Comparing aspirations: intercalibration of ecological status concepts across European lakes for littoral diatoms.

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Abstract

Eleven European countries participated in an exercise to harmonise diatom-based methods used for status assessment in lakes. Lakes were divided into low, medium and high alkalinity types for this exercise. However, it was not possible to perform a full intercalibration on low alkalinity lakes due to the short gradient and confounding factors. Values of the Trophie Index were computed for all samples in order that national datasets could all be expressed on a common scale. Not all participants had reference sites against which national methods could be standardised and, therefore, a Generalised Linear Modelling approach was used to control the effect of national differences in datasets. This enabled the high/good and good/moderate status boundaries to be expressed on a common scale and for deviations beyond $\pm$ 0.25 class widths to be identified. Those countries which had relaxed boundaries were required to adjust these to within $\pm$ 0.25 class widths whilst the intercalibration rules allowed those countries with more stringent boundaries to retain these. Despite biogeographical and typological differences between countries, there was broad agreement on the characteristics of high, good and moderate status diatom assemblages, and the exercise has ensured consistent application of Water Framework Directive assessments around Europe.

Key words: Water Framework Directive; lake; phytobenthos; diatoms; intercalibration; ecological status

Introduction

European environmental legislation such as the Water Framework Directive (WFD, European Union, 2000) operates within a system of governance known as “subsidiarity”, which leaves the details of implementation up to individual member states. As a result, some 297 different methods have been developed and adopted by member states to demonstrate compliance with the requirements set out in the WFD (Birk et al., 2012). In order to ensure that all member states have interpreted the Directive in a consistent manner, the WFD also stipulates that an intercalibration exercise should be performed. This aims to harmonise national approaches to defining those points along the ecological condition gradient which are most important, from the point of view of decision making within the WFD. Ensuring a consistent approach to these means that all member states of the EU share a common ambition, with respect to the state of surface water (Birk et al., 2013).

“Macrophytes and phytobenthos” is one of the biological quality elements (BQEs) whose condition contributes to evaluations of “ecological status” in rivers and lakes, with “good status” (equating to just a slight change from the unimpacted condition) being the target for all surface water bodies by 2015. In practice, most countries perform separate evaluations of macrophytes and phytobenthos, with diatoms being used widely as proxies of phytobenthos. An intercalibration of national methods for using diatoms in central European rivers was reported by Kelly et al. (2009); similar intercalibrations for rivers elsewhere in Europe were also performed, the outputs of which became legally-binding on the countries involved (European Commission, 2008).

A number of problems were encountered during this work, several of which were common to intercalibrations of other groups of organisms (Birk et al., 2013). These included agreeing unambiguous definitions of the unimpacted condition of rivers (Pardo et al., 2012; Kelly et al., 2012) as well as variation between national data sets, part of which mayreflect biogeography but differences in methodology may also play a role, despite all participants adhering to common

standards for sampling and analysis (CEN, 2003; 2004). Kahlert et al. (2012) noted variation between diatom analyses in a ring test persisted even after taxonomic harmonisation which may, under some circumstances, override the effect of continent scale biogeographical variation in determining monitoring outcomes (Kelly et al., 2012).

This paper describes an intercalibration exercise performed on diatom-based methods for assessing the ecological status of European lakes (defined as inland water bodies ≥50 hectares). The general approach was similar to that adopted for rivers (Kelly et al., 2009) but takes into account developments in the intercalibration procedures. National methods should be tuned to optimise the relationship between the diatom assemblage and pressure gradient for a country. However, because many of the species (or species aggregates) are widely distributed across Europe, there should be sufficient similarities between these individual relationships that a broader pressure response relationship should emerge. Put simply, we are asking whether biologists from Ireland and Slovenia (the north-westerly and south-easterly extremes of participants) could look down a microscope and arrive at similar judgements about the ecological status of a sample originating from Finland (the north-easterly extreme). If this is possible, then we can be confident that, for this group of organisms at least, the WFD is being implemented in a consistent manner across the EU.

