

Evidence

Assessment of ecological status in UK lakes using diatoms

Report – SC070034/TR3

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Miranda Kavanagh
Director of Evidence

Executive summary

1. This report describes an ecological classification tool for lakes based on diatoms, a key component of the biological quality element 'macrophytes and phytobenthos'.
2. A database of benthic diatom samples collected since 2003, specifically for development of the tool, was assembled. In total 1079 samples from 228 lakes were included in the database with matching environmental data. The dataset was divided into three lake types – low, medium and high alkalinity.
3. 'Reference sites', relatively unimpacted by human activity, were selected from this database using a range of criteria including evidence of degree of change based on palaeolimnological data, and were used to develop a type-specific reference typology.
4. A Lake Trophic Diatom Index (LTDI) was developed and ecological quality ratios (EQRs) were generated for each lake type that vary from ≥ 1 , where the diatom assemblage showed no impact, to (theoretically) 0, when the diatom assemblage was indicative of major anthropogenic activities.
5. The boundary between 'high' and 'good' status was defined as the 25th percentile of EQRs of all reference sites. The boundary between 'good' and 'moderate' status was set at the point at which nutrient-sensitive and nutrient-tolerant taxa were present in equal relative abundance. The 'moderate'/'poor' and 'poor'/'bad' boundaries were then defined by equal division of the remaining EQR gradient.
6. The reference data were employed to predict the 'expected' class at each site and these were compared with the classifications derived from the tool. For lakes identified as reference sites, 68% were classified as high with the remainder classified as good. For non-reference lakes the model predicted the majority of sites to be good or worse. The model appears to perform well.
7. The performance of the model was further tested by application to a set of 17 Cumbrian lakes for which classification based on a range of other tools was available. There was a tendency for the diatom tool classifications to be more relaxed than those using other tools, with 76% of lakes being placed in a higher status class than that indicated by the entire suite of biological metrics. The findings suggest that the reference community in low alkalinity lakes might be 'buffered' against change as the dominant taxa appear to be relatively tolerant to mild enrichment.
8. In an attempt to improve the predictions for low alkalinity lakes the response of surface sediment diatom assemblages along the nutrient gradient was explored, as these potentially contain a more diverse benthic diatom assemblage as well as planktonic taxa. However, a set of weighted averaging transfer functions offered no improvement over the diatom tool based on epilithic and epiphytic assemblages.
9. In conclusion, for low alkalinity lakes in particular, DALES (Diatoms for Assessing Lake/Loch Ecological Status) is more relaxed than other UK tools. However, DALES gives an accurate assessment of the condition of the littoral biofilm and thus provides a WFD-compliant tool to form part of the toolkit for classifying UK standing waters.

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Contents

| | | |
|----------|---|-----------|
| 1 | Introduction | 1 |
| 2 | Methods | 2 |
| 2.1 | Phytoplankton collection and identification | 2 |
| 2.2 | Environmental data | 2 |
| 2.3 | Sites | 3 |
| 2.4 | Defining reference lakes | 6 |
| 2.5 | Deriving a pressure metric: the Lake Trophic Diatom Index | 8 |
| 3 | Results | 9 |
| 3.1 | The Lake Trophic Diatom Index (LTDI) | 9 |
| 3.2 | Effect of season and substratum on LTDI | 11 |
| 3.3 | Calculation of expected LTDI at reference conditions and calculation of EQR | 12 |
| 3.4 | Defining the class boundaries | 12 |
| 3.5 | Predicted class status | 15 |
| 3.6 | Validation using the Cumbrian lakes dataset | 16 |
| 3.7 | Surface sediment analysis | 19 |
| 4 | Discussion | 21 |
| 4.1 | Definition of reference sites and ‘high status’ | 21 |
| 4.2 | Measuring deviations from reference conditions | 22 |
| 4.3 | Definition of ‘good status’ and the good/moderate boundary | 22 |
| 5 | Conclusion | 24 |
| | References | 25 |
| | Appendices | 30 |
| | Appendix A: Diatom full names and diatcodes | 30 |
| | Appendix B: Classification of lakes using the diatom tool | 31 |

1 Introduction

This study forms part of the DARLEQ project (Diatom Assessment of River and Lake Ecological Quality). It builds on the earlier DARES (Diatoms for Assessing River Ecological Status) and DALES (Diatoms for Assessing Lake/Loch Ecological Status) projects, which aimed at producing predictive models for evaluating ecological status based on benthic diatoms in UK rivers and lakes, respectively, as required by the European Union's Water Framework Directive (WFD) (European Union 2000). The overall objective of both projects was to develop a robust operational tool 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, Environment Agency Wales, the Scottish Environment Protection Agency (SEPA) and the Northern Ireland Environment Agency (NIEA).

This section provides an overview of the diatom-based tool for assessing ecological status in lakes. Phase 1 of this work (DALES) was the first attempt to define the benthic diatom flora of UK lakes that is expected in the absence of significant anthropogenic activity. It provides a foundation upon which the statutory agencies could start monitoring to assess the status of UK freshwaters, and a statistically sound basis for determining the need or otherwise of Programmes of Measures. However, the study highlighted a number of areas where this work could be developed including addition of further samples and refinement and improvement of the model itself (Kelly *et al.* 2007), and it is these developments that are reported here.

The definition of ecological status in Article 2 of the WFD refers to 'structure' and 'functioning' (European Union 2000). However, Annex V refers only to taxonomic composition and abundance for macrophytes and phytobenthos. Weighted-average metrics such as the Trophic Diatom Index (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. Although the TDI was developed for rivers, the same concept can, with recalibration, also be applied to lakes. This is considered preferable to the use of a diatom-based total phosphorus (TP) transfer function, which is already available for UK lakes (e.g. Bennion *et al.* 1996) as the latter does not express results in terms that are compatible with the ecological concepts at the heart of the WFD.

2 Methods

2.1 Phytobenthos collection and identification

Five cobbles were collected from the littoral zone of each lake away from inflow streams and obvious human impacts. The cobbles were placed into a tray with a little lake water and the top surface of each was brushed with a clean toothbrush in order to remove the biofilm (Kelly *et al.* 1998, CEN 2003). 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 (King *et al.* 2006). 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 (after Battarbee *et al.* 2001).

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). Intraspecific taxa were merged for those species where a preliminary examination of taxon–environment scatterplots suggested that the response of intraspecific taxa were not distinguishable from that of the species. For example, *Frustulia rhomboides* varieties were merged; *Cocconeis placentula* varieties were merged; *Fragilaria capucina* var. *capucina*, var. *rumpens* and var. *gracilis* were merged to *F. capucina*; *Synedra tenera* was merged to *S. nana*; fine and coarse forms of *Fragilaria vaucheriae* were merged; all forms of *Tabellaria flocculosa* were merged; all *Planothidium* species that were formerly varieties and subspecies of *Achnanthes lanceolata* were merged to *P. lanceolatum*; *Gomphonema angustum* and *G. pumilum* were merged. Small or benthic *Fragilaria* (*sensu lato*) refers to those *Fragilaria* spp. now considered to be separate genera: *Pseudostaurosira*, *Staurosira* and *Staurosirella*.

2.2 Environmental data

Environmental data linked to the diatom samples were extracted from databases held by the Environment Agency and SEPA. Ideally, the diatom data should have been matched with environmental data collected over the corresponding time periods. 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. 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 silica (SiO₂) and total oxidised nitrogen (TON) also have many missing values.

For simplicity, we have used TP to summarise the pressure gradient. In practice, TP and TN are highly correlated in this dataset ($r = 0.72$, $p < 0.001$) and there is evidence that some lakes, particularly upland, soft-waters, are N limited (Maberley *et al.* 2003).

2.3 Sites

The Phase 1 dataset consisted of 577 samples taken from the littoral zones of 177 English, Scottish and Welsh lakes during 2003–2004. In Phase 2, 502 additional samples were added to give a combined dataset of 1079 samples from 228 lakes. Although no data from Northern Ireland were included, the methods described here are not precluded from use there; the Lake Trophic Diatom Index has been implemented in the Republic of Ireland and Irish data will be included in the lake phytobenthos intercalibration exercise.

The sites represent a range of lake types. The reporting typology for ecoregion 18 (Great Britain – GB) divides lakes into 18 types based on the base status of their catchment geology and their mean depth (Phillips 2003). 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.* 2003b). 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).

However, for the purposes of this 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 (LA; <10 mg l⁻¹ CaCO₃), medium (MA; 10–50 mg l⁻¹ CaCO₃) and high alkalinity (HA; >50 mg l⁻¹ CaCO₃). There were three 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, and (iii) 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. Importantly, the first of these 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, indicating that the phytobenthos does not discriminate between deep, shallow and very shallow lake types. The typology also did not discriminate between humic and clear lakes but there was no indication in the results that lakes that were known to be humic responded differently to non-humic lakes.

The original dataset contained 51 samples from lakes with low pH (<5.5) and/or low alkalinity (<10 mg l⁻¹ CaCO₃). These sites are almost certainly under pressure from acidification and so these samples were removed to avoid the confounding effects of an acidity gradient on the nutrient tool development.

The distribution of sites and samples across the different lake typologies is shown in Table 2.1.

Table 2.1. The total number of samples (sites) in the lakes dataset by alkalinity and depth class

| | | Alkalinity class | | | |
|-------------|--------------|------------------|----------|----------|------------|
| | | Low | Medium | High | Total |
| Depth class | Very shallow | 87 (21) | 61 (13) | 209 (42) | 357 (76) |
| | Shallow | 227 (39) | 86 (21) | 110 (24) | 423 (84) |
| | Deep | 130 (25) | 35 (7) | 21 (5) | 186 (37) |
| | Unknown | 70 (20) | 6 (3) | 37 (8) | 113 (31) |
| | Total | 514 (105) | 188 (44) | 377 (79) | 1079 (228) |

2.3.1 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). In total, the dataset comprises 714 epilithon samples and 127 epiphyton samples (note that for some samples the substrate was not specified). The majority of epiphytic samples are from high alkalinity, very shallow lakes, such as the Norfolk Broads.

2.3.2 Season

For the purposes of this project, '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. Within the dataset there are a number of lakes where samples have been collected from three consecutive seasons. For example, 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).

The locations of the lakes are shown in Figure 2.1 and the environmental characteristics of each of the three lake types in the dataset are summarised in Figure 2.2. 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.

