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Assessment of ecological status in UK lakes using benthic diatoms

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Abstract: The European Union's Water Framework Directive (WFD) requires that all water bodies in Europe achieve good ecological status (GES) by 2015. We developed an ecological classification tool for UK lakes based on benthic diatoms, a key component of the biological-quality element *macrophytes and phytobenthos*. A database of 1079 epilithic and epiphytic diatom samples and matching environmental data was assembled from 228 UK lakes. The data set was divided into 3 lake types: low, medium, and high alkalinity. A lake trophic diatom index (LTDI) was developed based on modification of the trophic diatom index (TDI) for rivers, and ecological quality ratios (EQRs) were generated for each lake type. The high/good status boundary was defined as the 25th percentile of EQRs of all reference sites (identified based on independent sedimentary-diatom-assemblage data or catchment point-source and landuse data), whereas the good/moderate boundary 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 defined by equal division of the remaining EQR gradient. Samples from reference sites were used to predict the expected LTDI value for each sample, and these values were compared with the classifications derived from the LTDI. For lakes identified as reference sites, 68% were classified as having high status and 32% as having good. The model predicted 81% of nonreference lakes to have good or worse status. The model was applied to 17 English lakes (10 low- and 7 medium-alkalinity) for which classification based on other WFD tools was available. The classifications based on LTDI gave the same status (within 1 class) as other biological elements for 11 of the 17 lakes (65%). Thus, the LTDI gives a reliable assessment of the condition of the littoral biofilm and is a key component of a WFD-compliant tool kit for classifying UK standing waters.
Key words: diatoms, phytobenthos, lakes, Water Framework Directive, ecological status, nutrients, metrics, reference conditions

The European Union's Water Framework Directive (WFD; European Union 2000) now drives the management of surface waters throughout Europe. The goal is to achieve good ecological status (GES) of all waters by 2015. The structure and function of biological elements, such as fish, invertebrates, macrophytes, phytobenthos, and phytoplankton, are at the center of the assessments, and these data are supported by hydromorphology and physicochemistry. Ecological status is judged by the degree to which present-day conditions deviate from those expected in the absence of anthropogenic influence, termed *reference conditions*. Sites in which the various elements correspond totally or almost totally to reference conditions are classified as having high ecological status (HES), whereas 4 more categories—good (GES), moderate (MES), poor (PES), and bad (BES) ecological status—indicate the degree of deviation from the reference state. A key pressure on many lowland freshwater ecosystems in Europe is eutrophication.

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Therefore, great efforts have been made to develop tools that quantify the responses of a range of groups of organisms along nutrient gradients (Solheim and Gulati 2008, Birk et al. 2012).

Macrophytes and phytobenthos is one of the biological-quality elements that must be included in WFD assessments of ecological status of freshwaters. Diatoms are a major component of the phytobenthos in lotic and lentic systems, but only a few countries have produced WFD-compliant phytobenthos tools specifically for lakes although a number of authors have examined the relationships between taxonomic composition of benthic algal communities and environmental variables in lakes (e.g., King et al. 2000, Schoenfelder et al. 2002, Kitner and Poulíčková 2003, DeNicola et al. 2004, Denys 2004, 2006, 2007, Poulíčková et al. 2004, Gottschalk and Kahlert 2012). Schaumburg et al. (2004, 2007) used a variety of substrates from 100 lakes to develop a diatom index for 4 German lake types. This Diatom Index for Lakes (DI_{Seen}) combined the Trophic Index (TI) developed by Hofmann (1994) with a Quotient of Reference Species (RAQ). Stenger-Kovacs et al. (2007) used epiphytic diatom samples from 83 lowland, shallow lakes in Hungary and weighted averaging methods to develop the Trophic Diatom Index for Lakes (TDIL), which was applicable to 10 lake types. Bolla et al. (2010) noted that the Indice Biologique Diatomées (IBD) (Lenoir and Coste 1996) and TDIL had the highest correlations with chemistry in Lake Balaton, Hungary, and developed a multimetric index from these 2 indices. Most of these indices are mathematically identical to the transfer functions widely used in palaeolimnology to reconstruct variables such as total P (TP) (e.g., Bennion et al. 1996, Hall and Smol 2010). However, the objective of contemporary assessment is not to provide a proxy for a variable that can be measured relatively easily, but to distill biological information into a value that can be related to biological condition (Kelly 2011, 2013).

We describe a method for assessing ecological status of lakes in the UK based on the response of benthic diatom assemblages along a nutrient gradient. The work was undertaken as part of a larger project funded by the national agencies charged with implementation of the WFD. The overall objective of the lakes project was to develop a robust operational tool to enable prediction of ecological status based on the diatom assemblage present at any standing-water site in the UK. More specifically, the project team set out to: 1) gather existing and new data covering benthic diatoms and associated environmental data across the complete range of lake types in the UK into a database, 2) define the expected (reference condition) diatom assemblage at any site, 3) develop a model for assessing ecological status (expressed in terms of quantitative deviation from reference condition) along a nutrient pollution gradient, 4) develop a rationale for placing status class boundaries along this gradient, and 5) combine all of the above into a package to be used for routine assessment of water bodies.

METHODS

Sites and samples

A total of 1079 samples from the littoral zones of 228 lakes across England, Scotland, and Wales were analyzed. The sites represent a range of lake types (Table 1). The reporting typology for Great Britain defines 18 types based on catchment geology and mean depth (UK TAG 2003). However, for the purposes of this project, a simplified typology based on geology alone was used to divide lakes into 3 types: low (LA), medium (MA), and high alkalinity (HA). Depth was not incorporated for 3 reasons: 1) phytobenthos samples at the lake margins are unlikely to reflect differences in mean lake depth, 2) very low numbers of lakes in some types of sites were classified by depth and geology, and 3) full bathymetric surveys have not been carried out at all lakes, so for many water bodies, the mean depth is estimated or modeled based on the relationship between maximum and mean depth. A preliminary detrended correspondence analysis (DCA) of the diatom data for each lake type showed considerable overlap in the taxa present in the 3 depth classes for all lake types, indicating that the phytobenthos from the eulittoral zone does not discriminate between deep, shallow, and very shallow lake types. The typology also did not discriminate between humic and clear lakes, but no indication was found in the results that humic lakes responded differently than nonhumic lakes. The original data set contained 51 samples from lakes with low pH (<5.5) or very low alkalinity (<10 μeq/L). These sites were removed to avoid the confounding effects of an acidity gradient on development of the nutrient tool.

Most phytobenthos samples were collected from rocks and cobbles (epilithon), but such surfaces were scarce or absent at a number of lakes. In those lakes, samples were collected from plants (epiphyton) (see below). In total, the data set comprises 714 epilithon samples and 127 epiphyton samples. The exact substrate of the remaining samples was unknown because the information was not re-

Table 1. The total number of samples (sites) in the lakes data set by alkalinity and depth class.

	Alkalinity class			
Depth class	Low	Medium	High	Total
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)

corded. Most epiphytic samples were from very shallow HA lakes. The same location and substrate was sampled at each lake on each sampling occasion, such that multiple samples per lake are samples collected at different times. Spring (SP) samples were collected between March and May, summer (SU) samples between June and August, and autumn (AU) samples between September and November. Most samples were collected during spring, summer, and autumn 2004, with the largest number collected during spring 2004.