Methods

The EU intercalibration exercise

As intercalibration is a formal requirement of the WFD, a standard methodology, applicable to all types of water bodies and all BQEs (summarized in Birk et al., 2013) has to be adopted. Various options are available, depending on the similarities between national methods and the availability of reference sites. In the case of benthic diatoms, data are collected by very similar means by all participating countries, permitting an “indirect comparison” (Birk et al., 2013) whereby values computed using the national indices are each converted to a common metric. A regression between

national and common metrics then allows values for status class boundaries to be expressed on a common scale. Boundary bias is evaluated as the difference between the national boundary and the average of all participating countries and is regarded as acceptable if the national boundary falls within ± 0.25 class widths of the average. Countries whose boundaries are more than 0.25 class widths below the average must adjust these to be within ± 0.25 class widths; however, boundaries greater than 0.25 class widths above the average can be retained,.

Because ecological status is expressed as Ecological Quality Ratios (EQRs: defined as the observed state / expected state), there is an implicit assumption that all countries are able to make robust predictions of the expected (i.e. unimpacted) state for the water body in question. In practice, this has proved to be very difficult (see Pardo et al., 2012) and procedures have also been developed which allow comparisons in the absence of reference conditions. These are "alternative benchmarking" (when datasets were calibrated against a similar (low) level of impairment) and "continuous benchmarking", where biological differences between national datasets were established by regression analysis, and an appropriate offset applied to each national dataset to bring it into line (Birk et al., 2013).

Datasets

11 countries took part in this exercise (Table 1). Each submitted data from national monitoring or method development programs. Diatom samples were collected from the littoral zones of lakes, sampled from either submerged stones or macrophytes, adapting the principles of CEN (2003) to standing, rather than running waters (King et al., 2006); these were then processed in the laboratory to yield permanent slides from which at least 300 diatoms were named (mostly to species) and counted (CEN, 2004). Taxonomy was based on Krammer and Lange-Bertalot (1986-1991) and subsequent publications, following national conventions. As this paper does not directly compare composition, instead focusing on metric values, the taxonomic conventions described in Kelly and

Ector (2012) and Kahlert et al. (2012) are not necessary, and any systematic variation arising from different approaches to taxonomy will be included in the national offsets described below.

National methods fall into one of three types:

1. indices based on the weighted average equation of Zelinka & Marvan (1961) and optimised against a stressor gradient (e.g. Lake Trophic Diatom Index, LTDI, Bennion et al., 2014);

2. indices based on the relative proportions of taxa associated with unimpacted ("reference") and impacted conditions (e.g. PISIAD: VMM, 2009); and,

3. multimetrics based on a combination of these approaches (e.g. PHYLIB, Schaumburg et al., 2004).

Methods of Belgium (Flanders), Germany, Hungary, Ireland, Poland and UK were developed specifically for lakes whilst those of Finland, France, Sweden and Slovenia were originally developed for rivers but have statistically significant relationships with pressure gradients in lakes (e.g. Cellamare et al. 2012; Kahlert & Gottschalk, 2014). Further details of national methods can be found at www.wiser.eu/results/method-database and in Kelly (2013).

Lakes were classified into an appropriate "Geographical Intercalibration Group" and "Type" following Carvalho (2008). However, some factors used to define types (e.g. maximum depth) are less relevant for littoral-dwelling organisms and a simpler typology was adopted here, with lakes defined as either "low alkalinity (LA)" (< 0.2 meq L^{-1}), "moderate alkalinity (MA)" (≥ 0.2, < 1 meq L^{-1}) or "high alkalinity (HA)" (≥ 1 meq L^{-1}). .

Reference conditions

Lakes were deemed to be in reference condition if the following criteria applied:

1. no point sources of pollution;

2. population density < 15 people per square kilometre;

3. < 0.4% artificial land use within catchment;
4. < 20% agriculture in the catchment, not adjacent to lake (low-intensity stock raising on semi-natural landscapes excluded);
5. < 10% of lake shoreline is artificial;
6. no alteration of natural lake hydrology (i.e. no dams or similar structures);
7. no introduction of carp or other bottom-feeding fish;
8. no intensive (commercial) fishing.

Most of these criteria apply to whole lakes. For the agriculture and artificial shoreline criteria, samples were accepted if the sites were well away from such influence. The screening criteria make no explicit reference to aerial deposition of pollutants; however, those countries with lakes with very soft water did remove any which showed obvious signs of acidification.