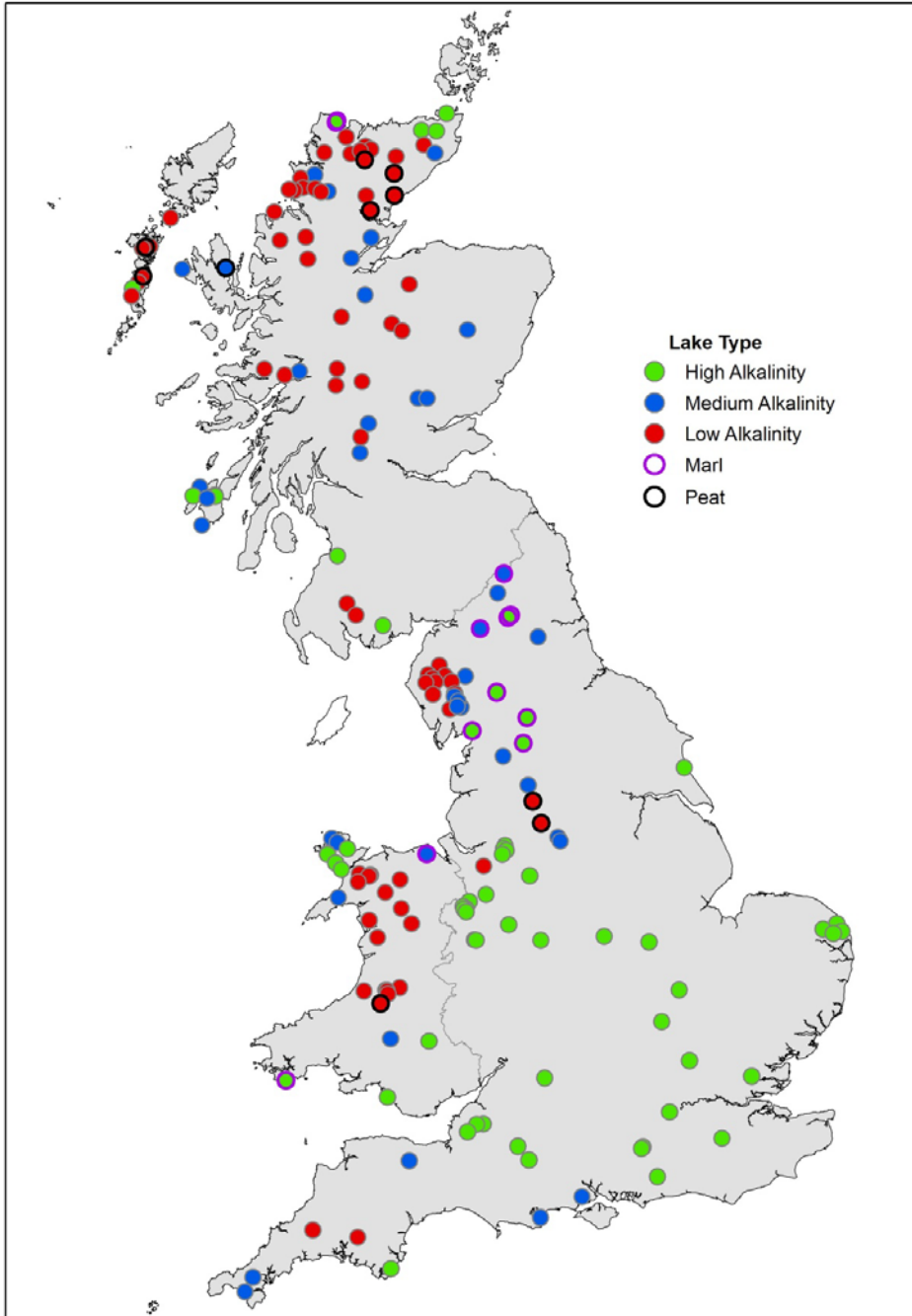


Figure 2.1 Map showing the location and type of lakes in the dataset

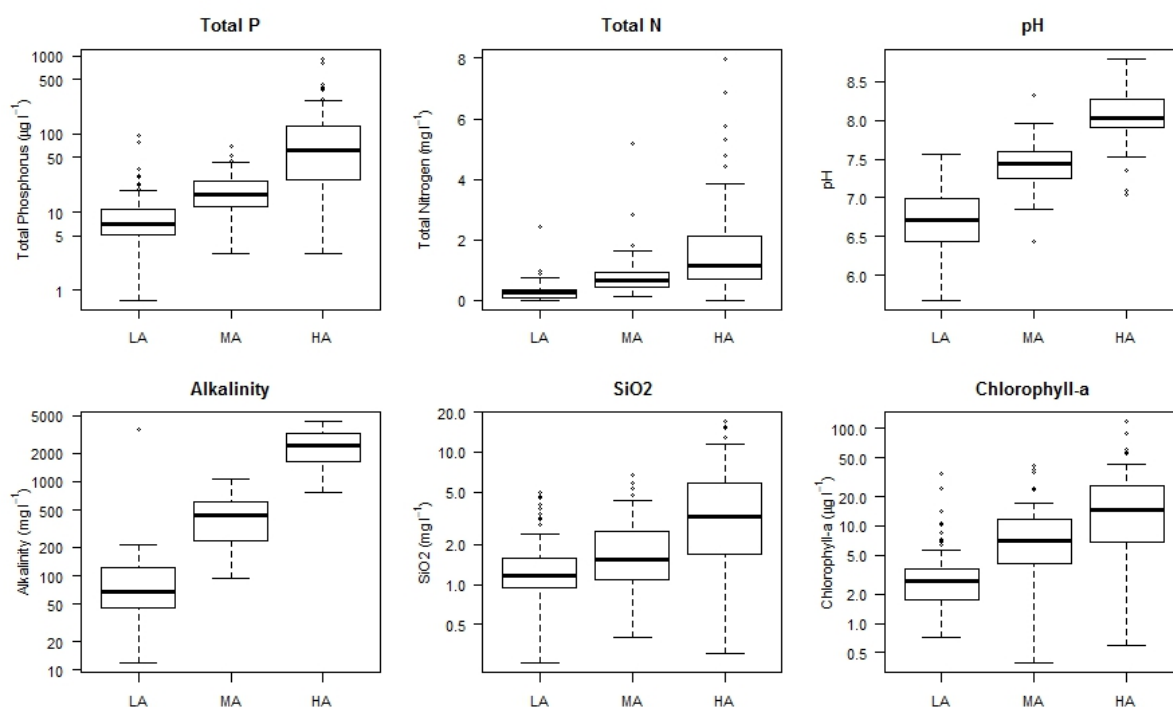


Figure 2.2 Boxplots showing the environmental characteristics of the three lake types (LA = low alkalinity, MA = medium alkalinity, HA = high alkalinity)

2.4 Defining reference lakes

The WFD requires that an assessment of 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. An understanding of the biota at reference condition is central, therefore, to the development of a WFD-compliant monitoring tool. The biota that is characteristic of reference conditions for a particular site defines the ‘expected’ biota for that site. 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).

A combination of the above methods was employed in order to identify a set of reference sites to assist in tool development. One data source was the set of reference lakes identified 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 and thus represent high status lakes in the context of their 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 (Carvalho *et al.* 2008). This list has formed the 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 (Phillips 2006). All of these lists were revisited and a final list of reference lakes was produced by Phillips in 2010 (unpublished).

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 south-eastern Ontario lakes (Reavie *et al.* 2002) and to assess ecological change in UK lakes (Bennion 2004, Bennion *et al.* 2004, Bennion and Simpson 2011). 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 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, as here. 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 sediments of shallow, high alkalinity lakes 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 lakes dataset identified using the various datasets above is given in Appendix B. A total of 35, 13 and 7 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. These data were used to develop an *a priori* status classification for the lakes in the dataset, such that lakes were classed either as reference, non-reference or unknown, the latter referring to lakes where insufficient data were available to determine their status.

2.5 Deriving a pressure metric: the Lake Trophic Diatom Index

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 similar to those described by Hill *et al.* (2000) and Walley *et al.* (2001) 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 and allocated to one of five sensitivity groups using the rescaling algorithm described above. The taxon TDI scores are numbered from 1 (low nutrient tolerance) to 5 (high nutrient tolerance).

3 Results

3.1 The Lake Trophic Diatom Index (LTDI)

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 ($r = 0.81$) between the CCA axis 1 species scores and LTDI species indicator values. Furthermore, there is a very strong correlation ($r = 0.95$) between the CCA axis 1 sample scores and LTDI scores for individual lake samples. 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 3.1, 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 TP.

Figure 3.1 (top) illustrates that for the low alkalinity group the diatoms exhibit little response along the TP gradient. *Achnantheidium minutissimum* type (ZZZ835) was abundant across the whole gradient. *Brachysira vitrea* (BR001A) and *Tabellaria flocculosa* (TA001A) also occurred in high relative abundances particularly at TP concentrations $>2 \mu\text{g l}^{-1}$ ($>0.3 \log_{10}\mu\text{g l}^{-1}$). 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 ($<50 \mu\text{g TP l}^{-1}$ or $<1.7 \log_{10}\mu\text{g l}^{-1}$). 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 *et al.* 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 and an increase in mesotrophic taxa (e.g. Bennion 1995). It is possible that there may be shifts in composition within the *A. minutissimum* and other species complexes that were masked by the merged taxonomy adopted here; however, the large number of inocula taxa with relatively broad niches in a lake littoral zone serve to 'buffer' the community against change. Moreover, low alkalinity lakes within the DALES dataset do not cover a long enough nutrient gradient for us to see any marked species turnover. The result is that, for some lakes, 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 (e.g. Bennion *et al.* 2000a, b, 2003a). However, this impact is not reflected in the benthic diatom community.

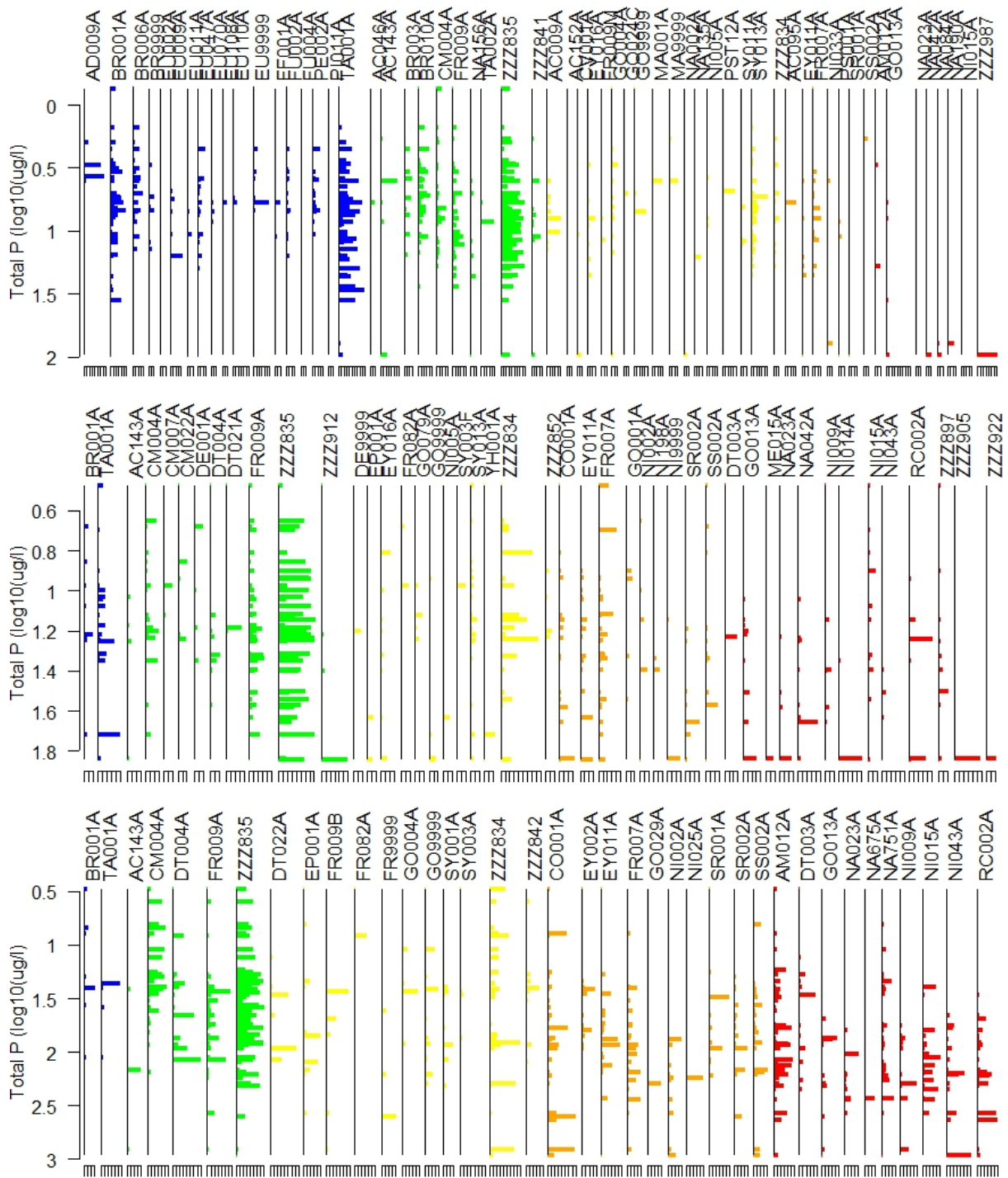


Figure 3.1 Diatom species distribution along the nutrient gradient (expressed as TP, for each type (low alkalinity, top; medium alkalinity, middle; high alkalinity, lower). Taxa are coded by LTDI groups, blue = 1, to red = 5. See Appendix A for key to taxon names

The medium alkalinity group (Figure 3.1 middle) was also dominated by *Achnanthes minutissimum* type (ZZZ835), and *Gomphonema angustum/pumilum* type (ZZZ834) 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 $\sim 20 \mu\text{g l}^{-1}$ ($> 1.3 \log_{10} \mu\text{g l}^{-1}$). The latter included *Gomphonema parvulum* (GO013A), *Navicula minima* (NA042A), *Planothidium lanceolatum* (ZZZ897) and *Nitzschia dissipata* (NI015A).