Phytobenthos collection and identification

Five cobbles were collected from the littoral zone of each lake away from inflow streams and obvious human influences. The top surface of each cobble was brushed with a clean toothbrush 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, 5 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). Stems were cut below water level (ideally from different individuals of the same species), and a plastic sampling bottle was placed upside down on the underwater stem. The stem was then cut below the mouth of the bottle, and the bottle plus stem turned upright. Diatoms were removed from the stem with vigorous brushing with a toothbrush. The resulting suspension was collected in a plastic bottle and fixed with Lugol's iodine. Samples were either digested in a saturated solution of potassium permanganate and concentrated HCl (Hendey 1974) or with H_2O_2 to remove organic material, and permanent slides were prepared using Naphrax® (refractive index = 1.74; Brunel Microscopes, Chippenham, UK) as a mountant (Battarbee et al. 2001).

At least 300 valves of nonplanktonic taxa were identified and counted at 1000× magnification (CEN 2004). The primary floras and identification guides used were Krammer and Lange-Bertalot (1986–2004) 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 taxon–environment scatterplots suggested that the responses of infraspecific taxa were not distinguishable from that of the species (Kelly et al. 2008a). The list of common diatom taxa and their authorities is given in Appendix S1.

Environmental data

Environmental data linked to the diatom samples were extracted from databases held by the UK regulatory agencies. For some samples, the timing of environmental data collection did not correspond with the timing of biological sampling, so environmental data were substituted from the nearest available time period. The environmental data set comprises mean annual values. Annual means were considered more robust than seasonal values because seasonal data were absent or patchy for a large number of lakes. The number of measurements contributing to annual means ranged from 1 to 45 (average 8). The number of samples and the seasonal distribution of these samples affected the robustness of annual mean values calculated for individual lakes. Data for total N (TN) were very limited, particularly for LA lakes, as were data for silica (SiO_2) and total oxidized N (TON). However, total P (TP) concentrations were available for all lakes, and TP and TN are highly correlated in this data set ($r = 0.72$, $p < 0.001$). Thus, TP was used to summarize the nutrient-pressure gradient.

Defining reference lakes

An understanding of the biota at reference condition is central to development of a WFD-compliant monitoring tool. In the absence of long-term data, reference conditions can be derived with a number of methods including spatial-state schemes, expert judgment, palaeolimnology, and modeling (WFD Annex V). A combination of these methods was used to identify a set of reference sites for use across a number of WFD projects. The main criterion was absence of anthropogenic influences, defined as absence of point-source inputs, <10% nonnatural land use, and <10 inhabitants/km^2 (Carvalho et al. 2008, Järvinen et al. 2013).

Palaeoecological data were used to validate reference lakes chosen by the above criteria. Data were collated from all UK lakes where palaeoecological diatom studies have been undertaken, and the top and bottom samples of a sediment core (assumed to represent the present day and reference conditions, respectively) were compared (Bennion and Simpson 2011). Examples do exist of lakes that first began to be enriched by human activity many centuries and even millennia ago, but an analysis of palaeoecological data from ~100 European lakes indicates that eutrophication for most lakes in Europe occurred from the middle-to-late 19th century (Battarbee et al. 2011). For the UK, ~1850 is considered a suitable date against which to assess impacts for lakes because this year represents a period before major industrialization and agricultural intensification (Battarbee 1999). Hence, the core sample dated to ~1850 or, for undated cores, the lowermost (i.e., oldest) sample was taken to represent the reference state (Bennion and Simpson 2011). Where statistically insignificant change in the diatom assemblages was observed between the reference and core top samples, sites were judged to be reference lakes. Nineteen, 11, and 5 reference lakes for LA, MA, and HA types, respectively, were identified by this method. Where palaeoecological data were not available, the landuse criteria were used to identify 16, 2, and 2 more reference lakes (LA, MA, and HA types, respectively).

A total of 35, 13, and 7 reference lakes were identified for LA, MA, and HA types, respectively. Few HA reference lakes exist, but this situation might be expected given their productive catchments and long history of impacts. These data were used to develop an a priori status classification for the lakes in the data set, so that lakes were classed either as reference (55 lakes) or nonreference (63 lakes). Where insufficient abiotic or palaeoecological data were available to evaluate lake status, a class was not assigned (110 lakes).

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

Weighted-average metrics, such as the Trophic Diatom Index (TDI; Kelly and Whitton 1995) offer a convenient means of summarizing information about taxonomic change in a single value. The TDI was developed for rivers, but the same concept can, with recalibration, be applied to lakes. This approach is preferable to using a diatom-based TP transfer function (e.g., Bennion et al. 1996) because TP transfer functions infer chemical variables and, hence, do not express results in terms that are compatible with the ecological concepts at the heart of the WFD. Therefore, the TDI developed for rivers (Kelly and Whitton 1995, Kelly et al. 2008a) was taken as the starting point for deriving a pressure metric for lakes because it is an existing expert system for phytobenthos in UK waters, is sensitive to the pressure of interest (nutrients), and is in keeping with the ecological structure and function concepts of the WFD.

A rescaling algorithm used by Kelly et al. (2008a), 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 data set and to adjust taxa to the lake nutrient gradient. First, a sample score was derived for all samples in the database as a weighted average of the original TDI taxon indicator values. Second, new taxon indicator values were calculated as a weighted average of the sample scores. This double-weighted-averaging calculation was repeated until the new taxon scores stabilized. The resulting index was termed the Lake Trophic Diatom Index (LTDI). As for rivers, all taxa were allocated to 1 of 5 sensitivity groups with this rescaling algorithm. The taxon LTDI scores are numbered from 1 (low nutrient tolerance) to 5 (high nutrient tolerance).

The assumption that the LTDI reflects the primary gradient in the data set was tested by canonical correspondence analysis (CCA) on the whole data set with nutrients as the only environmental variables. A comparison of CCA-axis-1 species scores and sample scores with LTDI scores provided an assessment of how well the new metric reflected the nutrient pressure. In addition, the relationships between LTDI groups and the nutrient gradient were examined separately for each lake type by plotting the relative abundances of the major taxa in the 5 LTDI groups along the nutrient gradient, expressed as TP, for each type. The relationship between the LTDI scores and nutrients for each lake type was further assessed via correlation.

Some authors (e.g., Blanco et al. 2004, Bolla et al. 2010) have advocated the use of metrics developed for rivers for assessment of ecological status in lakes, so the river variant of the TDI (Kelly et al. 2008a) also was computed on all the samples in the lake data set and the outputs were compared with the outputs from the LTDI. The expectation would be a high correlation between the 2 variants (slope = 1) if river and lake metrics performed similarly.

Calculation of expected LTDI at reference conditions and derivation of Ecological Quality Ratios (EQRs)

The median of the LTDI values for all samples from reference lakes in each type was used as the expected LTDI value for that type so that ecological status assessments could be presented as Ecological Quality Ratios (EQRs). An EQR was calculated for each sample in the data set as the ratio of observed to expected LTDI score, that is EQR = O/E, where O = (100 – observed LTDI) and E = (100 – expected LTDI). The rescaling (100 – n) was necessary because LTDI is a nutrient index in which low values imply good and high values imply poor quality, whereas the WFD requires high EQR values to indicate high status (implying low nutrient concentrations) and low values to indicate poor or bad status. The site EQR was calculated as the mean of all sample EQRs for that site.

