Good
11189	S	NC043120	Loch Osgaig	OSGA	L	S	L	0	0	High	1.10	High
11238	S	NC004125	Loch na Béiste	NABE	L	D	S	0	0	Good	1.04	High
11338	S	NC315109	Loch Ailsh	AILS	M	V	L	0	0	High	0.92	High
11355	S	NC262108	Loch Borralan	BORL	L	V	S	0	0	Good	0.76	Good
11611	S	NC852078	Loch Brora	BROR	L	S	L	1	0	High	0.95	High
11642	S	NC624074	Loch Craggie	CRA	L	V	L	0	0	High	0.95	High
12578	S	NH658955	Loch an Lagain	LAGN	L	V	S	1	0	Mod.	0.98	High
12733	S	NG885943	Loch na Béiste	BEIS	L	S	S	0	0	High	1.01	High
12978	S	NF827490	Loch Langabhat	LGBH	L	U	L	0	0	High	0.99	High
14057	S	NG985675	Loch Maree	MARE	L	D	L	0	0	High	0.99	High
14293	S	NH137743	Loch a Bhraoin	BHRA	L	S	L	0	0	N/A	1.03	High
14403	S	NH665736	Loch Achnacloich	ACHN	M	S	S	0	0	High	0.82	Good
15176	S	NF882669	Loch a' Bhuird	BHUI	L	S	S	0	0	N/A	1.13	High
15316	S	NF846663	Loch na Moracha	MORA	L	V	S	1	0	Mod.	0.96	High
15551	S	NF820651	Loch Tormasad	TORM	L	V	S	0	0	Mod.	0.93	High
16456	S	NH505574	Loch Ussie	USSI	M	V	L	0	0	Mod.	0.94	High
16530	S	NH152564	Loch Gowan	GOWA	L	V	S	0	0	N/A	0.97	High
17329	S	NG493493	Loch Fada	FADA	M	D	S	1	0	N/A	0.77	Good

WBID	UK area	Grid reference	Lake name	Site code	GB Lakes Typology					*A priori* status	EQR	Predicted status
					Alk	Depth	Size	Peat	Marl			
17514	S	NG144484	Loch Mór	LMOR	M	S	S	0	0	N/A	0.96	High
18305	S	NF822416	Caslub	CLUB	L	V	S	0	0	N/A	0.92	High
18682	S	NF789376	Loch Druidibeag	DRUI	L	S	L	0	0	High	1.03	High
18825	S	NH971361	Lochindorb	DORB	L	S	L	0	0	High	0.97	High
19170	S	NF740327	West Loch Ollay	OLAW	H	V	S	0	0	Good	0.94	High
19540	S	NH616277	Loch Ruthven	RUTV	M	S	L	0	0	Good	1.02	High
19593	S	NF734268	Loch Aird an Sgairbh	SGAE	L	V	S	0	0	N/A	0.79	Good
20633	S	NH425100	Loch Tarff	TARF	L	S	L	0	0	High	0.97	High
20860	S	NH830044	Loch Insh	INSH	L	S	L	0	0	Mod.	0.88	Good
21189	S	NO442995	Loch Kinnord	KINO	M	V	L	0	0	Good	0.91	High
21191	S	NN913990	Loch Einich	EINI	L	U	L	0	0	Good	1.07	High
22259	S	NN391681	Loch Ossian	OSSI	L	S	L	0	0	N/A	1.11	High
22308	S	NM808678	Loch Doilet	DOI	L	S	L	0	0	High	1.03	High
22395	S	NN087659	Lochan Lùnn Dà – Bhrà	LUNN	M	V	S	0	0	Mod.	0.85	Good
22577	S	NM968632	Loch nan Gabhar	GABH	L	V	S	0	0	High	1.02	High
22782	S	NN610580	Loch Rannoch	RANN	L	D	L	0	0	High	1.01	High
22839	S	NN380542	Loch Laidon	LAI	L	S	L	0	0	High	0.98	High
23557	S	NO042444	Loch of Craiglush	CRAI	M	S	S	0	0	High	1.04	High
23561	S	NO115442	Loch of Clunie	CLUN	M	S	L	0	0	Mod.	1.04	High
24132	S	NN640235	Loch Earn	EARN	M	D	L	0	0	Poor/bad	0.92	High