The most marked response along the nutrient gradient was exhibited by the high alkalinity group (Figure 3.1, bottom) where taxa in TDI groups 3, 4 and 5 occurred almost exclusively at TP concentrations $>30 \mu\text{g l}^{-1}$ ($>1.48 \log_{10}\mu\text{g l}^{-1}$).

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 3.1) 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 pH and alkalinity. This further explains why little taxon differentiation across the TP gradient was observed in Figure 3.1 (top). 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.

Table 3.1 Correlations between LTDI score for each sample and key chemical variables (\log_{10} transformed except pH), by lake type. All correlations significant ($p < 0.01$) except those asterisked

| | Alk | pH | Cond | Chl a | TP | TN | SiO ₂ |
|-------------------|------|------|------|-------|------|-------|------------------|
| Low alkalinity | 0.40 | 0.55 | 0.33 | 0.32 | 0.29 | 0.10* | 0.02* |
| Medium alkalinity | 0.29 | 0.36 | 0.28 | 0.41 | 0.46 | 0.62 | 0.17* |
| High alkalinity | 0.18 | 0.33 | 0.18 | 0.30 | 0.68 | 0.32 | 0.14* |

3.2 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) and most samples have been collected from the epilithon but at some sites epiphyton and/or epilithon samples have been collected. Variability in sampling season and habitat raises the important question of the effect of these variables on LTDI.

In order to examine seasonal effects data were extracted from all sites with spring, summer and autumn samples and the mean LTDI score was calculated for each season at each site. An analysis of variance was used, 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, samples were extracted for sites with both an epilithon and epiphyton sample and the mean LTDI score was calculated for each substratum at each site. There is a tendency for epilithon samples at sites with high LTDI scores to have slightly higher LTDI scores than epiphyton samples, although overall the mean difference between the paired samples of 2.3 LTDI units was not significant ($p = 0.18$, 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, therefore, these variables are not considered in subsequent model development.

3.3 Calculation of expected LTDI at reference conditions and calculation of EQR

The expected LTDI value was derived for each lake type as the median of the LTDI values of reference lakes for that type. The range of LTDI values for reference lakes belonging to each type is shown in Figure 3.2. The corresponding median values are 22, 35 and 42 for low, medium and high alkalinity types, respectively.

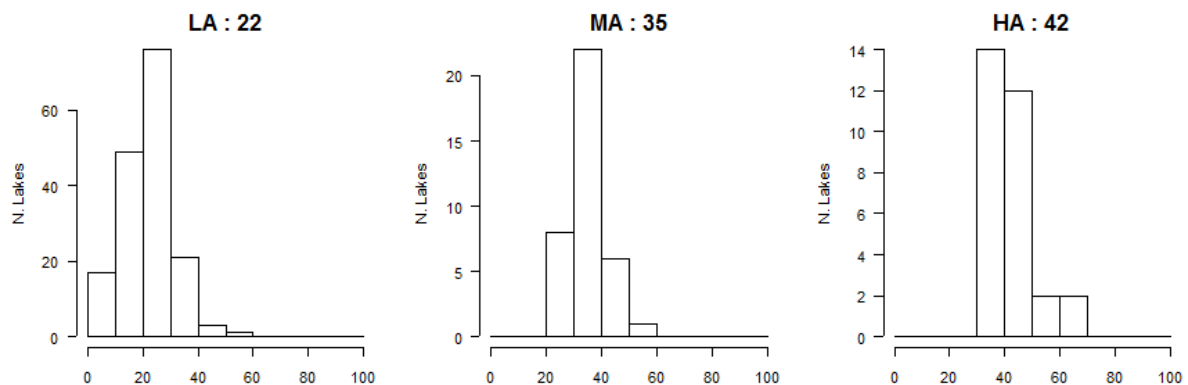


Figure 3.2 Histograms showing the distribution of LTDI values for samples from reference lakes of each type. The numbers in the titles are the median LTDI values of that subset. (LA = low alkalinity, MA = medium alkalinity, HA = high alkalinity, LTDI is on the x-axis)

EQR is then defined as the ratio of observed to expected LTDI score; that is, $EQR = O/E$ where $O = 100 - \text{observed LTDI}$ and $E = 100 - \text{expected LTDI}$. The rescaling $(100-n)$ is necessary as LTDI is a nutrient index where low values imply good quality and high values poor quality.

3.4 Defining the class boundaries

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 change along pressure gradients is gradual, whereas the WFD requires the delimitation of five distinct categories of ecological status. The option adopted in several EU 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.

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 markedly different structure to that found at HES. In both cases a relationship between the structure and functioning of an ecosystem is assumed. This can be explained by reference to the five taxon groups on which the TDI is based. Taxa are classified according to their nutrient sensitivity. Taxa that are associated with 'reference

conditions' tend to be found in the first three groups, but with group 2 (which includes taxa such as *Achnanthydium minutissimum* and *Fragilaria capucina*) predominating (Figure 3.3).

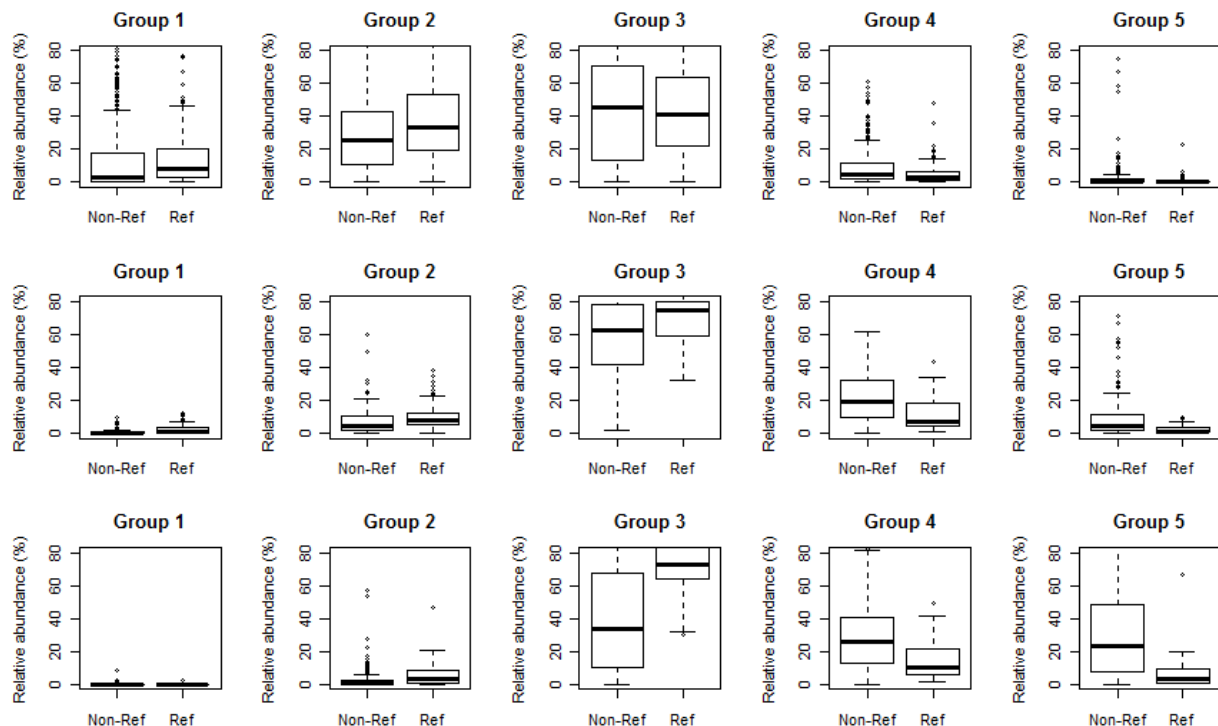


Figure 3.3 Boxplots comparing the distribution of LTDI taxon groups 1 to 5 between reference and non-reference sites, for each class. Top row = low alkalinity lakes, middle row = medium alkalinity lakes, bottom row = high alkalinity lakes)

However, as EQR decreases, so the proportion of individuals belonging to taxa in group 1 falls very steeply, and good status is characterised by a flora composed predominately of group 2 taxa, accompanied by a small number of group 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.

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 was divided into three equal portions. The main characteristics of poor ecological status (PES) and bad ecological status (BES) were that sensitive taxa were rare or absent and, as pressure levels increase, indifferent taxa also 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 filamentous algae such as *Cladophora* are likely to be abundant (Willby *et al.* 2009). There is usually also a relatively large content of abiotic material (although we do not have the data) and most of the diatoms will be motile, rather than sessile.

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. We then define the high/good boundary as the 25th percentile of the EQR values for reference sites (high status) in each type (Table 3.2).

Setting the boundary at the 25th percentile allows for the possibility that some of the sites identified as reference may be slightly impacted and so may have lower EQRs than true reference sites.

Table 3.2 Quantiles of EQRs for reference sites in each alkalinity type

| | 0 | 25 | 50 | 75 | 100 |
|---------------|------|------|-----|------|------|
| Low | 0.58 | 0.93 | 1.0 | 1.09 | 1.29 |
| Medium | 0.61 | 0.93 | 1.0 | 1.08 | 1.27 |
| High | 0.26 | 0.81 | 1.0 | 1.12 | 1.44 |

For the good/moderate boundary the DARES approach is also followed and is set at the cross-over between sensitive and tolerant taxon groups as described above. Inspection of the boxplots in Figure 3.3 (and more detailed scatterplots not shown) indicates that for the low and medium alkalinity types, LTDI species groups 1 and 2 differentiate reference from non-reference sites and can be considered sensitive taxa in these systems. Likewise, LTDI species groups 4 and 5 can be considered tolerant taxa indicative of disturbance. However, LTDI group 1 and 2 taxa are rare for the high alkalinity type, and in this type LTDI group 3 taxa can be considered sensitive. The good/moderate boundary is, therefore, defined as the cross-over between combined groups 1 and 2 (nutrient sensitive), and combined groups 4 and 5 (nutrient tolerant) for both the low and medium alkalinity types and as the cross-over between combined groups 1, 2, and 3, and combined groups 4 and 5 for the high alkalinity type.

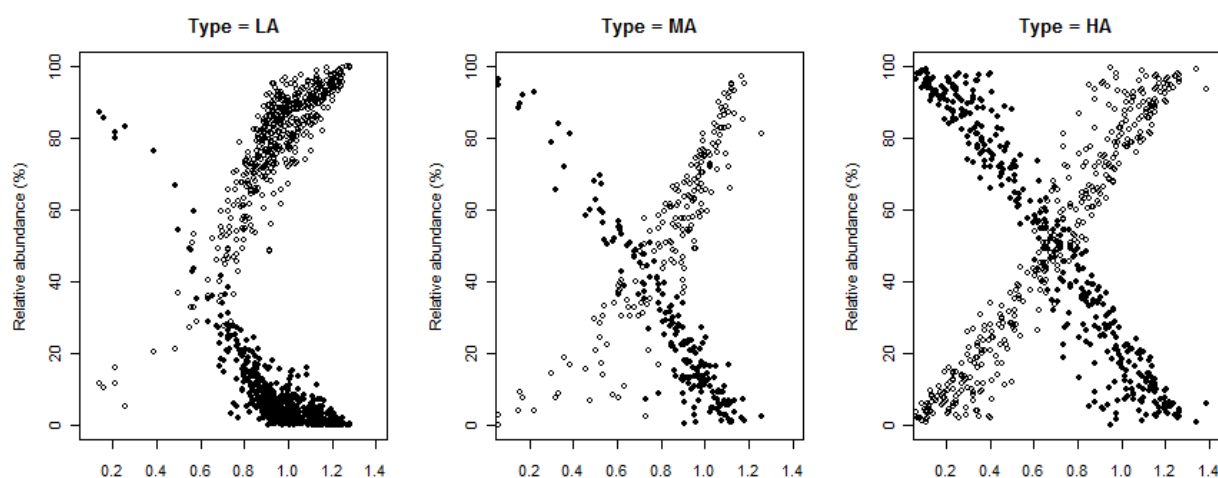


Figure 3.4 Abundances of sensitive (open circles) and tolerant (closed circles) taxa along the EQR gradient for the three alkalinity classes. (LA = low alkalinity, MA = medium alkalinity, HA = high alkalinity; EQR is on the x-axis)

The distribution of sensitive and tolerant taxon groups along the EQR gradient is shown in Figure 3.4. The cross-over point on these plots allows us to define the good/moderate boundary for each alkalinity type. The moderate/poor and other boundaries are then defined by equal division of the remaining EQR gradient. The resulting boundary values are shown in Table 3.3.