WFD normative definitions define the composition of the phytobenthos at GES as slightly changed from that expected at HES. Once the composition is moderately changed, the biota is at MES. Therefore, a rationale was needed to distinguish between a slight and a moderate change, given that change along pressure gradients is gradual, whereas the WFD requires delimitation of 5 distinct categories of ecological status. The procedure for defining the class boundaries for lakes follows the same approach as that for UK rivers (Kelly et al. 2008a). First, the LTDI scores were converted to EQR scores based on the expected LTDIs at reference condition. The high/good boundary was defined as the 25th percentile of the EQR values for reference sites in each type. Setting the boundary at the 25th percentile allowed for the possibility that some sites identified as reference might be slightly impacted and so might have lower EQRs than true reference sites. The GES/MES boundary was defined as the crossover from sensitive to tolerant taxon groups. This boundary represents the point at which taxa characteristic of reference conditions become subordinate to those associated with impacted conditions. Thus, our concept of MES is a biofilm with a significantly different structure than that at HES (Kelly et al. 2009). The remaining EQR gradient beyond GES/MES was divided into 3 equal parts.

Assessing model performance

The performance of the model was tested by comparing the status class predicted by the LTDI and the a priori classification of lakes in an independent data set. King et al. (2000) explored the relationship between epilithic algae and environmental variables at 17 lakes in the English Lake District (Cumbria) along a trophic gradient. Each lake was visited 3 times resulting in a total of 51 epilithic diatom samples. These data were harmonized with the UK lakes data set and the LTDI was applied. This set of lakes is useful for assessing the performance of the LTDI because the lakes are well studied LA and MA sites spanning a gradient of pressures for which a range of biological and chemical data are available. These data include EQRs based on TP, chlorophyll *a*, and O_2 concentrations (e.g., Phillips et al. 2008), and status classes based on macrophyte communities (Willby et al. 2009), the chironomid pupal exuvial technique (CPET) (Ruse 2010), and the chord distance from palaeoecological studies (Bennion and Simpson 2011). The evaluation followed the one-out-all-out principle as required by the WFD. The lowest EQR of those measured determined the classification. For example, for Derwent Water, diatoms suggest GES, but the final status is MES, determined by macrophytes.

All numerical analyses were done with R software for statistics and graphics (version 2.0-5; R Project for Statistical Computing, Vienna, Austria) with the *vegan* package for community ecology (Oksanen et al. 2012).

RESULTS

LTDI

CCA axis-1 species scores were strongly correlated (r = 0.81) with LTDI species indicator values, and CCA axis-1 sample scores were very strongly correlated (r = 0.95) with LTDI scores for individual lake samples. The responses of LTDI groups along the nutrient gradient differed among lake types. In LA lakes (Fig. 1A), diatoms showed little response along the TP gradient. *Achnanthidium minutissimum* type (ZZZ835) was abundant across the whole gradient. *Brachysira vitrea* sensu lato (BR001A) and *Tabellaria flocculosa* (TA001A) also occurred in high relative abundances, particularly at TP concentrations >2 μg/L (>0.3 $\log_{10}$[μg/L]). However, no taxa had a strong preference for higher TP concentrations. In MA lakes (Fig. 1B), *A. minutissimum* type (ZZZ835) also was dominant, and *Gomphonema angustum/pumilum* type (ZZZ834) was equally abundant in several lakes. Taxa showed a little more distinction along the TP gradient than for the LA lakes, with a notable decrease in representation of LTDI groups 1 and 2 taxa and a relative increase in LTDI groups 4 and 5 taxa when TP was >~20 μg/L (>1.3 $\log_{10}$[μg/L]). The latter included *Gomphonema parvulum* sensu lato (GO013A), *Eolimna minima* (NA042A) and *Staurosira elliptica* (SR002A). In HA lakes (Fig. 1C), the response along the nutrient gradient was marked. Taxa in LTDI groups 4 and 5, and to a lesser extent in group 3, occurred almost exclusively at TP concentrations >30 μg/L(>1.48 $\log_{10}$[μg/L]). The strength of the relationships between the LTDI score for each sample and the key chemical variables also varied among lake types (Table 2). LTDI score and nutrient variables were strongly correlated in MA and HA lakes (TP: r = 0.46 and 0.68, respectively), but weakly correlated in LA lakes (TP: r = 0.29), where the LTDI was more closely associated with pH and alkalinity (r = 0.55 and 0.40, respectively).

Defining the class boundaries

Median LTDI values for reference lakes belonging to each lake type (22, 35, and 42 for LA, MA, and HA lakes, respectively) (Fig. 2A–C) were used to represent the expected LTDI for the 3 lake types. For the LA and MA lakes, LTDI species groups 1 and 2 differentiated reference from nonreference sites and can be considered sensitive taxa in these systems (Fig. 3A, B). LTDI species groups 4 and 5 were more abundant in nonreference lakes and can be considered tolerant taxa indicative of disturbance (Fig. 3A, B). However, LTDI group 1 taxa were rare in HA lakes (Fig. 3C). In HA lakes, LTDI groups 2 and 3 taxa can be considered sensitive. Therefore, the GES/MES boundary was defined as the crossover between combined groups 1 and 2 (nutrient sensitive) and combined groups 4 and 5 (nutrient tolerant) for the LA and MA types (Fig. 4A, B) and as the crossover between combined groups 1, 2, and 3 and combined groups 4 and 5 for the HA type (Fig. 4C). The MES/PES and other boundaries were defined by equal division of the remaining EQR gradient. The resulting boundary value for the critical GES/MES boundary is 0.70 for all 3 lake types, and the values for the HES/GES boundary are 0.92, 0.95, and 0.92 for the LA, MA, and HA types, respectively (Table 3).

Predicted class status

A summary of the a priori status classes (reference, nonreference) vs model predictions for each lake type is presented in Table 4. For lakes identified as reference sites, the LTDI gave good predictions for all 3 lake types,