WBID	UK area	Grid reference	Lake name	Site code	GB Lakes Typology					*A priori* status	EQR	Predicted status
					Alk	Depth	Size	Peat	Marl			
24459	S	NN585130	Loch Lubnaig	LUBN	L	S	L	0	0	High	0.95	High
24919	S	NN580005	Lake of Menteith	MENT	M	S	L	0	0	Mod.	0.75	Good
25899	S	NR284727	Ardnave Loch	ARDN	M	V	S	0	0	Good	0.74	Good
26168	S	NR230657	Loch Gorm	GOR	H	D	L	0	0	N/A	1.04	High
26178	S	NR405662	Loch Ballygrant	BALG	H	S	S	0	0	Good	1.01	High
26217	S	NR408652	Loch Lossit	LOSS	H	D	S	0	0	Good	1.22	High
26257	S	NR341638	Loch Skerrols	SKEL	M	V	S	0	0	High	1.06	High
26944	S	NR301422	Loch Kinnabus	KINB	M	D	S	0	0	High	0.82	Good
27398	S	NS394173	Martnaham Loch	MARH	H	V	S	0	0	Mod.	0.35	Poor
27568	E	NT736033	Catcleugh Reservoir	CATC	M	S	L	0	1	N/A	0.94	High
27698	E	NY686876	Kielder Water	KIEL	M	D	L	0	0	N/A	0.86	Good
27948	S	NX470790	Loch Dee	LDE	L	S	L	0	0	Good	1.02	High
28130	S	NX541691	Loch Grannoch	LGR	L	S	L	0	0	Good	0.98	High
28165	E	NY770696	Greenlee Lough	GREE	M	V	S	0	0	Good	0.83	Good
28172	E	NY790697	Broomlee Lough	BROL	H	V	S	0	1	Good	0.94	High
28220	E	NY766679	Crag Lough	CRAZ	H	V	S	0	1	Good	0.82	Good
28336	S	NX765615	Carlingwark Loch	CARL	H	V	S	0	0	Poor/bad	0.33	Poor
28386	E	NY545587	Talkin Tarn	TALK	M	S	S	0	1	Mod.	0.65	Moderate
28519	E	NZ011522	Derwent Reservoir (North)	DERN	M	S	L	0	0	N/A	0.82	Good

WBID	UK area	Grid reference	Lake name	Site code	GB Lakes Typology					*A priori* status	EQR	Predicted status
					Alk	Depth	Size	Peat	Marl			
28847	E	NY214296	Bassenthwaite Lake	BASS	L	S	L	0	0	Mod.	0.81	Good
28955	E	NY425204	Ullswater	ULLS	M	D	L	0	0	High	0.96	High
28965	E	NY259209	Derwent Water	DERW	L	S	L	0	0	Good	0.90	High
28986	E	NY124217	Loweswater	LOWS	L	S	L	0	0	Mod.	0.92	High
29000	E	NY157188	Crummock Water	CRUM	L	D	L	0	0	High	0.94	High
29021	E	NY313162	Thirlmere	THIR	L	D	L	0	0	Good	0.97	High
29052	E	NY182157	Buttermere	BUTM	L	D	L	0	0	High	0.92	High
29062	E	NY110150	Ennerdale	ENN	L	D	L	0	0	Mod.	0.98	High
29178	E	NY677076	Sunbiggin Tarn	SUNB	H	S	S	0	1	Mod.	0.64	Moderate
29183	E	NY165060	Wastwater	WAST	L	D	L	0	0	High	0.96	High
29184	E	NY338065	Grasmere	GRAS	L	S	L	0	0	Mod.	0.87	Good
29222	E	NY333041	Elter Water	ELTW	M	S	S	0	0	Mod.	0.95	High
29233	E	SD392958	Windermere	WIND	M	D	L	0	0	Mod.	0.87	Good
29270	E	NY366004	Blelham Tarn	BLEL	M	S	S	0	0	Good	0.79	Good
29321	E	SD301940	Coniston Water	CONI	L	D	L	0	0	Mod.	0.88	Good
29328	E	SD358969	Esthwaite Water	ESTH	M	S	L	0	0	Poor/bad	0.63	Moderate
29479	E	SD918874	Semer Water	SEME	H	S	S	0	1	Good	0.62	Moderate
29647	E	SD477766	Hawes Water	HAWE	H	S	S	0	1	Mod.	1.02	High
29844	E	SD895667	Malham Tarn	MALH	H	V	L	0	1	Mod.	0.72	Good
30030	E	SD729553	Stocks Reservoir	STOR	M	S	L	0	0	N/A	0.70	Good

WBID	UK area	Grid reference	Lake name	Site code	GB Lakes Typology					*A priori* status	EQR	Predicted status
					Alk	Depth	Size	Peat	Marl			
30244	E	TA190470	Hornsea Mere	HORN	H	V	L	0	0	Poor/bad	0.27	Poor
30604	E	SD931329	Widdop Reservoir	WIDD	M	S	S	0	0	N/A	N/A	N/A
31104	E	SD970194	White Holme Reservoir	WHIR	L	V	S	1	0	N/A	1.12	High
31942	E	SE036018	Chew Reservoir	CHER	L	D	S	1	0	N/A	1.14	High
32359	E	SK170909	Derwent Reservoir (Midlands)	DERM	M	S	L	0	0	N/A	0.83	Good
32435	W	SH346898	Llyn Llygeirian	LLYG	M	V	S	0	0	Good	0.55	Moderate
32459	E	SK189877	Ladybower Reservoir (site A)	LADY	M	S	L	0	0	N/A	0.71	Good
32538	W	SH392866	Llyn Alaw	ALAW	M	V	L	0	0	Good	0.82	Good
32650	E	SJ744842	Rostherne Mere	ROST	H	S	S	0	0	Poor/bad	0.59	Moderate
32744	E	SJ732818	The Mere (Mere Mere)	MERE	H	V	S	0	0	Mod.	0.91	High
32761	W	SH474819	Llyn yr Wyth-Eidion	WYTH	H	S	S	0	0	High	1.03	High
32804	E	SJ755801	Tatton Mere	TATT	H	S	S	0	0	Mod.	0.36	Poor
32948	W	SH310775	Llyn Dinam	DINA	H	V	S	0	0	Poor/bad	0.35	Poor
32960	E	SJ723769	Tabley Mere	TABL	H	V	S	0	0	Mod.	0.49	Moderate
32961	W	SJ112772	Llyn Helyg	HELY	M	V	S	0	1	Mod.	0.61	Moderate
32968	W	SH313768	Llyn Penrhyn	PERH	H	V	S	0	0	Poor/bad	0.34	Poor
33337	W	SH378700	Llyn Coron	CORO	H	V	S	0	0	Mod.	0.39	Poor
33474	E	SJ575678	Oak Mere	OAK	L	V	S	0	0	Mod.	0.36	Poor