Table 3.3 EQR class boundaries for the three alkalinity types

| | H/G | G/M | M/P | P/B |
|---------------|------------|------------|------------|-------------------|
| Low | 0.92 | 0.70 | 0.46 | 0.23 [†] |
| Medium | 0.93* | 0.66** | 0.46 | 0.23 [†] |
| High | 0.92 | 0.70 | 0.46 | 0.23 [†] |

* 0.95 prior to intercalibration with other European Member States

** 0.70 prior to intercalibration with other European Member States

† The Poor/Bad boundary is not applied in UK classification. Sites are classified only as High, Good, Moderate and Poor for diatom status

3.5 Predicted class status

The predicted class status for each site in the DALES dataset based on the boundaries proposed above is given in Appendix B. A summary of the *a priori* status classes (reference, non-reference or unknown) versus model predictions for each lake type is presented in Table 3.4.

For lakes identified as reference sites the model gives good predictions for all three lake types, with the majority of samples from this set of lakes being classified as high or good, and 64% of sites being classified as high with the remainder classified as good. For non-reference sites the model performs reasonably well, predicting the majority of samples as good or worse. However, 25, 33 and 24% of the non-reference sites are classified as high status for the low, medium and high alkalinity lake types, respectively. This is not considered to be excessive as it is likely that in several cases there are reasons other than nutrients for why these are not reference sites, such as hydromorphological change.

Table 3.4 Model predictions of status class versus *a priori* reference status for each lake type, for samples (top) and sites (bottom)

| | High | Good | Moderate | Poor | Bad |
|------------------------|-------------|-------------|-----------------|-------------|------------|
| Samples | | | | | |
| Low alkalinity | | | | | |
| Reference | 152 | 41 | 3 | 0 | 0 |
| Non-reference | 30 | 48 | 6 | 2 | 1 |
| Unknown | 146 | 76 | 6 | 0 | 3 |
| Med. alkalinity | | | | | |
| Reference | 45 | 13 | 1 | 0 | 0 |
| Non-reference | 18 | 26 | 11 | 1 | 0 |
| Unknown | 23 | 29 | 11 | 4 | 6 |
| High alkalinity | | | | | |
| Reference | 35 | 9 | 8 | 1 | 0 |
| Non-reference | 30 | 23 | 26 | 45 | 25 |
| Unknown | 70 | 30 | 28 | 27 | 20 |

Table 3.4 continued

| | High | Good | Moderate | Poor | Bad |
|------------------------|------|------|----------|------|-----|
| Sites | | | | | |
| Low alkalinity | | | | | |
| Reference | 24 | 10 | 0 | 0 | 0 |
| Non-reference | 4 | 9 | 1 | 2 | 0 |
| Unknown | 42 | 16 | 1 | 0 | 0 |
| Med. alkalinity | | | | | |
| Reference | 10 | 3 | 0 | 0 | 0 |
| Non-reference | 4 | 8 | 0 | 0 | 0 |
| Unknown | 9 | 9 | 2 | 2 | 0 |
| High alkalinity | | | | | |
| Reference | 3 | 4 | 0 | 0 | 0 |
| Non-reference | 7 | 4 | 5 | 9 | 4 |
| Unknown | 12 | 11 | 8 | 4 | 6 |

3.6 Validation using the Cumbrian lakes dataset

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 were harmonised with the DALES dataset and the model was 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 spanning a gradient of pressures across low and medium alkalinities for which a range of biological and chemical data are available. This includes EQRs based on total phosphorus (TP), chlorophyll a and oxygen concentrations (e.g. Phillips *et al.* 2008), and status classes based on macrophyte communities (Willby *et al.* 2009), the chironomid pupal exuvial technique (CPET) method (Ruse 2002) and the chord distance from palaeoecological studies (Bennion 2004).

The results (Table 3.5) show that the DALES tool is good at correctly predicting lakes at high and good status but is poorer at predicting lakes classed by other methods as 'less than good' status, such as Esthwaite Water and Grasmere. If we assume that the lowest EQR determines the classification, as per the one-out-all-out approach, then overall, 11 out of 17 lakes (65%) were correct to within one class but there was a tendency for DALES results to be more relaxed than classifications using other tools, with 13 out of the 17 lakes (76%) being placed in a higher status class than that indicated by the entire suite of biological metrics. However, if the comparison is based on the mean class derived from either the full suite of tools or the biological metrics only, then the DALES tool fares somewhat better with only Ennerdale, of the lakes where sufficient data are available for comparison, being too relaxed using the latter criteria. There are two possible reasons for the apparently low thresholds. The first is that all of the lakes in this test set are low and medium alkalinity types and given the limitations of the DALES dataset for these types (particularly low alkalinity) it is not surprising that the tool fails to detect any nutrient impact. Secondly, the reference community in low alkalinity lakes might be 'buffered' against change as the dominant taxa in the biofilms (e.g. *Achnanthydium minutissimum*) are relatively tolerant to mild enrichment, as discussed above. In palaeoecological studies of low alkalinity lakes (e.g. Bennion *et al.* 2001), it is often the planktonic taxa that are responsible for changes observed in the sediment record rather than shifts within the non-planktonic component. 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, such as macrophytes, are showing signs of enrichment.

Table 3.5 Comparison of DARLEQ2 classification with other status assessments for lakes in the English Lake District

(blue = high status, green = good status, yellow = moderate status, orange = poor status, red = bad status)

| WBID | NAME | Type | Grid reference | | Max. depth (m) | Mean depth (m) | Alk. (mg/l) | MEI | Mean TP 2000 (µg/l) | Mean chl 2000 (µg/l) | Ref TP (µg/l) | Ref chl (µg/l) | P risk | EQR TP | Status based on TP | Status based on oxygen | EQR chl a | Status based on chl a | Status based on plants | Status based on CPET | Squared chord Distance from palaeo | Status based on DARLEQ2 |
|-------|--------------------|------|----------------|-------|----------------|----------------|-------------|-------|---------------------|----------------------|---------------|----------------|--------|--------|--------------------|------------------------|-----------|-----------------------|------------------------|----------------------|------------------------------------|-------------------------|
| 29183 | Wast Water | LAD | NY14973 | 05000 | 76 | 39.73 | 60 | 0.002 | 1 | 1.19 | 4 | 1.5 | No | 3.70 | Blue | Green | 1.23 | Blue | Blue | 15.1 | 0.44 | Blue |
| 29052 | Buttermere | LAD | NY19161 | 15423 | 28.6 | 16.60 | 64 | 0.004 | 1.4 | 1.59 | 5 | 1.9 | No | 3.40 | Blue | Blue | 1.18 | Blue | Blue | 14.1 | 0.48 | Blue |
| 29062 | Ennerdale Water | LAD | NY11232 | 14977 | 42 | 17.76 | 62 | 0.003 | 1.9 | 1.26 | 5 | 1.8 | Low | 2.44 | Blue | Blue | 1.45 | Blue | Green | 28.9 | 0.68 | Yellow |
| 29116 | Brothers Water | MAS | NY40215 | 13003 | 15 | 6.20 | 202 | 0.033 | 6.6 | 2.18 | 8 | 3.3 | | 1.28 | Blue | | 1.49 | Blue | | | | Blue |
| 29000 | Crummock Water | LAD | NY16276 | 18305 | 43.9 | 26.70 | 50 | 0.002 | 3.2 | 2.95 | 4 | 1.5 | No | 1.22 | Blue | Blue | 0.52 | Green | Green | 24.9 | 0.16 | Blue |
| 28965 | Derwent Water | LAS | NY26460 | 22727 | 22 | 5.50 | 127 | 0.023 | 7.5 | 4.73 | 8 | 3.0 | No | 1.03 | Blue | Yellow | 0.63 | Green | Yellow | 21.6 | 0.43 | Green |
| 29321 | Coniston Water | MAD | SD30971 | 95228 | - | 24.10 | 222 | 0.009 | 7.5 | 5.68 | 6 | 2.3 | Low | 0.80 | Blue | Blue | 0.41 | Yellow | Green | 37.6 | 0.73 | Yellow |
| 28955 | Ullswater | MAD | NY38736 | 18849 | 62.5 | 25.30 | 248 | 0.010 | 9.9 | 4.85 | 6 | 2.4 | No | 0.62 | Green | Blue | 0.49 | Yellow | Yellow | 26.5 | 0.28 | Blue |
| 29233 | Windermere N Basin | MAD | SD38929 | 96301 | 64 | 25.10 | 250 | 0.010 | 12.3 | 4.35 | 6 | 2.4 | High | 0.50 | Green | | 0.55 | Green | Yellow | 35.3 | | Green |
| 29233 | Windermere S Basin | MAD | SD37979 | 87002 | 64 | 16.8 | 250 | 0.015 | 13.9 | 6.42 | 7 | 2.7 | | 0.49 | Yellow | Green | 0.41 | Yellow | Yellow | 55.4 | 1.17 | Green |
| 28986 | Loweswater | LAS | NY12682 | 21216 | 16 | 8.37 | 185 | 0.022 | 16.4 | 9.62 | 8 | 2.9 | Mod | 0.47 | Yellow | | 0.31 | Yellow | | 47.2 | 0.54 | Green |
| 29197 | Rydal Water | LAS | NY35616 | 06343 | <10 | 5.30 | 160 | 0.030 | 18.1 | 6.53 | 8 | 3.2 | | 0.46 | Yellow | | 0.49 | Yellow | Orange | | | Green |
| 28847 | Bassenthwaite Lake | LAS | NY22196 | 27349 | 19 | 5.30 | 180 | 0.034 | 20.85 | 14.53 | 9 | 3.3 | Mod | 0.41 | Yellow | Orange | 0.23 | Orange | Red | 40.3 | 0.98 | Green |
| 29328 | Esthwaite Water | MAS | SD36341 | 96475 | 15.5 | 6.40 | 459 | 0.072 | 30.26 | 21.02 | 10 | 4.0 | High | 0.35 | Yellow | Orange | 0.19 | Orange | Green | | 1.34 | Blue |
| 29270 | Blelham Tarn | MAS | NY36803 | 00628 | 14.5 | 6.80 | 558 | 0.082 | 31.7 | 14.94 | 11 | 4.1 | | 0.34 | Yellow | Orange | 0.28 | Orange | Orange | | 0.33 | Green |
| 29184 | Grasmere | LAS | NY34273 | 06081 | 21.5 | 7.74 | 168 | 0.022 | 23.6 | 12.34 | 8 | 2.9 | High | 0.32 | Yellow | Orange | 0.24 | Orange | Yellow | | 0.65 | Blue |
| 29222 | Elterwater | LAVS | NY33769 | 03944 | 7.4 | 2.90 | 135 | 0.047 | 50.7 | 30.32 | 9 | 3.6 | | 0.18 | Red | Orange | 0.12 | Red | Orange | | 0.65 | Green |

3.7 Surface sediment analysis

In the current lakes tool, the ecological status predictions for several low alkalinity lakes provide a poor match with the *a priori* classification. For example 25 samples (three sites) are classed as high status but were deemed to be non-reference in the *a priori* classification. Similarly, in the application of the tool to the Cumbrian lakes dataset the diatom score gave a higher class prediction than those of other classification tools in a number of cases. This appears to be largely due to the apparent lack of sensitivity of the benthic diatoms to the nutrient gradient in this lake type and additionally to the confounding effects of alkalinity as discussed in Section 3.1. For example, the *Achnanthydium*-dominated assemblages found at high and good ecological status in these lakes is tolerant of a moderate amount of enrichment before it shows significant changes and consequently the tool itself is relatively insensitive to change at low and moderate pressure levels. The situation is complicated by the small number of low alkalinity lakes at moderate, poor or bad status.