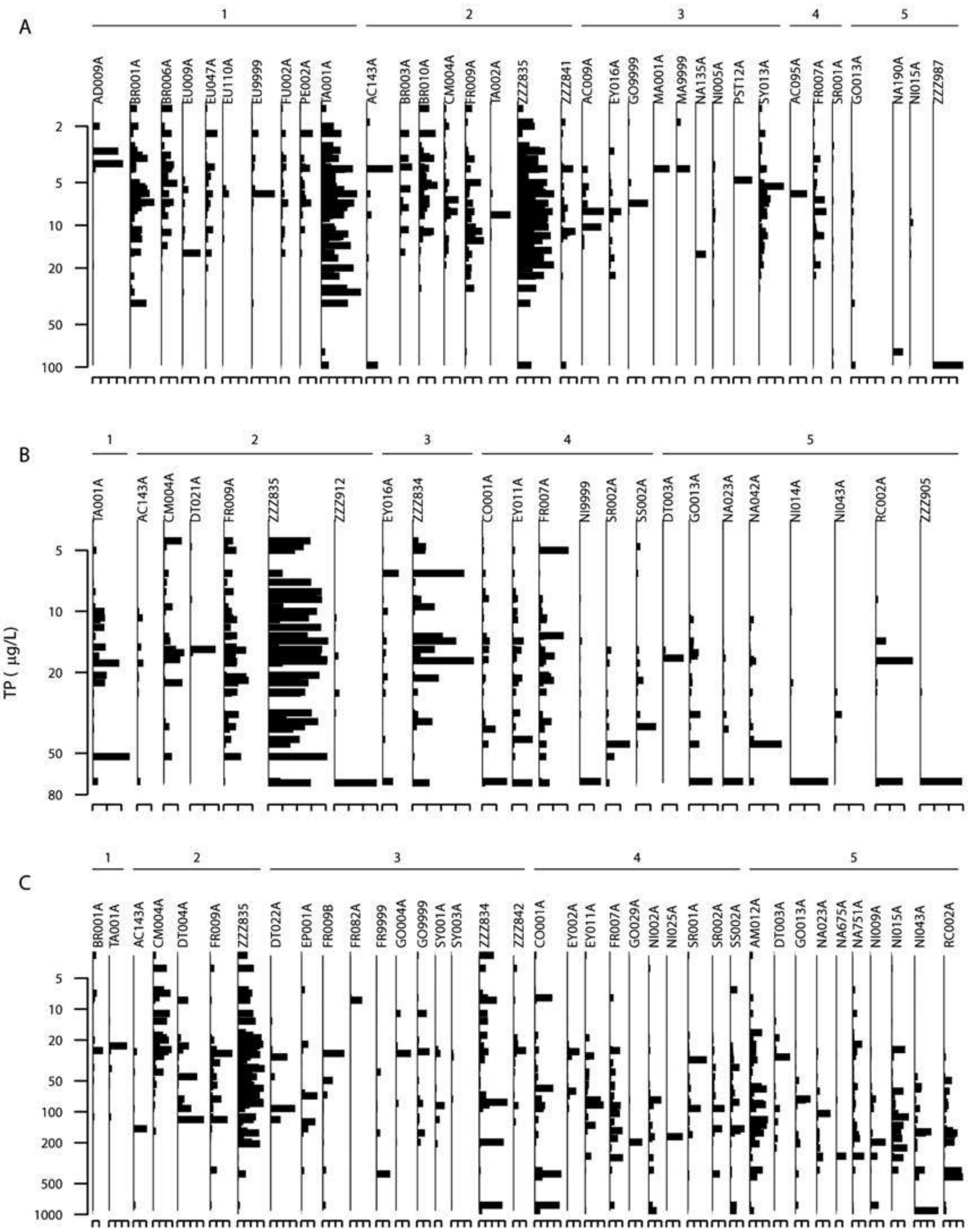

Figure 1. Diatom species distribution along the nutrient gradient (expressed as total P [TP]), for low alkalinity (LA) (A), medium alkalinity (MA) (B), and high alkalinity (HA) (C) lake types. Only those taxa with relative abundance >25% are shown for each lake type. Lines above plots indicate Lake Trophic Diatom Index (LTDI) group. Ticks on *x*-axes indicate units of 10% relative abundance. See Appendix S1 for key to taxon names.

with 68% of these sites classified as HES and the remainder classified as GES. For nonreference sites, the LTDI performed reasonably well, with 81, 78, and 83% of these sites classified as GES or worse for the LA, MA, and HA lake types, respectively.

Assessing model performance

A comparison of the LTDI classification results with status assessments based on other approaches, both biotic and abiotic, for 17 lakes in the English Lake District also indicated that the LTDI performed well (Table 5).

Table 2. Correlations between Lake Trophic Diatom Index (LTDI) score for each sample and key chemical variables ($\log_{10}[x]$-transformed except pH) by lake type. All correlations are significant ($p < 0.01$) except those indicated by asterisks. LA = low alkalinity, MA = medium alkalinity, HA = high alkalinity.

Type	Alkalinity	pH	Conductivity	Chlorophyll *a*	Total P	Total N	SiO_2
LA	0.40	0.55	0.33	0.32	0.29	0.10*	0.02*
MA	0.29	0.36	0.28	0.41	0.46	0.62	0.17*
HA	0.18	0.33	0.18	0.30	0.68	0.32	0.14*

Classifications based on the LTDI alone gave the same status (±1 class) as other approaches for 11 of 17 lakes (65%). Agreement with other tools was greatest for CPET (100% classified to within 1 class; 70% exact matches), followed by chlorophyll *a* (71, 41%), and macrophytes (60, 27%). The diatom classifications tended to be less stringent than those based on other tools and placed 13 of the 17 lakes (76%) in a higher status class than that indicated by the entire suite of biological metrics (Table 5). However, when the comparison was based on the mean class derived from either the full suite of tools or the biological metrics only, then only Ennerdale (of the lakes where sufficient data were available for comparison) was placed in a higher class using the LTDI, compared to placement using the whole suite of metrics.

Effect of substratum type on LTDI

The effect of substratum (sample habitat) on LTDI was examined for sites with both epilithon and epiphyton samples (33 sites). The mean LTDI score for each substratum was calculated for each site. Epilithon samples tended to have slightly higher LTDI scores than epiphyton samples at higher-LTDI sites, but overall, the mean difference between the paired samples (2.2 LTDI units) was not significantly different from 0 (paired *t*-test, $p = 1.84$). Given that the effects of substratum type on LTDI were not significant ($p > 0.05$), substratum type was not considered in subsequent model development.

Data were extracted for all sites with spring, summer, and autumn samples and the mean LTDI score was calculated for each season at each site to examine seasonal effects. Autumn samples had slightly higher LTDIs than summer or spring samples, but season did not significantly affect LTDI values (analysis of variance with Tukey's Honestly Significant Difference post hoc test). Season also was not considered in subsequent model development.

Comparison between lake and river TDIs

LTDI and river TDI values were strongly related ($r = 0.95$, $p < 0.01$; Fig. 5), but the slope of this relationship was 0.84 rather than 1.00, as might be expected. This relationship was used to calculate the rate of misclassification that would be achieved if the metrics were used as the basis for a categorical distinction between a water body complying or not with the WFD. An LTDI value of 60 equates to a river TDI of 50.4. In our data set, 88% of sites had the same categorical outcome (i.e., achieved GES or higher with both metrics or failed to achieve GES with both metrics). No obvious heteroscedasticity was observed in the relationship (no irregular or uneven spread of data points), so this rate of misclassification (proportion of sites misclassified by the river TDI) can be assumed to apply across the entire LTDI gradient.

DISCUSSION

Definitions of ecological status

DeNicola and Kelly (2014) pointed out that taxonomic metrics, such as the LTDI, have strong relationships with water chemistry but do not necessarily capture all aspects of ecological status and integrity and went on to discuss the problems associated with incorporating measures of diversity, biomass, and productivity into routine assessments. The LTDI is valuable only if used correctly. In the UK, it is used as part of a suite of assessment tools spanning several trophic levels for the basic task of assigning water bodies to the appropriate ecological status class. The lowest result from all the ecological components determines the final status class, so it is not necessary for all elements to reflect all pressures, although it is useful to understand confounding influences when interpreting results. Failure to achieve GES triggers further investigations, and at that stage, several of the methods suggested by DeNicola and Kelly (2014) may have value in untangling the various pressures from the causal thickets (sensu Wimsatt 1994) and deciding appropriate remedial action.