WBID	UK area	Grid reference	Lake name	Site code	GB Lakes Typology					*A priori* status	EQR	Predicted status
					Alk	Depth	Size	Peat	Marl			
33627	W	SH424648	Llyn Rhos-ddu	RHSD	H	V	S	0	0	N/A	0.57	Moderate
33730	W	SH569614	Llyn Padarn	PADA	L	S	L	0	0	Mod.	0.89	Good
33784	E	SJ944598	Rudyard Reservoir	RUDY	H	V	L	0	0	Mod.	0.19	Bad
33803	W	SH659604	Llyn Ogwen (A and B)	OGWE	L	V	S	0	0	Mod.	0.91	High
33836	W	SH645596	Llyn Idwal	IDWA	L	S	S	0	0	High	1.03	High
33962	W	SH898567	Llyn Alwen	ALWN	L	S	S	0	0	High	0.94	High
34002	W	SH560549	Llyn Cwellyn	CWEL	L	D	L	0	0	Mod.	0.92	High
34400	W	SH780463	Llyn Conwy	CON	L	S	S	0	0	High	0.97	High
34480	E	SJ588443	Comber Mere	COMB	H	S	L	0	0	Mod.	0.42	Poor
34622	W	SH402422	Llyn Glasfryn	GLFR	M	V	S	0	0	Poor/bad	0.57	Moderate
34780	W	SJ454395	Hanmer Mere	HANM	H	V	S	0	0	Mod.	0.61	Moderate
34987	W	SH905347	Llyn Tegid or Bala Lake	BALA	L	D	L	0	0	Mod.	0.75	Good
34990	E	SJ406349	The Mere, Ellesmere	ELLE	H	S	S	0	0	Poor/bad	0.32	Poor
35079	E	SJ433332	Colemere	COLE	H	S	S	0	0	Poor/bad	0.40	Poor
35091	E	SJ414329	Whitemere	WHIT	H	S	S	0	0	Mod.	0.24	Poor
35211	E	SJ430305	Crose Mere	CROS	H	S	S	0	0	Poor/bad	0.37	Poor
35561	W	SH648239	Llyn Bodlyn	BODL	L	S	S	0	0	High	0.98	High
35568	W	SH990213	Lake Vyrnwy/Llyn Efyrnwy	VERN	L	D	L	0	0	N/A	0.72	Good
35640	E	TG414222	Hickling Broad	HICK	H	V	L	0	0	Mod.	0.90	Good

WBID	UK area	Grid reference	Lake name	Site code	GB Lakes Typology					*A priori* status	EQR	Predicted status
					Alk	Depth	Size	Peat	Marl			
35724	E	SJ772204	Aqualate Mere	AQUA	H	V	L	0	0	Poor/bad	0.27	Poor
35953	E	TG308165	Wroxham Broad	WROX	H	V	S	0	0	Poor/bad	0.40	Poor
35981	E	TG464142	Rollesby Broad	ROLL	H	V	L	0	0	Poor/bad	0.53	Moderate
36202	E	TG388134	Upton Broad	UPTO	H	V	S	0	0	High	1.04	High
36331	E	SK545108	Cropston Reservoir	CROP	H	S	L	0	0	N/A	0.27	Poor
36405	W	ST850421	Tal-y-llyn Lake	TALY	L	V	L	0	0	High	0.82	Good
36479	E	SK936081	Rutland Water	RUTL	H	S	L	0	0	N/A	0.31	Poor
36523	E	SK034077	Chasewater	CHAS	H	S	L	0	0	N/A	0.65	Moderate
36544	E	SJ498080	Bomere Pool	BOME	H	S	S	0	0	Poor/bad	0.92	High
36566	E	SJ510078	Betton Pool	BETT	H	S	S	0	0	Poor/bad	0.32	Poor
38214	E	SN899691	Craig Goch Reservoir	GOCH	L	D	L	0	0	N/A	1.04	High
38310	E	TL148692	Grafham Water	GRAF	H	D	L	0	0	N/A	0.14	Bad
38390	W	SN783675	Llyn Teifi	TEIF	L	D	S	0	0	Good	1.01	High
38394	W	SN789675	Llyn Hîr	HIR	L	V	S	0	0	Good	0.95	High
38409	W	SN792671	Llyn Egnant	EGNA	L	S	S	0	0	High	0.94	High
38422	W	SN606670	Llyn Eiddwen	EIDD	L	V	S	0	0	Good	0.93	High
38525	W	SN800647	Llyn Gynon	GYN	L	V	S	0	0	Good	1.00	High
38907	W	SN743568	Llyn Berwyn	BER	L	S	S	1	0	High	0.94	High
39450	E	TL005428	Stewartby Lake	STBY	H	S	L	0	0	N/A	0.17	Bad
39967	E	SN828292	Usk Reservoir	USK	M	S	L	0	0	Good	0.90	High