In an attempt to improve the predictions for low alkalinity lakes an alternative approach was investigated based on the surface sediment samples collected at the same time as the littoral samples in 2005. This task explored whether the surface sediment diatom assemblages respond along the nutrient gradient and hence whether they can be employed to produce a more robust classification tool for nutrient pressure, particularly in low alkalinity lakes.

The full dataset contained 77 samples and 219 taxa across low, medium and high alkalinity sites. The TP concentrations ranged from 3 to 1026 $\mu\text{g l}^{-1}$, with a median of 18 $\mu\text{g l}^{-1}$, and a 75th percentile of 57 $\mu\text{g l}^{-1}$. A series of transfer functions based on weighted averaging (WA) were generated. Higher components in WA-partial least squares (WAPLS) were not significant. Models were developed for (i) all samples (Models 1 and 2), (ii) high and medium alkalinity lakes (Models 3 and 4), and (iii) low alkalinity lakes (Models 5 and 6). In each case two models were generated, one which includes all taxa in the surface sediment assemblage comprising non-planktonic and planktonic forms, and one with only the non-planktonic taxa included. Table 3.6 gives apparent and cross-validation performance statistics based on leave-one-out for the various TP models.

Table 3.6 Performance statistics of the various TP models based on the surface sediment dataset (RMSEP = root mean square error of prediction)

| | Apparent | | Cross-validation | |
|--|----------|----------------|------------------|----------------|
| | RMSEP | R ² | RMSEP | R ² |
| Model 1: All samples, all taxa | 0.28 | 0.78 | 0.37 | 0.63 |
| Model 2: All samples, non-planktonic taxa only | 0.30 | 0.75 | 0.39 | 0.59 |
| | | | | |
| Model 3: HA + MA sites, all taxa | 0.29 | 0.72 | 0.40 | 0.48 |
| Model 4: HA + MA sites, non-planktonic taxa only | 0.29 | 0.72 | 0.41 | 0.45 |
| | | | | |
| Model 5: LA sites, all taxa* | 0.17 | 0.78 | 0.34 | 0.14 |
| Model 6: LA sites, non-planktonic taxa only* | 0.17 | 0.77 | 0.35 | 0.10 |

*Models not significant at (p <0.05)

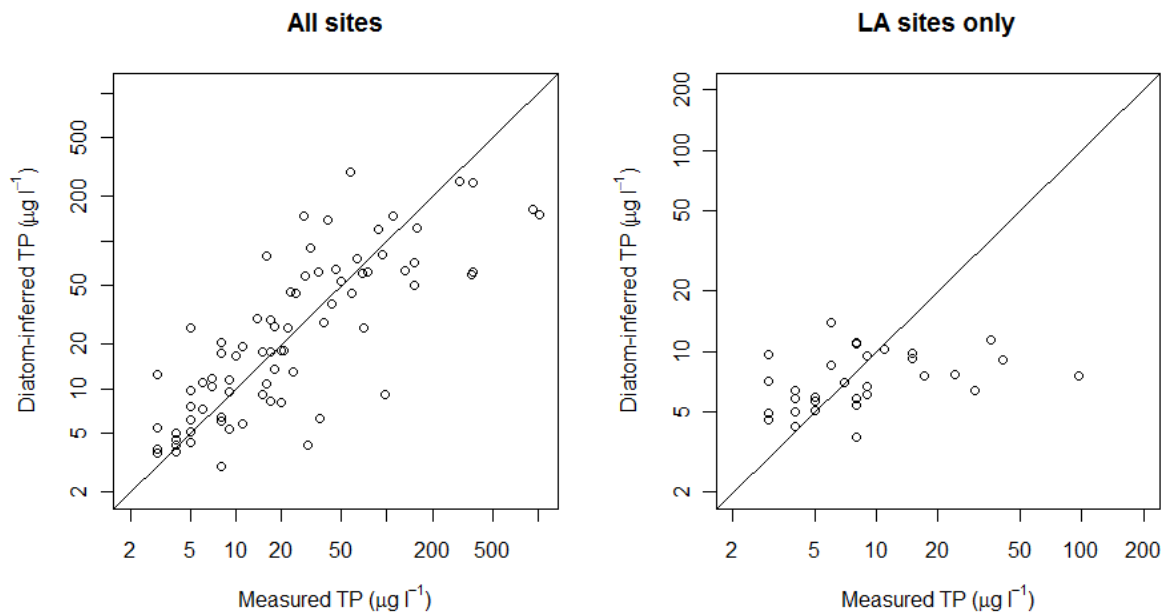


Figure 3.5 Plots of diatom-inferred versus measured TP under cross-validation for all sites (Model 1) and low alkalinity sites (Model 5)

The plots of diatom-inferred versus measured TP under cross-validation for all sites (Model 1) and low alkalinity sites (Model 5) are presented in Figure 3.5. The full model (all samples) shows good performance for all taxa (Model 1) and only a slight increase in prediction error when non-planktonic taxa only are included (Model 2). Prediction errors are broadly similar for all subset models (RMSEP 0.34 to 0.41) but the smaller gradient covered by the low alkalinity sites means that at this end of the gradient the model has virtually no predictive power. Therefore, the models based on the surface sediment data appear to offer no improvement over the diatom tool based on benthic assemblages.

4 Discussion

In contrast to rivers, modern periphyton communities have not been widely used in bioassessment of lakes. However, the advent of the WFD in 2000 required the development of lake classification tools based on phytobenthos. Several studies have employed existing diatom indices developed for rivers in order to classify lakes. For example, Ács *et al.* (2005) applied a range of benthic diatom indices to epiphyton (reed) samples from Lake Velence, a shallow lake in Hungary, using Omnidia software (Lecointe *et al.* 1993, 2008). The authors concluded that the index Indice Biologique Diatomées (IBD) (Lenoir and Coste 1996) was the best suited for classifying the ecological status of the lake and showed a significant ($p < 0.01$) negative correlation with TP. Similarly, Blanco *et al.* (2004) applied existing river indices to determine the trophic and saprobic state of six shallow Spanish lakes. A significant correlation was found between total nitrogen concentration and the diatom indices Specific Polluosensitivity Index (SPI) (Coste in CEMAGREF 1982) and IBD. They concluded that the two diatom indices were suitable for biomonitoring purposes in these lentic systems. It should be noted, however, that indices such as IBD and SPI are designed to indicate 'general pollution' and are optimised to saprobic and organic pollution. Recent experience from the lake diatom classification tool EU Member State intercalibration process suggests that only about half of the nutrient gradient is covered when these indices are applied to lakes. Furthermore, there is no apparent underlying ecological rationale for index selection; rather the index that has the highest correlation coefficient with measured nutrient variables is considered to be the most suitable.

While a number of authors have examined the relationships between taxonomic composition of benthic algal communities and measured environmental variables in lakes (e.g. King *et al.* 2000, Schoenfelder *et al.* 2002, Kitner and Pouličková 2003, DeNicola *et al.* 2004, Denys 2004, 2006, 2007, Pouličková *et al.* 2004), these studies were not specifically designed to produce indices for WFD purposes. More recently, several countries have produced WFD-compliant phytobenthos tools specifically for lakes and several more are in development. Schaumburg *et al.* (2004, 2007) employed a variety of substrates from 100 lakes to develop a diatom index for four German lake types, known as the Diatom Index for Lakes (DI_{Seen}) which combined the Trophic-Index (TI) developed by Hofmann (1994) with a Quotient of Reference Species (RAQ). They subsequently combined the diatom metric with one developed for macrophytes to give an overall assessment of lakes for the biological element macrophytes and phytobenthos. Stenger-Kovacs *et al.* (2007) developed the Trophic Diatom Index for Lakes (TDIL) in Hungary, applicable to ten lake types, using epiphytic diatom samples from 83 lowland, shallow lakes and weighted averaging methods. The TDIL was applied to a number of lakes which classified the ecological status as excellent (4), good (25), medium (21), tolerable (21) and bad (12) status. Bolla *et al.* (2010) analysed the applicability of different river and lake diatom indices for assessing the ecological status of Lake Balaton in Hungary. They concluded that IBD and TDIL were the best methods for assessing the water quality of Lake Balaton as determined by correlation between the various indices and measured chemical parameters. They subsequently developed a multimetric index from the two (Multimetric Index for Lake Balaton, $MIB = (IBD + TDIL)/2$).

4.1 Definition of reference sites and 'high status'

Some of the studies described above do not define the reference condition or explain how reference sites are selected, yet this is fundamental to WFD compliance. A notable exception is Denys (2003), who compared the present-day variation in

sedimentary and epiphytic diatoms of coastal dune wetlands with that of similar habitats occurring between c. 1850 and 1930 using diatom assemblages from recent samples and historical counterparts obtained from herbarium specimens of macrophytes. While this study was not designed to fulfil the requirements of the WFD, it does address the definition of reference conditions and illustrates the value of collection material as a valid and easily accessible source of historical information.

In DALES, reference sites were defined using a range of methods including spatial state schemes, palaeoecology, modelling and to a lesser extent expert judgement. Unlike rivers, lakes have the benefit of a sedimentary record that can be used to establish historical conditions directly (e.g. Bennion *et al.* 2004, Bennion and Simpson 2011) or can be used to assess whether lakes have undergone change in response to pressures, such as eutrophication, and thereby to determine which lakes might be considered as reference sites (e.g. Leira *et al.* 2006). For the purposes of this classification tool, a total of 35, 13 and 7 reference sites were generated for low, medium and high alkalinity lakes, respectively. While the number of reference sites was considered adequate for the former two lake types, unfortunately, there are few examples of high alkalinity reference lakes in the UK owing to the long history of impacts in lowland Britain and the productive nature of the lake catchments (e.g. Bennion *et al.* 2004). We must accept, therefore, that our definition of reference condition for the latter lake type is less robust than for the low and medium alkalinity sites.

4.2 Measuring deviations from reference conditions

A modification of the TDI (Kelly and Whitton 1995) termed the Lake TDI (LTDI) was developed as the basis for the lakes diatom tool. This well established weighted-average metric has the advantage of easy conversion to an EQR. Nonetheless the approach is limited in 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 are assessed by the present EQR. This reflects the primary concerns of the UK regulatory agencies, and the underlying principles used to develop the present model provides a framework from which separate metrics could be developed for assessing other pressures, such as acidity. It is anticipated that the Diatom Acidification Metric (DAM) tool recently developed for streams (Environment Agency, SC070034/TR2) will be tested on a set of soft water lakes in the near future.

4.3 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, but the terms of the WFD must 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, 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 these can be inferred from the taxonomic composition of benthic diatoms: see Kelly *et al.* 2008b, 2009).

The group of 'sensitive' taxa in the lakes dataset, and similarly in rivers (Kelly *et al.* 2008a) is dominated largely by *Achnanthydium 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 a concept of 'good status' can embrace the possibility of short-term, low impact pollution events that affect the flora but from which recovery can be rapid. A switch to a biofilm dominated by more 'nutrient demanding' species will occur with increasing enrichment of key nutrients and these species will proliferate, outcompeting the more sensitive 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 thick biofilms. The success of adnate (attached) species, such as *Achnanthydium minutissimum* and *Cocconeis placentula*, may also be compromised as they experience light and nutrient limitation. However, these species can still occur 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*).