Intercalibration process

The same principle was adopted here as for the river phytobenthos intercalibration exercise, with an "intercalibration metric" calculated on all national datasets to allow national boundaries to be converted, via linear regression, to a common scale. For the river phytobenthos intercalibration exercise, the phytobenthos intercalibration metric (Kelly et al., 2009) was the average of two widely-used metrics: the Indice de Polluosensibilité Specifique (IPS: Coste in CEMAGREF, 1982) and Trophieindex (TI: Rott *et al.*, 1999). However, the IPS is effective over a wide range of water quality, extending into highly "saprobic" conditions, rarely found in lakes. In practice, only about half the IPS scale was used and the IPS component of the metric did not improve its discrimination power and sensitivity, compared to TI alone. For this reason, the lake phytobenthos intercalibration metric is based on the TI alone.

An Ecological Quality Ratio (TI_EQR) was calculated as (4 - observed TI)/(4 - expected TI) for each sample, where “expected TI” was the average of national mean values of the TI for all countries with reference sites, as defined above. A separate expected TI was calculated for each of the three types using TI = 1.02 for low alkalinity (LA) lakes, 1.38 for moderate alkalinity (MA) lakes and 1.88 for high alkalinity (HA) lakes. Because the TI has a scale that increases with nutrient enrichment, actual values of the TI had to be subtracted from the maximum possible value (4) in order to ensure that low TI values (reflecting low nutrients) equated to high status.

Three basic options for intercalibration are explained in Birk et al. (2013). In situations such as this, where there are insufficient references and benchmark sites, Birk et al. (2013) recommend continuous benchmarking. The principle of this approach is that all national regression curves (national metric versus pressure gradient) are adjusted to a common regression curve for all data together (Böhmer et al 2012). One of the statistical models that can be used in the continuous benchmarking is the Generalized Linear Model (GLM). This is aflexible generalization of ordinary linear regression which allows for response variables that have other than a normal distribution error distribution. It is also allow the use of categorical variables when building the model, allowing us to include “member state” as a nominal random variable along with log TP as a continuous covariate.

Continuous benchmarking was done using the GLM function in SPSS Statistics version 17.0 (SPSS Inc. 2008). In the model, TI_EQR was used as a dependent variable, member state as a random variable and the logarithmic value of total phosphorus (log TP) as the covariate. Analyses were conducted separately for HA and MA lakes. No analyses were performed for LA lakes for reasons described below.

Each TI_EQR value could now be adjusted by the appropriate offset and the regression between the national metric and the adjusted TI_EQR and the high/good and good/moderate status class boundaries converted to TI_EQR. These could then be compared and, where necessary, adjusted to ensure that all boundaries complied with the rules of the intercalibration exercise (see above).

Distribution of taxa between status classes

The association of taxa with particular status classes was investigated by Indicator Species Analysis (Dufrène & Legendre 1997) implemented in PC-ORD 5.0 (McCune & Mefford 1999). This method calculates the proportional abundance (specificity) and frequency (fidelity) of a taxon in a group of samples and their product as a percentage Indicator Value (IV). To assess the statistical significance of the highest IV among groups, it is compared to the results for a large number of randomized data sets. Separate IV analyses were performed for MA and HA lakes.

Results

Reference conditions

Lakes at reference conditions were not evenly distributed between either countries or types. Two participants, BE-FL and HU, had no reference sites at all whilst, for other countries, the number of lakes which fulfilled the criteria was low, particularly for MA lakes where there were, on average, only 2.6 lakes per country (excluding those with no reference sites). More reference sites were available for LA and HA lakes, with averages of 8.5 and 9.8 respectively. Most countries included multiple samples from water bodies into their datasets, using average values. A few only had a single sample per water body whilst two (France and Slovnia) had so few lakes that multiple samples per lake were all treated separately. Again, MA lakes had the fewest reference samples per country, with just 5.2, whilst LA and HA lakes had 40 and 30 samples per country respectively. Overall, the shortcomings of the reference dataset led to a decision to adopt continuous benchmarking rather than attempt to use reference conditions as a benchmark.

Regressions

In order to successfully intercalibrate a national method there needs to be a significant($P \leq 0.05$) relationship between the national metric and both the intercalibration metric and the pressure

213 gradient (expressed here as log total phosphorus, TP: Table 2). For low alkalinity lakes, the
214 relationship with the pressure gradient was significant for all countries except Sweden; however, the
215 data cloud has a "Y"-shape (Fig. 1): the upper branch shows little response to increasing nutrient
216 levels, whilst the lower branch shows decreasing TI-EQR values as TP increases. Preliminary
217 investigations suggest that this is not easily explainable by typological factors (both branches include
218 strongly humic lakes) but the "upper" group tends to have lower pH (6-6.4) than the "lower" group
219 (pH 6.5-6.9 – based on FI data).