WBID	UK area	Grid reference	Lake name	Site code	GB Lakes Typology					*A priori* status	EQR	Predicted status
					Alk	Depth	Size	Peat	Marl			
40067	W	SO132265	Llangorse Lake	LLAN	H	V	L	0	0	Poor/bad	0.33	Poor
40755	E	TL230109	Stanborough Lake	STAB	H	V	VS	0	0	N/A	0.38	Poor
41427	E	TQ742982	Hanningfield Reservoir	HANN	H	S	L	0	0	N/A	0.20	Bad
41559	E	SU063968	Cotswold WP Lake No. 12	COTS	H	V	S	0	0	N/A	0.58	Moderate
41602	W	SR976946	Lily Ponds (Bosh Cent)	BOSH	H	V	S	0	1	Mod.	0.78	Good
42170	W	SS796815	Kenfig Pool	KENF	H	V	S	0	0	Mod.	0.65	Moderate
42639	E	TQ072696	Queen Mary Reservoir (D)	QUEE	H	S	L	0	0	N/A	0.19	Bad
43096	E	ST563605	Chew Valley Lake	CHEW	H	S	L	0	0	N/A	0.21	Bad
43135	E	ST514596	Blagdon Lake	BLAG	H	S	L	0	0	N/A	0.40	Poor
43348	E	ST447536	Cheddar Reservoir	CHED	H	V	L	0	0	N/A	0.98	High
43602	E	TQ498481	Bough Beech Reservoir	BOUG	H	S	L	0	0	N/A	0.18	Bad
43909	E	ST850421	Shearwater Lake	SHEA	H	V	S	0	0	Good	0.78	Good
43943	E	SU859414	Frensham Little Pond	PFRE	H	V	S	0	0	Mod.	0.74	Good
44031	E	SU845401	Frensham Great Pond	FREN	H	V	S	0	0	Mod.	0.56	Moderate
44471	E	SS972304	Wimbleball	WIMB	M	S	L	0	0	N/A	0.80	Good
44518	E	ST937311	Fonthill Lake	FONT	H	V	VS	0	0	Mod.	0.41	Poor
45108	E	SU974175	Burton Mill Pond	BURT	H	V	S	0	0	Good	0.72	Good
45652	E	SU367016	Hatchet Pond	HATC	M	V	VS	0	0	Good	0.81	Good

WBID	UK area	Grid reference	Lake name	Site code	GB Lakes Typology					*A priori* status	EQR	Predicted status
					Alk	Depth	Size	Peat	Marl			
46102	E	SZ029846	Little Sea Mere	LITT	M	V	S	0	0	High	0.93	High
46232	E	SX194745	Dozmary Pool	DOZM	L	V	S	0	0	Poor/bad	0.72	Good
46279	E	SX556685	Burrator Reservoir	BURR	L	S	L	0	0	N/A	0.90	Good
46472	E	SX824435	Slapton Ley	SLT	H	V	L	0	0	Poor/bad	0.72	Good
46501	E	SW713362	Stithians Reservoir	STIT	M	S	L	0	0	N/A	0.97	High
46556	E	SW648248	The Loe	TLOE	M	S	L	0	0	Mod.	0.21	Bad

Lakes are ordered by WBID. The areas E, S and W refer to UK lake locations – England, Scotland and Wales respectively. H, M and L correspond to 'high', 'medium' and 'low' alkalinity (Alk) waters respectively and D, S and V correspond to 'deep', 'shallow' and 'very shallow' lake depths. In relation to lake size, L, S and VS correspond to 'large', 'small' and 'very small'. Lake altitude (Alt) is classified as either High, Mid or Low. '*A priori*' status is current status based on existing chemical, non-diatom biological and palaeoecological evidence. See chapter 5 for derivation of EQRs and predicted status.

Appendix 2: Summary of samples in the DALES dataset. Lakes are ordered by typology