The lack of response of the low alkalinity models deserves closer examination. One possibility is that the pressure–response relationship was compromised by the merging of taxa to form aggregates such as *Achnanthydium minutissimum sensu lato*. There is evidence that taxa within the *A. minutissimum* complex have distinct ecological preferences (Potapova and Hamilton 2007) and, therefore, greater taxonomic discrimination may have given a stronger relationship with the pressure gradient than was observed here. Another possibility is that the scarcity of impacted low alkalinity lakes, a lake type that tends to occur in areas where agricultural productivity is low and where there are few settlements, means that we have been unable to capture the full gradient of response. Over the range we have studied, a 'generalist' taxon such as *A. minutissimum* is not outcompeted by nutrient-tolerant taxa.

The results of the Cumbrian lakes validation exercise (Table 3.5) indicates that, for low alkalinity lakes in particular, DALES is more relaxed than other UK tools. Rather than reflecting an inadequacy in the DALES model, it is considered that this is more likely to reflect an accurate assessment of the condition of the littoral biofilm which is, essentially, a robust assemblage of organisms that can adapt to slightly increased levels of pressure. Assemblages dominated by *Achnanthydium minutissimum* often indicate disturbance and/or grazing (Biggs *et al.* 1998), and hence the persistence of this taxon suggests that slightly increased levels of pressure do not lead to fundamental alterations in the energy flow through the ecosystem and ecological functioning, in this part of the biota at least, has not measurably changed. When viewed on a wider scale, a method based on littoral phytobenthos will only give a partial view of the status of the whole lake ecosystem and lake management will be based on data from more than one biological quality element.

5 Conclusion

The project has successfully developed a diatom model, the LTDI, for assessing ecological status of lakes along a nutrient gradient and a rationale for placing status class boundaries along this gradient for three lake types. Two-thirds of all lakes identified as reference sites were classified as high with the remainder classified as good, while for non-reference lakes the model predicted the majority of sites to be good or worse. The model, therefore, performs well. However, for low alkalinity lakes in particular, DALES appears to be more relaxed than other UK tools, but this may be largely due to the apparent lack of sensitivity of the benthic diatoms to nutrient pressure in this lake type, and additionally to the confounding effect of their relationship with alkalinity. Furthermore, the scarcity of impacted low alkalinity lakes makes it difficult to capture the full gradient of response. Nevertheless, DALES gives a reasonable assessment of the condition of the littoral biofilm and makes a valuable contribution to the classification toolkit for UK standing waters.

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Appendices

Appendix A: Diatom full names and diatcodes

| Code | Name | Code | Name |
|--------|--|--------|---|
| AC009A | <i>Achnanthes recurvata</i> | GO079A | <i>Gomphonema procerum</i> |
| AC046A | <i>Achnanthes altaica</i> | GO9999 | <i>Gomphonema</i> sp. |
| AC095A | <i>Achnanthes minuscula</i> | MA001A | <i>Mastogloia smithii</i> |
| AC143A | <i>Achnanthes oblongella</i> | MA9999 | <i>Mastogloia</i> sp. |
| AC152A | <i>Achnanthes carissima</i> | ME015A | <i>Melosira varians</i> |
| AD009A | <i>Achnantheidium microcephalum</i> | NA002A | <i>Navicula jaernefeltii</i> |
| AM012A | <i>Amphora pediculus</i> | NA023A | <i>Navicula gregaria</i> |
| BR001A | <i>Brachysira vitrea</i> | NA042A | <i>Navicula minima</i> |
| BR003A | <i>Brachysira serians</i> | NA084A | <i>Navicula atomus</i> |
| BR006A | <i>Brachysira brebissonii</i> fo. <i>brebissonii</i> | NA135A | <i>Navicula tenuicephala</i> |
| BR010A | <i>Brachysira neoexilis</i> | NA156A | <i>Navicula leptostriata</i> |
| BR9999 | <i>Brachysira</i> sp. | NA190A | <i>Navicula agrestis</i> |
| CM004A | <i>Cymbella microcephala</i> fo. <i>microcephala</i> | NA675A | <i>Navicula tenelloides</i> |
| CM007A | <i>Cymbella cymbiformis</i> | NA751A | <i>Navicula cryptotenella</i> |
| CM022A | <i>Cymbella affinis</i> | NI002A | <i>Nitzschia fonticola</i> |
| CO001A | <i>Cocconeis placentula</i> | NI005A | <i>Nitzschia perminuta</i> |
| CV001A | <i>Cavinula cocconeiformis</i> | NI009A | <i>Nitzschia palea</i> |
| DE001A | <i>Denticula tenuis</i> | NI014A | <i>Nitzschia amphibia</i> |
| DE9999 | <i>Denticula</i> sp. | NI015A | <i>Nitzschia dissipata</i> |
| DT003A | <i>Diatoma vulgare</i> | NI025A | <i>Nitzschia recta</i> |
| DT004A | <i>Diatoma tenue</i> | NI033A | <i>Nitzschia paleacea</i> |
| DT021A | <i>Diatoma mesodon</i> | NI043A | <i>Nitzschia inconspicua</i> |
| DT022A | <i>Diatoma moniliformis</i> | NI198A | <i>Nitzschia lacuum</i> |
| EP001A | <i>Epithemia sorex</i> | NI9999 | <i>Nitzschia</i> sp. |
| EU002A | <i>Eunotia pectinalis</i> | PE002A | <i>Peronia fibula</i> |
| EU009A | <i>Eunotia exigua</i> | PI011A | <i>Pinnularia microstauron</i> |
| EU011A | <i>Eunotia rhomboidea</i> | PS001A | <i>Pseudostaurosira brevistriata</i> |
| EU047A | <i>Eunotia incisa</i> | RC002A | <i>Rhoicosphenia abbreviata</i> |
| EU070A | <i>Eunotia bilunaris</i> | SR001A | <i>Staurosira construens</i> |
| EU108A | <i>Eunotia intermedia</i> | SR002A | <i>Staurosira elliptica</i> |
| EU110A | <i>Eunotia minor</i> | SS002A | <i>Staurosirella pinnata</i> |
| EU9999 | <i>Eunotia</i> sp. | SY001A | <i>Synedra ulna</i> |
| EY002A | <i>Encyonema caespitosum</i> | SY003A | <i>Synedra acus</i> |
| EY011A | <i>Encyonema minutum</i> | SY003F | <i>Synedra acus</i> var. <i>delicatissima</i> |
| EY016A | <i>Encyonema silesiacum</i> | SY011A | <i>Synedra delicatissima</i> |
| FF001A | <i>Fragilariforma virescens</i> | SY013A | <i>Synedra tenera</i> |
| FR007A | <i>Fragilaria vaucheriae</i> | TA001A | <i>Tabellaria flocculosa</i> |
| FR009A | <i>Fragilaria capucina</i> | TA002A | <i>Tabellaria fenestrata</i> |
| FR009B | <i>Fragilaria capucina</i> var. <i>mesolepta</i> | YH001A | <i>Ctenophora pulchella</i> |
| FR009M | <i>Fragilaria capucina</i> var. <i>distans</i> | ZZZ834 | <i>Gomphonema angustum/pumilum</i> type |
| FR082A | <i>Fragilaria incognita</i> | ZZZ835 | <i>Achnantheidium minutissimum</i> type |
| FR9999 | <i>Fragilaria</i> sp. | ZZZ841 | <i>Fragilariforma exigua</i> |
| FU002A | <i>Frustulia rhomboides</i> | ZZZ842 | <i>Fragilaria perminuta</i> |
| FU004A | <i>Frustulia amphipleuroides</i> | ZZZ852 | <i>Psammothidium helveticum</i> |
| GO001A | <i>Gomphonema olivaceum</i> | ZZZ897 | <i>Planothidium lanceolatum</i> |
| GO004A | <i>Gomphonema gracile</i> | ZZZ905 | <i>Planothidium delicatulum</i> |
| GO013A | <i>Gomphonema parvulum</i> | ZZZ912 | <i>Achnantheidium biasoletiana</i> |
| GO024C | <i>Gomphonema clevei</i> | ZZZ922 | <i>Planothidium</i> sp. |
| GO029A | <i>Gomphonema clavatum</i> | ZZZ987 | <i>Navicula</i> [small species] |

Appendix B: Classification of lakes using the diatom tool

Reference?: denotes whether the site is considered to be in reference condition – N = No, Y = Yes, UN = unknown

Numbers in the last five columns refer to the number of samples classified

| Name | Alk. type | Reference? | Site status | Mean EQR | Bad | Poor | Moderate | Good | High |
|---|-----------|------------|-------------|----------|-----|------|----------|------|------|
| Bassenthwaite Lake | LA | N | Good | 0.75 | 0 | 0 | 1 | 4 | 4 |
| Derwent Reservoir (Midlands) | LA | N | Good | 0.82 | 0 | 0 | 0 | 1 | 0 |
| Grasmere | LA | N | Good | 0.75 | 0 | 1 | 1 | 6 | 0 |
| Lettercraffoe | LA | N | Good | 0.84 | 0 | 0 | 0 | 3 | 0 |
| Llyn Cwellyn | LA | N | Good | 0.89 | 0 | 0 | 1 | 3 | 5 |
| Llyn Eiddwen | LA | N | High | 0.98 | 0 | 0 | 0 | 2 | 4 |
| Llyn Padarn | LA | N | Good | 0.83 | 0 | 0 | 0 | 9 | 1 |
| Llyn Tegid or Bala Lake | LA | N | Good | 0.74 | 0 | 0 | 4 | 6 | 0 |
| Loch Dee | LA | N | High | 1.10 | 0 | 0 | 0 | 0 | 13 |
| Loch Grannoch | LA | N | High | 1.20 | 0 | 0 | 0 | 0 | 5 |
| Loch Insh | LA | N | Good | 0.93 | 0 | 0 | 0 | 6 | 4 |
| Loch Lomond @ Tarbet Isle | LA | N | Good | 0.79 | 0 | 0 | 0 | 2 | 0 |
| Lower Tamar Lake | LA | N | Moderate | 0.60 | 0 | 0 | 2 | 0 | 0 |
| Loweswater | LA | N | Good | 0.88 | 0 | 0 | 0 | 10 | 0 |
| Oak Mere | LA | N | Poor | 0.39 | 0 | 1 | 0 | 0 | 0 |
| Upper Tamar Lake | LA | N | Poor | 0.39 | 1 | 0 | 1 | 0 | 0 |
| Angle Tarn | LA | UN | Good | 0.91 | 0 | 0 | 0 | 1 | 0 |
| Bleaberry Tarn | LA | UN | High | 1.24 | 0 | 0 | 0 | 0 | 1 |
| Burrator Reservoir | LA | UN | High | 1.03 | 0 | 0 | 0 | 0 | 3 |
| Cam Loch | LA | UN | Good | 0.78 | 0 | 0 | 1 | 1 | 1 |
| Caslub | LA | UN | Good | 0.77 | 0 | 0 | 1 | 2 | 0 |
| Coniston Water | LA | UN | Good | 0.83 | 0 | 0 | 2 | 5 | 3 |
| Craig Goch Reservoir | LA | UN | High | 1.15 | 0 | 0 | 0 | 0 | 4 |
| Crummock Water | LA | UN | Good | 0.87 | 0 | 0 | 0 | 8 | 1 |
| Derwent Water | LA | UN | Good | 0.84 | 0 | 0 | 0 | 10 | 0 |
| Dozmary Pool | LA | UN | Moderate | 0.56 | 2 | 0 | 2 | 3 | 0 |
| Llyn y Fan Fawr | LA | UN | Good | 0.92 | 0 | 0 | 0 | 1 | 0 |
| Greendale Tarn | LA | UN | High | 1.11 | 0 | 0 | 0 | 0 | 1 |
| Lake Vyrnwy/Llyn Efyrynwy | LA | UN | High | 1.03 | 0 | 0 | 0 | 0 | 4 |
| Llyn Berwyn | LA | UN | Good | 0.81 | 1 | 0 | 0 | 1 | 2 |
| Llyn Bodlyn | LA | UN | High | 1.24 | 0 | 0 | 0 | 0 | 2 |
| Llyn Conwy | LA | UN | High | 1.08 | 0 | 0 | 0 | 0 | 1 |
| Llyn Egnant | LA | UN | High | 1.28 | 0 | 0 | 0 | 0 | 3 |
| Llyn Gynon | LA | UN | High | 1.17 | 0 | 0 | 0 | 0 | 9 |
| Llyn Hir | LA | UN | High | 1.15 | 0 | 0 | 0 | 1 | 8 |
| Loch a'Bhraoin | LA | UN | High | 1.13 | 0 | 0 | 0 | 0 | 5 |
| Loch a'Bhuird | LA | UN | High | 1.16 | 0 | 0 | 0 | 0 | 3 |
| Loch a Bhraoin (WFD), W of Boat House, Garve | LA | UN | High | 1.15 | 0 | 0 | 0 | 0 | 6 |
| Loch an Lagain | LA | UN | High | 1.02 | 0 | 0 | 0 | 0 | 2 |
| Loch Ascaig | LA | UN | High | 1.05 | 0 | 0 | 0 | 1 | 2 |
| Loch Ba near Gruline House outfall, Gruline, Isle of Mull | LA | UN | Good | 0.93 | 0 | 0 | 0 | 1 | 3 |
| Loch Borralan | LA | UN | Good | 0.81 | 0 | 0 | 0 | 3 | 0 |
| Loch Brora | LA | UN | High | 0.96 | 0 | 0 | 0 | 2 | 2 |
| Loch Coulside | LA | UN | High | 1.04 | 0 | 0 | 0 | 0 | 3 |
| Loch Culag | LA | UN | Good | 0.93 | 0 | 0 | 0 | 1 | 2 |
| Loch Culaidh | LA | UN | High | 1.02 | 0 | 0 | 0 | 1 | 2 |
| Loch Dee | LA | UN | High | 1.16 | 0 | 0 | 0 | 0 | 13 |
| Loch Dee @ SW shore | LA | UN | High | 1.10 | 0 | 0 | 0 | 0 | 5 |
| Loch Einich | LA | UN | High | 1.09 | 0 | 0 | 0 | 0 | 1 |
| Loch Frisa near outfall (Swq Site) | LA | UN | High | 1.02 | 0 | 0 | 0 | 1 | 2 |
| Loch Gowan | LA | UN | High | 1.07 | 0 | 0 | 0 | 0 | 3 |