In our study, reference sites were identified based on evidence from contemporary monitoring, palaeoecology, modeling, and to a lesser extent, expert judgment. 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 identify those lakes that might be considered as reference sites (e.g., Leira et al. 2006). For the purpose of developing the LTDI, 35, 13, and 7 reference sites were identified for LA, MA and HA lake types, respectively. The number of reference sites was considered adequate for LA and MA lakes, but few examples of HA reference lakes exist in the UK because of the long history of

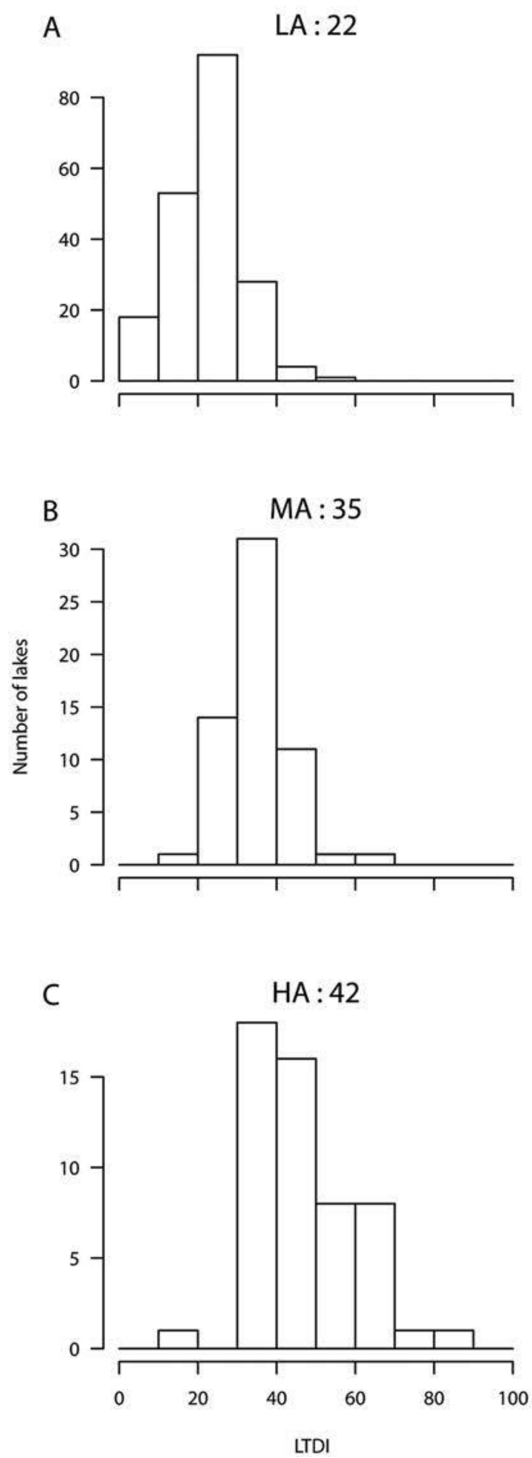

Figure 2. Histograms showing the distribution of Lake Trophic Diatom Index (LTDI) values for samples from low alkalinity (LA) (A), medium alkalinity (MA) (B), and high alkalinity (HA) (C) reference lakes. The numbers in the titles are the median LTDI values for that lake type.

impacts in lowland Britain and the productive nature of the lake catchments (e.g., Bennion and Simpson 2011). Therefore, our definition of reference condition is less robust for HA lakes than for LA and MA lakes.

Defining GES is, in many ways, more problematic than defining HES largely because the normative definitions for GES and MES allow a wide scope for interpretation. The point at which the assemblage ceases to be *slightly* changed and becomes *moderately* changed is the critical point beyond which a water body needs remedial measures to achieve GES, but the terms of the WFD must be translated into objective and defensible concepts. No absolute justification exists for placement of the GES/MES boundary on the ecological status gradient, and several methods have been proposed (see Davies and Jackson 2006, Brucet et al. 2013). We chose to use the point at which the taxa tolerant to nutrients (which are scarce in pristine environments) become relatively more abundant than the number of taxa that are sensitive to nutrients (which tend to be most common in pristine environments) (Kelly et al. 2009). This method is the *paired metric approach* outlined by Brucet et al. (2013) and reflects structural and ecophysiological changes in the phytobenthos, insofar as these can be inferred from the taxonomic composition of benthic diatoms (Kelly et al. 2008b, 2009).

When the taxa are classified according to their nutrient sensitivity, those associated with reference conditions tend to be found in the first 3 LTDI groups, with group 2 predominating. However, as EQR decreases, the proportion of individuals belonging to taxa in group 1 falls steeply and GES is characterized by a flora composed predominantly of group-2 taxa, accompanied by a small number of group-1 taxa. As the EQR decreases further (i.e., the pressure increases), an increasing proportion of indifferent (group 3) and tolerant (groups 4 and 5) taxa occur. Another characteristic is that the proportion of motile taxa is low at reference conditions, and this proportion increases as EQR decreases. A few motile taxa (e.g., *Navicula angusta*) are characteristic of HES and GES, but these taxa rarely are abundant, whereas at lower EQR values, motile taxa, such as *Nitzschia* spp., often constitute >60% of all individuals in a sample. Some of this difference is driven by biomass because thick periphyton mats in higher-nutrient lakes favor motile species (Yallop and Kelly 2006). Moreover, eutrophic lakes are more likely to have fine sediments, which provide habitat for motile taxa. Consequently, the biofilm found at MES or worse is very different from that described at HES.

The group of sensitive taxa in the lakes data set and in rivers (Kelly et al. 2008a) is dominated largely by *A. minutissimum* sensu lato and *Fragilaria capucina* sensu lato, whereas the tolerant category is dominated by *Amphora*