WBID	Area	GridRef	Lake name	Site code	Typology		SU03		AU03		SP04		SU04		AU04	
					Alk	Depth	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant
2144	S	NC390679	Loch Croispol	CROI	H	D	1	1	1	1	1					
2161	S	NC381668	Loch Borralie	BORR	H	D	1	1	1	1	1					
26168	S	NR230657	Loch Gorm	GOR	H	D					1		1		1	
26217	S	NR408652	Loch Lossit	LOSS	H	D					1		1		1	
38310	E	TL148692	Grafham Water	GRAF	H	D					1				1	
2358	S	ND072602	Loch Calder	CALR	H	S	1	1	1		1					
26178	S	NR405662	Loch Ballygrant	BALG	H	S					1		1		1	
29178	E	NY677076	Sunbiggin Tarn	SUNB	H	S					1		1		1	
29479	E	SD918874	Semer Water	SEME	H	S			1		1		1		1	
29647	E	SD477766	Hawes Water	HAWE	H	S			1		1			1	1	
32650	E	SJ744842	Rostherne Mere	ROST	H	S						1		1		1
32761	W	SH474819	Llyn yr Wyth-Eidion	WYTH	H	S								1		1
32804	E	SJ755801	Tatton Mere	TATT	H	S					1		1		1	1
34480	E	SJ588443	Comber Mere	COMB	H	S					1	1	1	1	1	1
34990	E	SJ406349	The Mere, Ellesmere	ELLE	H	S			1		1		1		1	
35079	E	SJ433332	Colemere	COLE	H	S					1		1		1	
35091	E	SJ414329	Whitemere	WHIT	H	S					1		1		1	
35211	E	SJ430305	Crose Mere	CROS	H	S			1		1		1		1	
36331	E	SK545108	Cropston Reservoir	CROP	H	S					1		1		1	
36479	E	SK936081	Rutland Water	RUTL	H	S					1		1			
36523	E	SK034077	Chasewater	CHAS	H	S			1		1		1		1	
36544	E	SJ498080	Bomere Pool	BOME	H	S					1				1	

WBID	Area	GridRef	Lake name	Site code	Typology		SU03		AU03		SP04		SU04		AU04	
					Alk	Depth	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant
36566	E	SJ510078	Betton Pool	BETT	H	S			1		1		1		1	
39450	E	TL005428	Stewartby Lake	STBY	H	S					1		1		1	
41427	E	TQ742982	Hanningfield Reservoir	HANN	H	S					1		1		1	
42639	E	TQ072696	Queen Mary Reservoir (D)	QUEE	H	S					1		1		1	
43096	E	ST563605	Chew Valley Lake	CHEW	H	S			1		1		1		1	
43135	E	ST514596	Blagdon Lake	BLAG	H	S			1		1		1		1	
43602	E	TQ498481	Bough Beech Reservoir	BOUG	H	S					1	1	1		1	
2088	S	ND271736	Loch of Mey	MEY	H	V					1		1			
2499	S	ND189596	Loch Scarmclate	SCAM	H	V					1		1	1	1	
19170	S	NF740327	West Loch Ollay	OLAW	H	V					1		1		1	
27398	S	NS394173	Martnaham Loch	MARH	H	V					1		1		1	
28172	E	NY790697	Broomlee Lough	BROL	H	V			1		1		1		1	
28220	E	NY766679	Crag Lough	CRAZ	H	V			1		1		1		1	
28336	S	NX765615	Carlingwark Loch	CARL	H	V	1	1	1	1	1	1				
29844	E	SD895667	Malham Tarn	MALH	H	V			1				1			
30244	E	TA190470	Hornsea Mere	HORN	H	V			1						1	
32744	E	SJ732818	The Mere (Mere Mere)	MERE	H	V					1	1				1
32948	W	SH310775	Llyn Dinam	DINA	H	V			1				1		1	
32960	E	SJ723769	Tabley Mere	TABL	H	V						1		1		1
32968	W	SH313768	Llyn Penrhyn	PERH	H	V								1		1
33337	W	SH378700	Llyn Coron	CORO	H	V				1		1	1	1	1	
33627	W	SH424648	Llyn Rhos-ddu	RHSD	H	V				1				1		1
33784	E	SJ944598	Rudyard Reservoir	RUDY	H	V					1		1		1	

WBID	Area	GridRef	Lake name	Site code	Typology		SU03		AU03		SP04		SU04		AU04	
					Alk	Depth	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant
34780	W	SJ454395	Hanmer Mere	HANM	H	V			1			1		1		1
35640	E	TG414222	Hickling Broad	HICK	H	V						1		1		1
35724	E	SJ772204	Aqualate Mere	AQUA	H	V						1		1		1
35953	E	TG308165	Wroxham Broad	WROX	H	V					1	1	1	1		1
35981	E	TG464142	Rollesby Broad	ROLL	H	V					1		1	1	1	
36202	E	TG388134	Upton Broad	UPTO	H	V						1		1		1
40067	W	SO132265	Llangorse Lake	LLAN	H	V			1		1				1	
40755	E	TL230109	Stanborough Lake	STAB	H	V					1		1		1	
41559	E	SU063968	Cotswold WP Lake No. 12	COTS	H	V					1					
41602	W	SR976946	Lily Ponds (Bosh Cent)	BOSH	H	V			1		1		1		1	
42170	W	SS796815	Kenfig Pool	KENF	H	V			1		1		1		1	
43348	E	ST447536	Cheddar Reservoir	CHED	H	V						1		1	1	
43909	E	ST850421	Shearwater Lake	SHEA	H	V					1		1		1	
43943	E	SU859414	Frensham Little Pond	PFRE	H	V						1		1		1
44031	E	SU845401	Frensham Great Pond	FREN	H	V				1		1		1		1
44518	E	ST937311	Fonthill Lake	FONT	H	V					1		1		1	
45108	E	SU974175	Burton Mill Pond	BURT	H	V				1		1		1		1
46472	E	SX824435	Slapton Ley	SLT	H	V			1		1					
8751	S	NC210245	Loch Assynt	ASSY	M	D					1		1		1	
17329	S	NG493493	Loch Fada	FADA	M	D					1		1		1	
24132	S	NN640235	Loch Earn	EARN	M	D					1		1			
26944	S	NR301422	Loch Kinnabus	KINB	M	D					1		1		1	
27698	E	NY686876	Kielder Water	KIEL	M	D					1		1		1	