| Name | Alk. type | Reference? | Site status | Mean EQR | Bad | Poor | Moderate | Good | High |
|--|-----------|------------|-------------|----------|-----|------|----------|------|------|
| Loch Grannoch @ east shore | LA | UN | High | 1.20 | 0 | 0 | 0 | 0 | 3 |
| Loch Insh | LA | UN | Good | 0.83 | 0 | 0 | 0 | 6 | 4 |
| Loch Insh (in loch) SWC | LA | UN | Good | 0.92 | 0 | 0 | 0 | 5 | 1 |
| Loch Katrine 80M West Eil | LA | UN | High | 0.99 | 0 | 0 | 0 | 0 | 3 |
| Loch Laidon | LA | UN | High | 1.23 | 0 | 0 | 0 | 0 | 2 |
| Loch Langabhat | LA | UN | High | 1.04 | 0 | 0 | 0 | 0 | 3 |
| Loch Lee (EU), Glenesk, Brechin | LA | UN | High | 0.97 | 0 | 0 | 0 | 3 | 2 |
| Loch Lomond, W of Ross Priory, Alexandria | LA | UN | Good | 0.77 | 0 | 0 | 0 | 2 | 0 |
| Loch Meadie | LA | UN | High | 1.05 | 0 | 0 | 0 | 0 | 6 |
| Loch Morlich (in loch) SWC | LA | UN | Good | 0.92 | 0 | 0 | 0 | 3 | 2 |
| Loch Muick (in loch) SWC | LA | UN | High | 1.06 | 0 | 0 | 0 | 0 | 1 |
| Loch na Beiste | LA | UN | High | 1.18 | 0 | 0 | 0 | 0 | 6 |
| Loch na Moracha | LA | UN | High | 1.02 | 0 | 0 | 0 | 0 | 3 |
| Loch Ness (SWCL) | LA | UN | Good | 0.85 | 0 | 0 | 0 | 5 | 1 |
| Loch Ossian | LA | UN | High | 1.15 | 0 | 0 | 0 | 0 | 3 |
| Loch Sgealtair | LA | UN | Good | 0.72 | 0 | 0 | 1 | 4 | 0 |
| Loch Sionascaig | LA | UN | High | 1.04 | 0 | 0 | 0 | 0 | 3 |
| Loch Syre | LA | UN | High | 1.08 | 0 | 0 | 0 | 0 | 2 |
| Loch Tay @ lochside south of bridge (Bankside) | LA | UN | Good | 0.81 | 0 | 0 | 0 | 3 | 0 |
| Loch Tormasad | LA | UN | Good | 0.90 | 0 | 0 | 0 | 2 | 2 |
| Loch Urrahag Lewis | LA | UN | Good | 0.88 | 0 | 0 | 0 | 4 | 2 |
| Stoney Tarn | LA | UN | High | 1.18 | 0 | 0 | 0 | 0 | 1 |
| Tal-y-llyn | LA | UN | Good | 0.83 | 0 | 0 | 0 | 4 | 1 |
| Thirlmere | LA | UN | High | 0.93 | 0 | 0 | 0 | 2 | 3 |
| Buttermere | LA | Y | Good | 0.91 | 0 | 0 | 0 | 5 | 1 |
| Ennerdale | LA | Y | Good | 0.85 | 0 | 0 | 2 | 4 | 4 |
| Llyn Idwal | LA | Y | High | 1.04 | 0 | 0 | 0 | 0 | 7 |
| Llyn Ogwen | LA | Y | High | 1.02 | 0 | 0 | 0 | 1 | 11 |
| Llyn Teifi | LA | Y | High | 1.18 | 0 | 0 | 0 | 0 | 7 |
| Loch Doilet | LA | Y | High | 1.18 | 0 | 0 | 0 | 0 | 4 |
| Loch Doilet @ southern shore, N of B-road | LA | Y | High | 1.17 | 0 | 0 | 0 | 0 | 6 |
| Loch Druidbeag | LA | Y | High | 0.95 | 0 | 0 | 0 | 1 | 1 |
| Loch Druidibeg South Uist SWCL | LA | Y | High | 1.00 | 0 | 0 | 0 | 0 | 6 |
| Loch Glascarnoch (SWCL) | LA | Y | High | 1.02 | 0 | 0 | 0 | 2 | 10 |
| Loch Hope | LA | Y | High | 1.10 | 0 | 0 | 0 | 0 | 3 |
| Loch Hope SWCL | LA | Y | High | 1.02 | 0 | 0 | 0 | 1 | 5 |
| Loch Laggan S of Aberarder | LA | Y | High | 1.12 | 0 | 0 | 0 | 0 | 4 |
| Loch Lubnaig | LA | Y | High | 0.94 | 0 | 0 | 0 | 5 | 4 |
| Loch Lubnaig at delta on E shore | LA | Y | High | 0.93 | 0 | 0 | 0 | 5 | 3 |
| Loch Maree | LA | Y | High | 1.02 | 0 | 0 | 0 | 0 | 8 |
| Loch Naver | LA | Y | High | 1.07 | 0 | 0 | 0 | 0 | 3 |
| Loch Naver at Grummore SWCL | LA | Y | High | 1.06 | 0 | 0 | 0 | 0 | 6 |
| Loch Osgaig | LA | Y | High | 1.06 | 0 | 0 | 0 | 0 | 5 |
| Loch Osgaig SWCL | LA | Y | High | 1.05 | 0 | 0 | 0 | 0 | 12 |
| Loch Stack | LA | Y | High | 0.99 | 0 | 0 | 0 | 0 | 3 |
| Loch Stack SWCL | LA | Y | High | 1.00 | 0 | 0 | 0 | 1 | 5 |
| Loch Treig | LA | Y | Good | 0.82 | 0 | 0 | 0 | 1 | 0 |
| Loch Venachar near dam wall | LA | Y | Good | 0.89 | 0 | 0 | 0 | 7 | 2 |
| Lochindorb | LA | Y | High | 0.98 | 0 | 0 | 0 | 1 | 3 |
| Lochindorb | LA | Y | Good | 0.91 | 0 | 0 | 0 | 4 | 1 |
| Wastwater | LA | Y | Good | 0.91 | 0 | 0 | 1 | 3 | 4 |
| Loch Craggie | LA | Y | Good | 0.89 | 0 | 0 | 0 | 2 | 0 |
| Loch Loyal | LA | Y | Good | 0.91 | 0 | 0 | 0 | 2 | 1 |
| Loch Lubnaig | LA | Y | Good | 0.92 | 0 | 0 | 0 | 5 | 4 |
| Loch na Beiste | LA | Y | High | 1.18 | 0 | 0 | 0 | 0 | 6 |
| Loch nan Gabhar | LA | Y | High | 1.04 | 0 | 0 | 0 | 0 | 3 |
| Loch Rannoch | LA | Y | Good | 0.84 | 0 | 0 | 0 | 3 | 0 |
| Loch Tarff | LA | Y | Good | 0.91 | 0 | 0 | 0 | 3 | 1 |