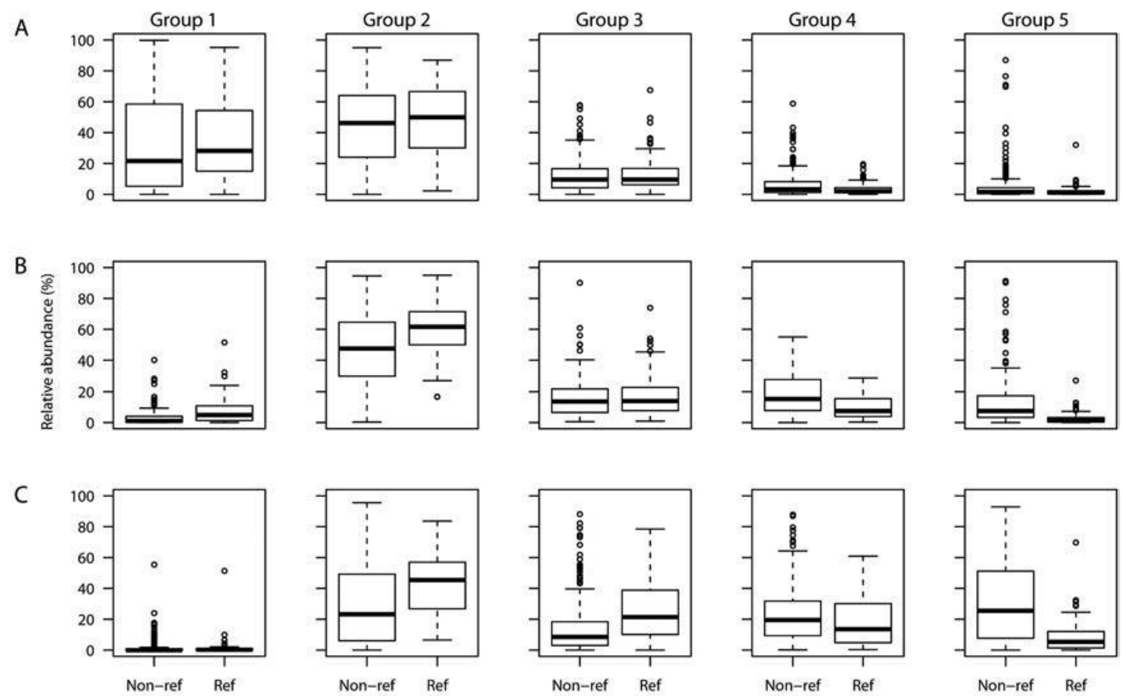

Figure 3. Box-and-whisker plots comparing the distribution of Lake Trophic Diatom Index (LTDI) taxon groups 1 to 5 between reference (ref) and nonreference sites (non-ref) for low alkalinity (LA) (A), medium alkalinity (MA) (B), and high alkalinity (HA) (C) lakes. Lines in boxes show medians, box ends are quartiles, whiskers extend to 1.5 times the interquartile range, and circles show outliers.

pediculus, *Navicula*, and *Nitzschia* spp. Therefore, a number of pollution-tolerant taxa are found at the lower end of GES. In low numbers, such taxa are a natural part of the biota and a concept of GES can embrace the possibility of short-term, low-impact events that affect the flora but from which recovery can be rapid. A switch to a biofilm dominated by nutrient-tolerant species occurs with increasing enrichment of key nutrients, and only those species with specialized mechanisms to exploit such conditions proliferate, out-competing the nutrient-sensitive species. The marked increase in motile species (e.g., *Navicula gregaria* and *Navicula dissipata*) at the GES/MES interface may be explained by their ability to exploit resources unavailable to those occupying a fixed position within productive biofilms. Naviculoids are light–space specialists requiring relatively high concentrations of N and P, and together with nitzschioids, can overgrow less-competitive taxa (Fairchild et al. 1985, Carrick and Lowe 1988). The success of adnate species, such as *A. minutissimum* sensu lato and *Cocconeis placentula* sensu lato, may be compromised as they experience light and possibly nutrient limitation. These species may still occur as epiphytes on filamentous algae, such as *Cladophora*, removing them from the constraints that develop within thicker biofilms. However, nutrient limitation within the *A. minutissimum* complex has been questioned (Fairchild et al. 1985) because it is a P specialist capable of obtaining resources at lower concentrations than its rivals within the biofilms. Under enriched conditions, the most prolific of the sessile diatoms are often found as epiphytes (e.g., *Cocconeis pediculus*, *Rhoicosphenia abbreviata*), which together with *Epithemia adnata* have been identified as N specialists because they possess N-fixing cyanobacterial endosymbionts that can dominate in conditions of low N:P (DeYoe et al. 1992, Marks and Lowe 1993, Gottschalk and Kahlert 2012).

Model performance

Comparison of LTDI outputs with the a priori classification indicates that the model discriminates reference from nonreference lakes with good accuracy. However, 19, 22, and 17% of the nonreference sites were classified as HES for the LA, MA, and HA lake types, respectively. This degree of difference is not considered excessive because several of these sites were classified as nonreference sites for reasons other than nutrient pressures (e.g., alteration of hydrologic regime). Similarly, the mismatch between classifications based on diatoms and those based

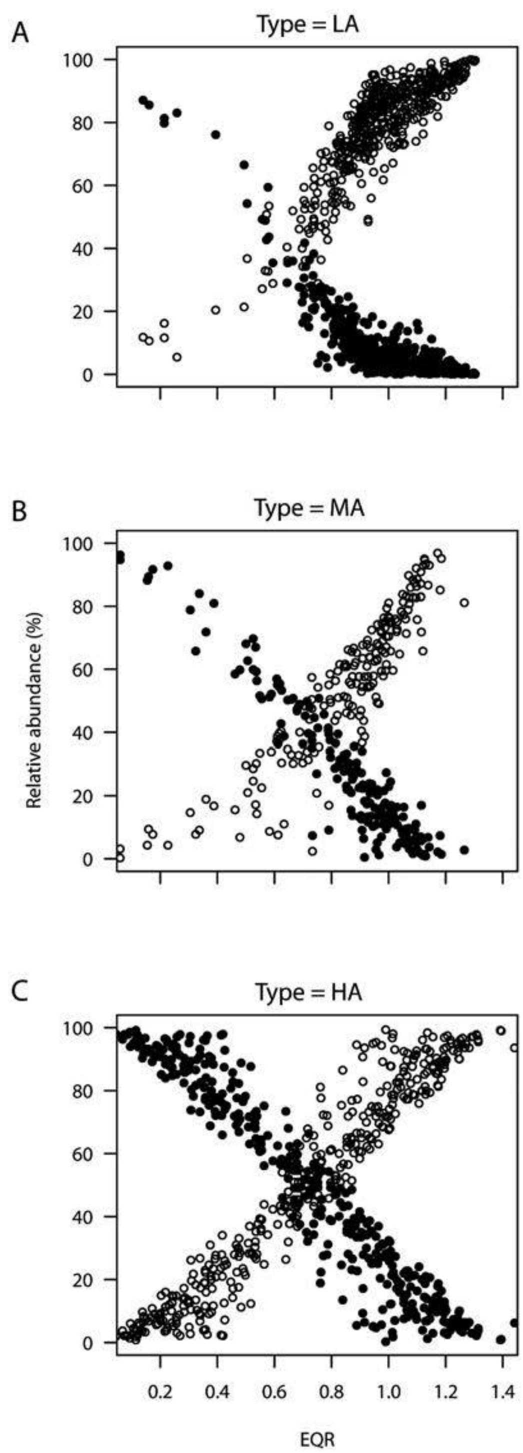

Figure 4. Abundances of nutrient sensitive (open circles) and tolerant (closed circles) diatom taxa along the Ecological Quality Ratio (EQR) gradient for low alkalinity (LA) (A), medium alkalinity (MA) (B), and high alkalinity (HA) (C) lakes. The good/moderate boundary is defined as the crossover between combined groups 1 and 2 (nutrient sensitive), and combined groups 4 and 5 (nutrient tolerant) for the LA and MA types and as the crossover between combined groups 1, 2, and 3, and combined groups 4 and 5 for the HA type.

on other groups of biota used in the Cumbrian lakes reflects responses to other pressures (acidification, hydromorphological, recreational), whose effects will be expressed differently by each element of biological quality. In fact, very high agreement among classifications based on different metrics would suggest redundancy among metrics, which is clearly not the case. Our results show that the LTDI predictions match the predictions made from other evidence for lakes at HES and GES, but diatom and other methods agree less for lakes classified by the other methods as having less-than-good status (e.g., Esthwaite Water and Grasmere; Table 5).