WBID	Area	GridRef	Lake name	Site code	Typology		SU03		AU03		SP04		SU04		AU04	
					Alk	Depth	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant
28955	E	NY425204	Ullswater	ULLS	M	D					1		1		1	
29233	E	SD392958	Windermere	WIND	M	D					1		1		1	
5714	S	ND177415	Loch Rangag	RANG	M	S					1		1		1	
14403	S	NH665736	Loch Achnacloich	ACHN	M	S					1	1	1		1	
17514	S	NG144484	Loch Mór	LMOR	M	S					1		1		1	
19540	S	NH616277	Loch Ruthven	RUTV	M	S	1	1	1		1					
23557	S	NO042444	Loch of Craiglush	CRAI	M	S	1	1	1	1	1					
23561	S	NO115442	Loch of Clunie	CLUN	M	S	1	1	1		1	1				
24919	S	NN580005	Lake of Menteith	MENT	M	S			1		1		1			
27568	E	NT736033	Catcleugh Reservoir	CATC	M	S					1		1		1	
28386	E	NY545587	Talkin Tarn	TALK	M	S					1		1		1	
28519	E	NZ011522	Derwent Reservoir (North)	DERN	M	S					1		1		1	
29222	E	NY333041	Elter Water	ELTW	M	S					1		1		1	
29270	E	NY366004	Blelham Tarn	BLEL	M	S						1		1	1	
29328	E	SD358969	Esthwaite Water	ESTH	M	S			1		1		1		1	
30030	E	SD729553	Stocks Reservoir	STOR	M	S					1		1		1	
30604	E	SD931329	Widdop Reservoir	WIDD	M	S										
32359	E	SK170909	Derwent Reservoir (Midlands)	DERM	M	S					1					
32459	E	SK189877	Ladybower Reservoir (site A)	LADY	M	S					1					
39967	E	SN828292	Usk Reservoir	USK	M	S					1		1		1	
44471	E	SS972304	Wimbleball	WIMB	M	S					1	1				
46501	E	SW713362	Stithians Reservoir	STIT	M	S					1		1		1	
46556	E	SW648248	The Loe	TLOE	M	S			1		1		1		1	

WBID	Area	GridRef	Lake name	Site code	Typology		SU03		AU03		SP04		SU04		AU04	
					Alk	Depth	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant
11338	S	NC315109	Loch Ailsh	AILS	M	V					1		1		1	
16456	S	NH505574	Loch Ussie	USSI	M	V					1	1				1
21189	S	NO442995	Loch Kinnord	KINO	M	V	1	1	1	1						
22395	S	NN087659	Lochan Lùnn Dà – Bhrà	LUNN	M	V					1		1		1	
25899	S	NR284727	Ardnave Loch	ARDN	M	V					1		1		1	
26257	S	NR341638	Loch Skerrols	SKEL	M	V					1		1		1	
28165	E	NY770696	Greenlee Lough	GREE	M	V			1		1		1		1	
32435	W	SH346898	Llyn Llygeirian	LLYG	M	V			1		1		1		1	
32538	W	SH392866	Llyn Alaw	ALAW	M	V					1		1		1	
32961	W	SJ112772	Llyn Helyg	HELY	M	V								1		1
34622	W	SH402422	Llyn Glasfryn	GLFR	M	V					1		1		1	
45652	E	SU367016	Hatchet Pond	HATC	M	V			1		1				1	
46102	E	SZ029846	Little Sea Mere	LITT	M	V				1		1		1		1
2490	S	NC463548	Loch Hope	HOPL	L	D					1		1		1	
3904	S	NC621475	Loch Loyal	LOYA	L	D					1		1		1	
6234	S	NC863390	Loch Culaidh	CULH	L	D					1		1		1	
8945	S	NC849255	Loch Ascaig	ASCA	L	D					1		1		1	
10786	S	NC114139	Loch Sionascaig	SION	L	D					1		1		1	
11238	S	NC004125	Loch na Béiste	NABE	L	D					1		1		1	
14057	S	NG985675	Loch Maree	MARE	L	D					1				1	
22782	S	NN610580	Loch Rannoch	RANN	L	D			1		1					
29000	E	NY157188	Crummock Water	CRUM	L	D					1		1		1	
29021	E	NY313162	Thirlmere	THIR	L	D					1		1		1	