220 For moderate alkalinity lakes, all relationships between national metrics and pressure variables were
221 significant with the exception of Germany and Italy, both of which had only very small datasets
222 spanning a small part of the total gradient for this particular type. The relationship between TI-EQR
223 and log TP is significant for France only if Lac Carcans-Hourtin is excluded. This is a lowland shallow
224 reference lake albeit with both relatively high TP (and a high N:P ratio) and very high values for TI-
225 EQR. Overall, there is some heteroscedasticity in the relationship (Fig. 2a), with a wide range of
226 values of TI_EQR recorded at low pressure, and a possible response threshold at about 10 µg L^{-1} TP.
227 However, few countries had data that spanned the whole gradient and there are few sites with >100
228 µg L^{-1} TP.

229 All relationships between diatom metrics and log TP in high alkalinity lakes were significant, again
230 with the exception of Italy, probably due to either the small size of its national dataset or typological
231 differences (HA lakes submitted by Italy were mainly large and deep and volcanic in nature). Samples
232 from Slovenia are clustered at the top left hand corner of the graph (Fig. 3a), whilst there are also a
233 number of outliers for Poland which cannot be explained by any typological factors.

234 The weak relationships, and suspicions of confounding factors, within the low alkalinity dataset led to
235 no further action at this point. Generalised linear models were calculated for the moderate and high
236 alkalinity datasets, in order that offsets could be calculated which would account for variability
237 introduced into the regressions by "national" effects (Table 3). Figs. 2b and 3b show these effects for

moderate and high alkalinity respectively. For moderate alkalinity, subtracting the offset improved the fit of the whole dataset to log TP from R^2 = 0.33 to 0.43 whilst, for high alkalinity, the improvement was from R^2 = 0.56 to 0.62. Lac Carcans-Hourtin remained an outlier in the moderate alkalinity dataset, as did some Polish sites in the high alkalinity dataset, even after adjustments, whilst Slovenian sites moved closer to the main trend of the dataset.

Intercalibration

Having established relationships between each national method and the intercalibration metric, using the offset to account for national differences, the next stage was to convert national boundaries for high/good and good/moderate status to equivalent values of the TI-EQR, then to examine the deviation of these from the common view (expressed as the mean of the TI-EQRs for all participating countries, Table 4). For high/good status in moderate alkalinity lakes, Belgium (Flanders) had highly precautionary boundaries whilst Sweden and UK had relaxed boundaries (where each country is allowed $\pm$ 0.25 class deviation). For good/moderate status, Belgium (Flanders) and Ireland are both stringent whilst Finland is relaxed (Fig. 4a & b). Countries are allowed to retain stringent boundaries, but those with relaxed boundaries must adjust these to within $\pm$ 0.25 class widths. Those countries with stringent and relaxed boundaries therefore examined their national datasets to ensure that outcomes were robust. In the case of Ireland, for example, the data spanned a short gradient, mostly at high and good status, and the Irish dataset was therefore supplemented with data from UK lakes to produce a dataset spanning a longer gradient in order to check calculations. The Irish boundary was, however, still precautionary, even after this and both they and Belgium (Flanders) exerted their right to retain these values. The implications of these decisions on those countries with relaxed boundaries was examined but even if both Belgium (Flanders) and Ireland had adjusted their boundaries, Finland, Sweden and UK would still have relaxed boundaries and, as a result, all made adjustments in order to bring their boundaries into line. France, Germany and Italy

were excluded from the final intercalibration of MA lakes due to the small size of their national datasets and, in the case of France and Italy, possible typological issues.

A similar process was enacted for high alkalinity lakes (Fig. 5a & b). Here, Italy was again excluded due to the small size of the dataset and possible typological issues. Hungary and Poland were excluded from the calculation of the average position of the boundary as some aspects of their methods did not comply with the agreed procedures though, once this had been established, the position of their boundaries were assessed relative to this mean view. Slovenia was stringent for the high/good boundary whilst Germany, Slovenia and UK had stringent good/moderate boundaries whilst Poland had relaxed boundaries for H/G and G/M and Hungary had a relaxed G/M boundary only. Again, an iterative process was undertaken to ensure that the relationships for each participating country were robust, and testing the consequences of adjusting stringent boundaries downwards before Hungary and Poland adjusted their boundaries to within $\pm$ 0.25 classes.