One possible explanation for the lower stringency of the diatom metric than of some other UK lake tools is that the pressure–response relationship was compromised by merging taxa to form aggregates, such as *A. minutissimum* sensu lato. Some evidence shows that taxa in the *A. minutissimum* complex can be assigned to morphological groups, each with differing ecological preferences, though these morphological groups are not discontinuous (Potapova and Hamilton 2007). Thus, greater taxonomic discrimination might give stronger relationships with the pressure gradient than we observed. However, in the absence of ecophysiological studies on individual species within this complex, the effects of pressure-related and nonpressure-related covariables cannot be disentangled. Therefore, we suggest that, given present knowledge, the aggregate provides a better estimate of the functioning of the biofilm (see below).

Another possible explanation is the scarcity of impacted LA lakes. Such lakes tend to occur in areas where agricultural productivity is low and settlements are few. Thus, we were unable to capture the full response gradient. Over the range studied, a generalist taxon, such as *A. minutissimum*, may not be out-competed by nutrient-tolerant taxa. Palaeoecological work at LA lakes indicates that subtle shifts in planktonic diatoms suggest the early stages of enrichment rather than changes in nonplanktonic diatoms (Bennion et al. 2004). 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 assemblage, such as a decrease in oligotrophic and an increase in mesotrophic taxa (e.g., Bennion 1995). The high numbers of inocula of taxa, such as *A. minutissimum*, in a lake littoral zone would buffer it against change, so in our data set, the LA lakes do not cover a long enough nutrient gradient for us to observe marked species turnover. However, changes might be observed in the total biomass of the periphyton. The result is that, for some lakes, diatom metrics are likely to underestimate the degree of ecological change attributable to nutrient enrichment. For example, palaeoecological studies and other ecological data suggest that Loweswater, Bassenthwaite Lake, and Grasmere, 3 of

Table 3. Ecological Quality Ratio (EQR) class boundaries for the 3 alkalinity types. LA = low alkalinity, MA = medium alkalinity, HA = high alkalinity. H = high, G = good, M = moderate, P = poor, B = bad ecological status.

Type	H/G	G/M	M/P	P/B
LA	0.92	0.70	0.46	0.23
MA	0.95	0.70	0.46	0.23
HA	0.92	0.70	0.46	0.23

the Cumbrian lakes included in the validation study, are not in their former oligotrophic state and have experienced enrichment (Bennion et al. 2000). However, this impact is not reflected in the epilithic diatom assemblage. This means that the littoral biofilm can be said to be at GES even though other components of the lake ecosystem, such as macrophytes, are showing signs of enrichment. Rather than reflecting an inadequacy in the diatom model, we think that the LTDI is a reliable 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 *A. minutissimum* often indicate disturbance or grazing (Biggs et al. 1998), and hence the persistence of this taxon suggests that slightly increased pressure does not lead to fundamental alterations in energy flow through the ecosystem. Hence, ecological functioning, in this part of the biota at least, has not measurably changed. This interpretation continues the theme outlined by DeNicola and Kelly (2014) of focusing on the role of phytobenthos in delivering ecosystem services rather than simply trying to optimize pressure–response relationships.

Consideration also should be given to the different exposure to nutrients experienced within periphyton. Phytoplankton in the water column typically are in more-direct contact with nutrients than are periphyton (Cattaneo 1987, Lowe 1996). Steep resource gradients have been measured in periphyton (Bothwell 1988), and water chemistry in periphyton may differ from that in the water column (Revsbech and Jørgensen 1986). The thickness of the boundary layer overlying the periphyton affects rates of diffusion. In areas of low turbulence, e.g., among aquatic plant beds, this layer may be much thicker than in areas of higher turbulence. The degree of nutrient limitation may depend on the efficiency of nutrient recycling by the bacterial component and potential pathways from overlying water and the hyporheic zone (Wetzel 1993). Thus, it might be hypothesized that the periphyton responses in littoral regions may lag behind those of phytoplankton. Exceptions to this pattern may occur in areas of localized nutrient loading to littoral areas, in which case the phytobenthos might act as an early warning system (Hawes and Smith 1992). The lack of a response by the LA periphyton also could be related to C limitation in LA lakes (Fairchild and Sherman 1993). Despite these concerns, a gathering body of evidence suggests that indices derived from benthic assemblages may be more effective and sensitive measurements of eutrophication than their phytoplanktic counterparts, particularly in sites experiencing increased shore degradation (Kann and Falter 1989, Lambert et al. 2008).

We found no significant differences between LTDI values derived with epilithon or epiphyton at 33 sites, a result corroborating findings from comparable measurements undertaken in rivers (Yallop et al. 2009). Further, Kitner and Poulíčková (2003) measured no significant differences in trophic indices derived from epilithon, epiphyton, and epipelon in samples from shallow lakes.

The UK plant metric (LEAFPACS) and the LTDI provided the same status in only 4 of the 17 lakes in the English Lake District, whereas the LTDI indicated a higher status than LEAFPACS in 11 other lakes. Indices derived from macrophytes might not be a direct indication of water-column nutrient concentrations because most macrophytes derive most of their nutrients from the sediments, whereas their epiphytes rely on nutrients in the littoral water column and are considered the primary scavengers of nutrients from the water column (Wetzel 2001). The shorter generation time for diatoms means they provide an indication of increasing or decreasing water quality over time scales of weeks, whereas macrophytes, with longer generation times and lower rates of dispersal, are slower to respond (Schneider et al. 2012). Schneider et al. (2012) suggested that differences in assessments between biotic indices based on diatoms and those based on macrophytes are expected in ecosystems subjected to environmental change, and they postulated that differences between indices inform us of ecosystem stability. In some lakes, we recorded either no difference (Wastwater,

Table 4. Model predictions of ecological status class vs a priori reference status for each lake type indicating number of sites in each class.

Type	High	Good	Moderate	Poor	Bad
Low alkalinity					
Reference	24	11	0	0	0
Nonreference	3	10	1	2	0
Medium alkalinity					
Reference	10	3	0	0	0
Nonreference	4	8	4	2	0
High alkalinity					
Reference	3	4	0	0	0
Nonreference	5	6	5	8	5

Table 5. Comparison of diatom classification results with other status assessments for 17 lakes in the English Lake District. H = high, G = good, M = moderate, P = poor, B = bad, LA = low alkalinity, MA = medium alkalinity, HA = high alkalinity, D = deep, S = shallow, vS = very shallow, 2000 = year of sampling, Ref = reference, TP = total P, Chl *a* = chlorophyll *a*, CPET = chironomid pupal exuvial technique, Palaeo = palaeoecological study, LTDI = Lake Trophic Diatom Index, WBID = Waterbody ID as used in the UK Lakes database. Type is formatted as a combination of alkalinity and lake depth.