WBID	Area	GridRef	Lake name	Site code	Typology		SU03		AU03		SP04		SU04		AU04	
					Alk	Depth	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant
29052	E	NY182157	Buttermere	BUTM	L	D			1		1		1		1	
29062	E	NY110150	Ennerdale	ENN	L	D			1		1		1		1	
29183	E	NY165060	Wastwater	WAST	L	D			1		1		1		1	
29321	E	SD301940	Coniston Water	CONI	L	D			1		1		1		1	
31942	E	SE036018	Chew Reservoir	CHER	L	D					1					
34002	W	SH560549	Llyn Cwellyn	CWEL	L	D			1		1		1		1	
34987	W	SH905347	Llyn Tegid or Bala Lake	BALA	L	D			1		1		1		1	
35568	W	SH990213	Lake Vyrnwy/Llyn Efyrnwy	VERN	L	D					1		1		1	
38214	E	SN899691	Craig Goch Reservoir	GOCH	L	D			1		1		1		1	
38390	W	SN783675	Llyn Teifi	TEIF	L	D					1		1		1	
5222	S	NC502410	Loch Meadie	MEAH	L	S					1		1		1	
5350	S	NC288424	Loch Stack	STAK	L	S					1		1		1	
6405	S	NC614364	Loch Naver	NAVE	L	S					1		1		1	
10934	S	NC213134	Cam Loch	CAM	L	S	1		1		1					
11189	S	NC043120	Loch Osgaig	OSGA	L	S	1	1	1	1	1					
11611	S	NC852078	Loch Brora	BROR	L	S					1		1		1	
12733	S	NG885943	Loch na Béiste	BEIS	L	S					1		1		1	
14293	S	NH137743	Loch a Bhraoin	BHRA	L	S	1	1	1	1	1					
15176	S	NF882669	Loch a' Bhuird	BHUI	L	S					1		1		1	
18682	S	NF789376	Loch Druidibeag	DRUI	L	S					1		1			
18825	S	NH971361	Lochindorb	DORB	L	S	1	1	1		1					
20633	S	NH425100	Loch Tarff	TARF	L	S	1	1	1		1					
20860	S	NH830044	Loch Insh	INSH	L	S	1	1	1		1					

WBID	Area	GridRef	Lake name	Site code	Typology		SU03		AU03		SP04		SU04		AU04	
					Alk	Depth	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant
22259	S	NN391681	Loch Ossian	OSSI	L	S	1		1		1					
22308	S	NM808678	Loch Doilet	DOI	L	S	1		1		1	1				
22839	S	NN380542	Loch Laidon	LAI	L	S					1		1			
24459	S	NN585130	Loch Lubnaig	LUBN	L	S		1	1		1					
27948	S	NX470790	Loch Dee	LDE	L	S	1	1	1	1	1	1				
28130	S	NX541691	Loch Grannoch	LGR	L	S	1	1	1		1	1				
28847	E	NY214296	Bassenthwaite Lake	BASS	L	S			1		1		1		1	
28965	E	NY259209	Derwent Water	DERW	L	S			1		1		1		1	
28986	E	NY124217	Loweswater	LOWS	L	S			1		1		1		1	
29184	E	NY338065	Grasmere	GRAS	L	S			1		1		1		1	
33730	W	SH569614	Llyn Padarn	PADA	L	S			1		1x2		1		1	
33836	W	SH645596	Llyn Idwal	IDWA	L	S					1		1		1	
33962	W	SH898567	Llyn Alwen	ALWN	L	S			1				1		1x2	
34400	W	SH780463	Llyn Conwy	CON	L	S					1		1		1	
35561	W	SH648239	Llyn Bodlyn	BODL	L	S					1		1		1	
38409	W	SN792671	Llyn Egnant	EGNA	L	S					1		1		1	
38907	W	SN743568	Llyn Berwyn	BER	L	S					1		1		1	
46279	E	SX556685	Burrator Reservoir	BURR	L	S					1		1		1	
12978	S	NF827490	Loch Langabhat	LGBH	L	U					1		1		1	
21191	S	NN913990	Loch Einich	EINI	L	U	1									
4204	S	ND090482	Loch Meadie	MEAD	L	V					1		1		1	
4974	S	NC661448	Loch Syre	SYRE	L	V					1		1		1	
5307	S	NC580435	Loch Coulside	COUL	L	V					1		1		1	

WBID	Area	GridRef	Lake name	Site code	Typology		SU03		AU03		SP04		SU04		AU04	
					Alk	Depth	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant	Rock	Plant
9669	S	NC097216	Loch Culag	CULA	L	V					1		1		1	
11355	S	NC262108	Loch Borralan	BORL	L	V					1		1		1	
11642	S	NC624074	Loch Craggie	CRA	L	V					1		1		1	
12578	S	NH658955	Loch an Lagain	LAGN	L	V					1		1		1	
15316	S	NF846663	Loch na Moracha	MORA	L	V					1		1		1	
15551	S	NF820651	Loch Tormasad	TORM	L	V					1		1		1	
16530	S	NH152564	Loch Gowan	GOWA	L	V					1		1		1	
18305	S	NF822416	Caslub	CLUB	L	V					1		1		1	
19593	S	NF734268	Loch Aird an Sgairbh	SGAE	L	V					1		1		1	
22577	S	NM968632	Loch nan Gabhar	GABH	L	V					1		1		1	
31104	E	SD970194	White Holme Reservoir	WHIR	L	V							1		1	
33474	E	SJ575678	Oak Mere	OAK	L	V								1		
33803	W	SH659604	Llyn Ogwen (A and B)	OGWE	L	V					1x2		1		1	
36405	W	ST850421	Tal-y-llyn Lake	TALY	L	V					1		1		1	
38394	W	SN789675	Llyn Hîr	HIR	L	V					1		1		1	
38422	W	SN606670	Llyn Eiddwen	EIDD	L	V			1		1		1		1	
38525	W	SN800647	Llyn Gynon	GYN	L	V			1		1		1			
46232	E	SX194745	Dozmary Pool	DOZM	L	V					1		1		1	

Lakes are ordered by typology, where H, M and L correspond to 'high', 'medium' and 'low' alkalinity waters respectively and D, S and V correspond to 'deep', 'shallow' and 'very shallow' lake depths. SP, SU and AU correspond to the seasons in which phytobenthos samples were taken – 'spring', 'summer' and 'autumn' respectively. 03 and 04 correspond to the years in which samples were taken, i.e. 2003 and 2004 respectively. The areas E, S and W refer to UK lake locations – England, Scotland and Wales respectively.