| Name | Alk. type | Reference? | Site status | Mean EQR | Bad | Poor | Moderate | Good | High |
|---|-----------|------------|-------------|----------|-----|------|----------|------|------|
| Loch Tarff @ S shore, S of Eilean Ban Island, Fort Augustus | LA | Y | High | 0.94 | 0 | 0 | 0 | 2 | 4 |
| Bassenthwaite Lake | MA | N | High | 0.98 | 0 | 0 | 1 | 4 | 4 |
| Derwent Reservoir, Midlands | MA | N | High | 0.99 | 0 | 0 | 0 | 0 | 1 |
| Derwent Reservoir, Northern | MA | N | Good | 0.82 | 0 | 0 | 0 | 3 | 0 |
| Elterwater | MA | N | High | 1.06 | 0 | 0 | 0 | 0 | 2 |
| Esthwaite Water | MA | N | Good | 0.73 | 0 | 0 | 6 | 2 | 2 |
| Greenlee Lough | MA | N | Good | 0.75 | 0 | 0 | 3 | 2 | 5 |
| Hatchet Pond | MA | N | Good | 0.91 | 0 | 0 | 0 | 3 | 2 |
| Lake of Menteith | MA | N | Good | 0.76 | 0 | 0 | 1 | 2 | 0 |
| Lake of Menteith at Outlet | MA | N | Good | 0.82 | 0 | 0 | 1 | 2 | 1 |
| Loch Earn | MA | N | Good | 0.89 | 0 | 0 | 0 | 1 | 1 |
| Loch of Clunie | MA | N | High | 0.98 | 0 | 0 | 0 | 2 | 3 |
| Windermere | MA | N | Good | 0.78 | 0 | 1 | 2 | 5 | 3 |
| Ardnave Loch | MA | UN | Good | 0.75 | 0 | 0 | 1 | 2 | 0 |
| Catcleugh Reservoir | MA | UN | High | 1.05 | 0 | 0 | 0 | 1 | 2 |
| Kielder Water | MA | UN | High | 0.99 | 0 | 0 | 0 | 1 | 3 |
| Ladybower Reservoir (site A) | MA | UN | Good | 0.79 | 0 | 0 | 1 | 1 | 1 |
| Llyn Alaw | MA | UN | Good | 0.76 | 0 | 0 | 1 | 1 | 1 |
| Llyn Glasfryn | MA | UN | Poor | 0.46 | 1 | 0 | 2 | 0 | 0 |
| Llyn Helyg | MA | UN | Moderate | 0.61 | 0 | 0 | 1 | 1 | 0 |
| Llyn Llygeirian | MA | UN | Moderate | 0.67 | 0 | 1 | 4 | 3 | 0 |
| Loch Fada | MA | UN | Good | 0.77 | 0 | 0 | 1 | 2 | 0 |
| Loch Mór | MA | UN | Moderate | 0.68 | 0 | 1 | 0 | 1 | 1 |
| Loch na Muilne @ NW shore | MA | UN | High | 0.94 | 0 | 0 | 0 | 0 | 2 |
| Loch Ruthven | MA | UN | High | 0.93 | 0 | 0 | 0 | 2 | 2 |
| Loch Skerrols | MA | UN | High | 1.03 | 0 | 0 | 0 | 0 | 3 |
| Loch Tay @ Dalerb picnic site, Kenmore | MA | UN | Good | 0.76 | 0 | 0 | 1 | 0 | 1 |
| Lochan Lunn Da-Bhra | MA | UN | Good | 0.84 | 0 | 0 | 0 | 3 | 0 |
| St Marys Loch at outlet | MA | UN | High | 1.05 | 0 | 0 | 0 | 0 | 2 |
| Stithians Reservoir | MA | UN | High | 1.04 | 0 | 0 | 0 | 0 | 4 |
| Stocks Reservoir | MA | UN | Good | 0.75 | 0 | 0 | 1 | 4 | 0 |
| Talkin Tarn | MA | UN | Moderate | 0.65 | 0 | 0 | 2 | 1 | 0 |
| The Loe | MA | UN | Poor | 0.25 | 5 | 3 | 1 | 0 | 0 |
| Usk Reservoir | MA | UN | High | 0.96 | 0 | 0 | 0 | 1 | 2 |
| Wimbleball | MA | UN | High | 0.93 | 0 | 0 | 0 | 1 | 1 |
| Loch Achnaclaich | MA | Y | High | 0.95 | 0 | 0 | 0 | 2 | 2 |
| Loch Ailsh | MA | Y | High | 1.05 | 0 | 0 | 0 | 0 | 3 |
| Loch Assynt | MA | Y | High | 1.02 | 0 | 0 | 0 | 0 | 3 |
| Loch Kinnabus | MA | Y | Good | 0.78 | 0 | 0 | 0 | 3 | 0 |
| Loch Kinord | MA | Y | High | 1.03 | 0 | 0 | 0 | 0 | 4 |
| Loch Kinord (in loch) SWC | MA | Y | High | 1.03 | 0 | 0 | 0 | 1 | 5 |
| Loch of Craiglush | MA | Y | High | 1.04 | 0 | 0 | 0 | 0 | 5 |
| Loch Ussie | MA | Y | High | 0.96 | 0 | 0 | 0 | 1 | 2 |
| Loch Ussie SWCL | MA | Y | High | 1.00 | 0 | 0 | 0 | 1 | 5 |
| Blelham Tarn | MA | Y | Good | 0.77 | 0 | 0 | 1 | 2 | 0 |
| Little Sea Mere | MA | Y | High | 1.06 | 0 | 0 | 0 | 0 | 7 |
| Loch Rangag | MA | Y | Good | 0.92 | 0 | 0 | 0 | 2 | 1 |
| Ullswater | MA | Y | High | 1.03 | 0 | 0 | 0 | 1 | 8 |
| Aqualate Mere | HA | N | Poor | 0.27 | 2 | 1 | 0 | 0 | 0 |
| Betton Pool | HA | N | Bad | 0.23 | 2 | 2 | 0 | 0 | 0 |
| Bomere Pool | HA | N | High | 0.94 | 0 | 0 | 0 | 1 | 1 |
| Bosherston Lily Ponds (Central) | HA | N | Good | 0.90 | 0 | 0 | 1 | 2 | 6 |
| Bosherston Lily Ponds (Eastern Arm) | HA | N | Good | 0.73 | 0 | 0 | 1 | 0 | 1 |
| Bosherston Lily Ponds (Western Arm) | HA | N | High | 0.94 | 0 | 0 | 0 | 1 | 1 |
| Carlingwark Loch | HA | N | Poor | 0.28 | 2 | 4 | 0 | 0 | 0 |
| Colemere | HA | N | Bad | 0.21 | 2 | 1 | 0 | 0 | 0 |
| Comber Mere | HA | N | Moderate | 0.46 | 1 | 2 | 0 | 0 | 1 |
| Croise Mere | HA | N | Poor | 0.40 | 1 | 4 | 2 | 0 | 0 |

| Name | Alk. type | Reference? | Site status | Mean EQR | Bad | Poor | Moderate | Good | High |
|--|-----------|------------|-------------|----------|-----|------|----------|------|------|
| Frensham Little Pond | HA | N | High | 0.92 | 0 | 0 | 0 | 2 | 1 |
| Greenlee Lough | HA | N | High | 0.99 | 0 | 0 | 3 | 2 | 5 |
| Hanmer Mere | HA | N | Poor | 0.42 | 0 | 5 | 2 | 1 | 0 |
| Hickling Broad | HA | N | High | 1.01 | 0 | 0 | 0 | 1 | 4 |
| Hornsea Mere | HA | N | Bad | 0.22 | 4 | 2 | 0 | 0 | 0 |
| Kenfig Pool | HA | N | Good | 0.78 | 0 | 0 | 6 | 2 | 2 |
| Llangorse Lake | HA | N | Poor | 0.41 | 0 | 5 | 2 | 0 | 0 |
| Llyn Dinam | HA | N | Poor | 0.37 | 0 | 8 | 1 | 0 | 0 |
| Llyn Penrhyn | HA | N | Poor | 0.34 | 1 | 1 | 0 | 0 | 0 |
| Loch Leven from jetty near sluices | HA | N | Good | 0.81 | 0 | 0 | 0 | 5 | 1 |
| Rollsby Broad | HA | N | Moderate | 0.57 | 0 | 1 | 2 | 1 | 0 |
| Rostherne Mere | HA | N | Moderate | 0.48 | 1 | 3 | 1 | 2 | 0 |
| Semer Water | HA | N | Moderate | 0.67 | 0 | 0 | 2 | 2 | 0 |
| Slapton Ley | HA | N | Good | 0.88 | 0 | 0 | 1 | 0 | 1 |
| Tatton Mere | HA | N | Poor | 0.34 | 3 | 3 | 1 | 0 | 1 |
| The Mere (Mere Mere) | HA | N | Good | 0.77 | 0 | 2 | 0 | 3 | 2 |
| The Mere, Ellesmere | HA | N | Bad | 0.21 | 2 | 2 | 0 | 0 | 0 |
| Whitemere | HA | N | Bad | 0.15 | 3 | 0 | 0 | 0 | 0 |
| Wroxham Broad | HA | N | Moderate | 0.51 | 2 | 3 | 1 | 0 | 2 |
| Aboyne Loch at Boat House (SWC) | HA | UN | Good | 0.81 | 0 | 0 | 0 | 2 | 0 |
| Blagdon Lake | HA | UN | Poor | 0.40 | 1 | 3 | 2 | 0 | 0 |
| Bough Beech Reservoir | HA | UN | Bad | 0.19 | 2 | 2 | 0 | 0 | 0 |
| Broomlee Lough | HA | UN | High | 1.12 | 0 | 0 | 0 | 0 | 9 |
| Chasewater | HA | UN | Moderate | 0.64 | 0 | 1 | 2 | 2 | 1 |
| Cheddar Reservoir | HA | UN | High | 1.05 | 0 | 0 | 0 | 1 | 2 |
| Chew Valley Lake | HA | UN | Bad | 0.15 | 3 | 1 | 0 | 0 | 0 |
| Cotswold WP | HA | UN | Poor | 0.45 | 0 | 1 | 0 | 0 | 0 |
| Crag Lough | HA | UN | High | 1.09 | 0 | 0 | 0 | 2 | 5 |
| Cropston Reservoir | HA | UN | Poor | 0.27 | 1 | 2 | 0 | 0 | 0 |
| Fonthill Lake | HA | UN | Moderate | 0.52 | 0 | 2 | 0 | 1 | 0 |
| Grafham Water | HA | UN | Bad | 0.10 | 2 | 0 | 0 | 0 | 0 |
| Hanningfield Reservoir | HA | UN | Bad | 0.10 | 3 | 0 | 0 | 0 | 0 |
| Hawes Water | HA | UN | High | 1.13 | 0 | 0 | 0 | 1 | 9 |
| Llyn Coron | HA | UN | Poor | 0.45 | 0 | 6 | 3 | 0 | 0 |
| Llyn Llygeirian | HA | UN | Good | 0.70 | 0 | 1 | 4 | 3 | 0 |
| Llyn Rhos-ddu | HA | UN | Good | 0.74 | 0 | 0 | 1 | 2 | 0 |
| Loch Ballygrant | HA | UN | High | 1.10 | 0 | 0 | 0 | 0 | 3 |
| Loch Borrallie | HA | UN | High | 0.94 | 0 | 0 | 0 | 2 | 3 |
| Loch Croispol | HA | UN | Good | 0.85 | 0 | 0 | 1 | 1 | 3 |
| Loch Gorm | HA | UN | High | 0.98 | 0 | 0 | 0 | 0 | 3 |
| Loch Gorm near outflow (Swq Site) | HA | UN | High | 1.21 | 0 | 0 | 0 | 0 | 3 |
| Loch Lossit | HA | UN | High | 1.29 | 0 | 0 | 0 | 0 | 3 |
| Loch of Mey | HA | UN | Moderate | 0.70 | 0 | 0 | 1 | 1 | 0 |
| Loch of Spiggie, South Mainland (SWC) | HA | UN | Good | 0.73 | 0 | 0 | 0 | 2 | 0 |
| Loch of Strathbeg @ S shore Boat House, W of Rattray | HA | UN | Good | 0.76 | 0 | 0 | 1 | 4 | 0 |
| Loch Ore @ yacht jetty near outlet | HA | UN | Good | 0.80 | 0 | 0 | 1 | 1 | 2 |
| Malham Tarn | HA | UN | High | 0.98 | 0 | 0 | 1 | 2 | 6 |
| Martnaham Loch | HA | UN | Poor | 0.40 | 0 | 3 | 0 | 0 | 0 |
| Queen Mary Reservoir (D) | HA | UN | Bad | 0.14 | 2 | 1 | 0 | 0 | 0 |
| Rescobie Loch (Bankside) | HA | UN | Good | 0.76 | 0 | 0 | 2 | 2 | 1 |
| Rudyard Reservoir | HA | UN | Poor | 0.45 | 2 | 2 | 0 | 0 | 1 |
| Rutland Water | HA | UN | Poor | 0.26 | 0 | 2 | 0 | 0 | 0 |
| Shearwater Lake | HA | UN | Good | 0.81 | 0 | 0 | 2 | 2 | 1 |
| Sowley Pond | HA | UN | Moderate | 0.51 | 0 | 1 | 2 | 0 | 0 |
| Stanborough Lake | HA | UN | Poor | 0.45 | 2 | 0 | 2 | 1 | 0 |
| Stewartby Lake | HA | UN | Bad | 0.18 | 2 | 1 | 0 | 0 | 0 |
| Sunbiggin Tarn | HA | UN | Moderate | 0.68 | 0 | 0 | 3 | 2 | 0 |
| Swannay Loch | HA | UN | High | 0.93 | 0 | 1 | 0 | 2 | 4 |

| Name | Alk. type | Reference? | Site status | Mean EQR | Bad | Poor | Moderate | Good | High |
|---------------------|------------------|-------------------|--------------------|-----------------|------------|-------------|-----------------|-------------|-------------|
| Tabley Mere | HA | UN | Moderate | 0.50 | 0 | 1 | 2 | 0 | 0 |
| West Loch Ollay | HA | UN | High | 0.99 | 0 | 0 | 0 | 1 | 2 |
| Loch Calder | HA | Y | High | 1.03 | 0 | 0 | 0 | 0 | 16 |
| Loch Harray | HA | Y | Good | 0.80 | 0 | 0 | 2 | 1 | 3 |
| Upton Broad | HA | Y | High | 1.04 | 0 | 0 | 0 | 1 | 7 |
| Burton Mill Pond | HA | Y | Good | 0.82 | 0 | 0 | 2 | 1 | 1 |
| Frensham Great Pond | HA | Y | Good | 0.71 | 0 | 1 | 3 | 0 | 3 |
| Llyn yr Wyth-Eidion | HA | Y | High | 1.18 | 0 | 0 | 0 | 0 | 2 |
| Loch Scamclate | HA | Y | Good | 0.83 | 0 | 0 | 3 | 4 | 3 |

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