										Ecological status based on:						
WBID	Name	Type	Maximum depth (m)	Mean depth (m)	Alkalinity (mg/L)	Mean TP 2000 (µg/L)	Mean Chl *a* 2000 (µg/L)	Ref TP (µg/L)	Ref Chl *a* (µg/L)	TP	O_2	Chl *a*	Plants	CPET	Palaeo	LTDI
29183	Wast Water	LAD	76	39.73	60	1	1.19	4	1.5	H	G	H	H	H	H	H
29052	Buttermere	LAD	28.6	16.60	64	1.4	1.59	5	1.9	H	H	H	H	H	H	H
29062	Ennerdale Water	LAD	42	17.76	62	1.9	1.26	5	1.8	H	H	H	G	G	M	H
29116	Brothers Water	MAS	15	6.20	202	6.6	2.18	8	3.3	H		H				H
29000	Crummock Water	LAD	43.9	26.70	50	3.2	2.95	4	1.5	H	H	G	G	H	H	G
28965	Derwent Water	LAS	22	5.50	127	7.5	4.73	8	3.0	H	M	G	M	H	G	G
29321	Coniston Water	MAD	56.1	24.10	222	7.5	5.68	6	2.3	H	H	M	G	G	M	G
28955	Ullswater	MAD	62.5	25.30	248	9.9	4.85	6	2.4	G	H	M	M	H	H	H
29233	Windermere N Basin	MAD	64	25.10	250	12.3	4.35	6	2.4	G		G	M	G		G
29233	Windermere S Basin	MAD	64	16.8	250	13.9	6.42	7	2.7	M	G	M	M	G	P	G
28986	Loweswater	LAS	16	8.37	185	16.4	9.62	8	2.9	M		M		G	M	G
29197	Rydal Water	LAS	<10	5.30	160	18.1	6.53	8	3.2	M		M	P			G
28847	Bassenthwaite Lake	LAS	19	5.30	180	20.9	14.53	9	3.3	M	P	P	B	G	M	G
29328	Esthwaite Water	MAS	15.5	6.40	459	30.3	21.02	10	4.0	M	P	P	G		M	H
29270	Blelham Tarn	MAS	14.5	6.80	558	31.7	14.94	11	4.1	M	P	M	P		G	G
29184	Grasmere	LAS	21.5	7.74	168	23.6	12.34	8	2.9	M	P	P	M		M	H
29222	Elterwater	LAvS	7.4	2.90	135	50.7	30.32	9	3.6	B	P	B	P		M	G

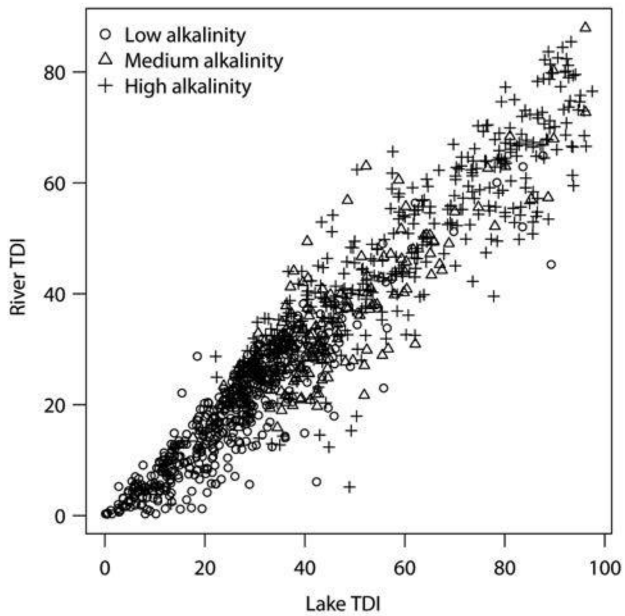

Figure 5. Relationship between Trophic Diatom Index (TDI) lake and river metrics for low (n = 514), medium (n = 188), and high (n = 377) alkalinity lakes.

Buttermere), or only 1-status-class difference (Ennerdale Water, Crummock Water) between status indices obtained from diatoms, plants, chironomid exuviae, palaeoindicators, and other metrics, including chlorophyll a or TP (Table 5). In other lakes (e.g., Bassenthwaite Lake, Windermere South Basin) these indices spanned up to 4 status classes, indicating greater instability.

A reduction in external nutrient loading, in many cases, might not lead to the return of macrophyte domination in lakes because of a number of stabilizing factors that operate in the turbid state. Delayed responses might result from internal P loading (Scheffer et al. 1997). Therefore, reduction in nutrient loading might bring about an improvement in the littoral flora before any noticeable change occurs in the macrophyte assemblage. Use of both metrics is recommended to provide more information about the potential for ecosystem recovery.

The strong agreement between results of assessments based on diatoms and those based on chironomids may reflect the tight coupling between benthic diatoms and chironomid grazers (Maasri et al. 2008, Tarkowska-Kukuryk 2013). The data set used for these comparisons was small, and the association may be a by-product of 2 independent strong correlations with the underlying TP gradient, but the possibility of a functional link between the 2 groups suggests that the outcome of assessing both components is more than the sum of assessing 2 separately.

Do diatoms respond differently in lakes and rivers?

Some authors have shown that diatom metrics developed for rivers are strongly correlated with pressure gradients in lakes (Blanco et al. 2004, Bolla et al. 2010, Cellamere et al. 2012). This outcome leads to questions regarding the need for distinct lake-diatom assessment tools. Cantonati and Lowe (2014) highlighted similarities in the physical and biological pressures encountered by biofilms in the littoral zones of lakes and in rivers. In our study, the TDI metric calibrated on a river data set (Kelly et al. 2008a) was strongly correlated with the LTDI but had a distinctly different response when applied to our lake data set. This difference was manifested in a lower slope (<1) over the alkalinity spectrum of lake types (Fig. 5). One possible explanation for this is that the river TDI was calibrated against a longer nutrient gradient, so the range of river TDI values is compressed relative to lake TDI values. Because UK lakes have fewer point-source discharges of organic matter and their littoral regions have longer residence times compared with rivers, taxa strongly associated with organic pollution (e.g., *Nitzschia palea*, *Fistulifera saprophila*) are less likely to thrive. Blanco et al. (2004), Bolla et al. (2010), and Cellamere et al. (2012) all used metrics developed for rivers, but many of their sites were compressed into the upper half of the scale of the metrics. The river TDI used in our study fared better, perhaps because of its origin as an index of inorganic, rather than organic, enrichment (Kelly and Whitton 1995). Some taxa do appear to be more strongly associated with either lakes (e.g., *Epithemia* spp., some *Cymbella* species) or rivers (*Hannaea arcus*), but most diatoms are opportunistic and exploit the similarities between lake littoral zones and river beds (Cantonati and Lowe 2014). Thus, river metrics do offer a potential alternative to lake-specific metrics (Kahlert and Gottschalk 2014). However, the effects of eutrophication on littoral assemblages in lakes are manifested partly through the shading brought about by increased phytoplankton biomass, with possibilities of subtle interactions that would be overlooked through use of a river metric.

Conclusions

A diatom model, the LTDI, and a rationale for placing status-class boundaries has been successfully developed for assessing ecological status of 3 lake types (LA, MA, HA) along a nutrient gradient. Two-thirds of all lakes identified as reference sites were classified by the LTDI as HES and the rest as GES, whereas the model predicted most nonreference sites as GES or worse. Therefore, the model performs well. However, for LA lakes, the LTDI appears to be less stringent than other UK tools, possibly because of an apparent lack of sensitivity of the benthic diatoms to nutrient pressure in this lake type and the confounding relationship with alkalinity. Furthermore, the scarcity of impacted LA lakes prevented us from capturing the full gradient of response. Nevertheless, the LTDI gives a reasonable assessment of the condition of the littoral biofilm and makes a valuable contribution to the classification toolkit for UK standing waters. It provides a foundation upon which the statutory agencies can monitor to assess the status of UK freshwaters and a statistically sound basis for identifying the need or otherwise for Programmes of Measures. A full description of the method described here is available at www.wfduk.org.

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