Appendix 3: Sites identified as potential reference lakes in the DALES dataset

WBID	Lake name	Typology		Chord distance	Depth of reference sample (cm)	Core dated?/ Dating method	Potential reference sites		
		Alk	Depth				Ref Palaeo	Ref MEI	Ref GIG
32761	Llyn yr Wyth-Eidion	H	S	0.319	5	None	1	1	0
2499	Loch Scarmclate	H	V				0	1	0
36202	Upton Broad	H	V	0.421	50	R	1	1	1
44031	Frensham Great Pond	H	V	0.156	30	SCPs	1	0	0
45108	Burton Mill Pond	H	V	0.257	70	SCPs	1	0	0
8751	Loch Assynt	M	D				0	1	0
26944	Loch Kinnabus	M	D	0.418	20		1	0	0
28955	Ullswater	M	D	0.277	20	SCPs	1	0	0
14403	Loch Achnacloich	M	S	0.336	40	None	1	0	0
23557	Loch of Craiglush	M	S	0.324	84	None	1	0	0
29270	Blelham Tarn	M	S	0.332	30	SCPs	1	0	0
11338	Loch Ailsh	M	V	0.415	40	None	1	1	0
16456	Loch Ussie	M	V	0.438	90	R	1	1	0
21189	Loch Kinnord	M	V	0.301	40	R	1	0	0
46102	Little Sea Mere	M	V	0.317	56	SCPs	1	0	0
2490	Loch Hope	L	D	0.403	20	None	1	0	0
3904	Loch Loyal	L	D				0	1	1
8945	Loch Ascaig	L	D	0.318	20	None	1	0	0
11238	Loch na Béiste	L	D	0.266	29	None	1	0	0
14057	Loch Maree	L	D	0.129	35	R	1	1	1
22782	Loch Rannoch	L	D	0.253	3	R	1	1	0
29000	Crummock Water	L	D	0.160	36	SCPs	1	0	0
29052	Buttermere	L	D	0.476	24	SCPs	0	1	1
29183	Wastwater	L	D	0.436	15	R	0	1	1
5222	Loch Meadie	L	S				0	1	1
5350	Loch Stack	L	S				0	1	1
6405	Loch Naver	L	S				0	1	1
10934	Cam Loch	L	S				0	1	1
11189	Loch Osgaig	L	S				0	1	1
11611	Loch Brora	L	S				0	1	1
12733	Loch na Béiste	L	S	0.403	22	None	1	0	0
18682	Loch Druidibeag	L	S	N/A			0	1	0
18825	Lochindorb	L	S				0	1	1
20633	Loch Tarff	L	S	0.265	19	None	1	0	0
22308	Loch Doilet	L	S	0.349	36	R	1	0	0
22839	Loch Laidon	L	S	0.427	26	R	1	0	0
24459	Loch Lubnaig	L	S	0.204	32	R	1	1	1
33836	Llyn Idwal	L	S	0.314	24	SCPs	1	1	0

WBID	Lake name	Typology		Chord distance	Depth of reference sample (cm)	Core dated?/ Dating method	Potential reference sites		
		Alk	Depth				Ref Palaeo	Ref MEI	Ref GIG
11642	Loch Craggie	L	V	0.269	30	None	1	1	0
12578	Loch an Lagain	L	V	0.418	20	None	1	0	0
22577	Loch nan Gabhar	L	V	0.307	15		1	0	0
36405	Tal-y-llyn Lake	L	V	0.359	20	None	1	0	0
12978	Loch Langabhat	L	U				0	1	0

Dating method: R refers to 'radiometric' dating methods and SCPs refers to the 'spheroidal carbonaceous particle' dating techniques.

Potential reference sites were selected using one or more of the following techniques:

a) **Ref Palaeo** palaeolimnological methods. Grey shading indicates possible reference sites that have been identified by palaeoecological methods. However there is uncertainty in the 'reference' status of these lakes because either palaeo records are based on short sediment cores, or other available evidence is conflicting and suggests non-reference status or the squared chord distance is slightly higher than the 2.5th percentile (0.39).

b) **Ref MEI** the GB calibrated morphoedaphic index (MEI).

c) **Ref GIG** results from the EU Rebecca project intercalibration exercise.

'Chord distance' is a statistical measure that can be used to assess the degree of floristic change between reference and present day sediment core samples. The 2.5th percentile (score < 0.39) is used to define reference sites, i.e. those with low floristic change between reference and present day samples. See chapter 5 for a detailed description of the chord distance measure.

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Published by:

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