Distribution of taxa between status classes

Most of the abundant taxa were found across the EQR gradient, albeit with some clear patterns in relative abundance emerging between status classes for both types which were reflected by significant indicator values (Tables 5 & 6). *Achnanthidium minutissimum sensu lato*, for example, is the most commonly recorded taxon in the database, often forming more than 40% of the total in high and good status sites, but declining in relative abundance as EQR decreased, and there were few sites with >20% *A. minutissimum sensu lato* at moderate status or below. Other taxa with a predominately high/good distribution included *Brachysira microcephala/ vitrea* (more abundant in MA than in HA lakes), *Gomphonema angustum sensu lato* and *Tabellaria flocculosa* (the latter, again, more common in MA than in HA lakes).

Other taxa which tended to increase as EQR decreased were *Amphora pediculus*, *Cocconeis placentula sensu lato., Gomphonema parvulum* and *Nitzschia amphibia*.

Discussion

General comments

The hypothesis outlined in the Introduction appears to hold: this study shows good pan-European agreement in response of diatoms to the predominant eutrophication gradient, with about half of total variation being explained by a simple linear regression between a common index (TI-EQR) and log total phosphorus. There is still scope for local fine-tuning of indices but the relationship is strong enough to allow valid comparisons to be made between countries, a point also made by Blanco et al. (2013).

Though the Annex V of the WFD refers to the assessment of macrophytes **and** phytobenthos in lakes, only 11 of the 27 member states of the EU took part in this intercalibration exercise. Of the others, three include filamentous algae in their macrophyte survey methods whilst the remainder do not consider phytobenthos at all (Kelly, 2013). Several countries argued that their macrophyte assessment systems were adequate to fulfil their obligations, although few presented any data to support this assertion (Poikane, 2013).

Parker and Maberley (2000) present a convincing study of the benefits of phosphorus reduction in Windermere (UK), by evaluating changes in filamentous algae in the littoral zone; it is sobering to realise that such obvious changes would not just be missed by the 12 assessment systems that have no consideration of phytobenthos at all, but also by five of the countries included in this exercise but who lack parallel assessment of filamentous algae in their macrophyte assessment systems.

Use of metrics developed for rivers in lakes

Four countries involved in this exercise used metrics originally developed for rivers as part of their assessment of ecological status in lakes. Whilst the strongest correlations with the pressure gradient were for the Belgian (Flanders) metric developed specifically for lakes (Table 2), strong correlations were also observed in some cases when metrics originally developed for rivers were used in lakes.

For example, Finland explained over 70% of the variation in the main pressure gradient in MA lakes using the IPS, designed originally for use in rivers (Coste, in CEMAGREF, 1982). This relationship is stronger than that for several metrics developed specifically for lakes (Table 2) though other factors including the length of the gradient interact to determine the apparent strength of these relationships. Several other studies have also demonstrated strong relationships between diatom metrics originally developed for rivers to lake environment (Poulíčková et al., 2004; Kitner & Poulíčková, 2003; Blanco et al., 2004; Cejudo-Figueiras et al., 2010; Ács et al 2005; Bolla et al. 2010). Many of the taxa encountered during this study (Table 5) are also common in rivers, reflecting the similarities in physical, chemical and biological stresses encountered by diatoms in the littoral zones of lakes and in benthic habitats in rivers (Cantonati & Lowe, 2014; Kahlert & Gottschalk, 2014). However, there are also limitations associated with the use of metrics developed for rivers, and some diatom species do have distinct preferences for lakes over rivers. Cejudo-Figueiras et al. (2011) noted that one of the indices they tested (CEC; Descy & Coste 1991) does not include *Aulacoseira subarctica, Fragilaria bicapitata* or *Navicula cryptocephala,* all of which are typical of shallow lakes of NW Spain. Kitner & Poulíčková (2003) also encountered problems when using the TI in Czech fishponds, noting that the absence of some taxa from this metric led to overestimations of lake quality. It is also difficult to disentangle issues regarding the taxa which contribute to river versus lake metrics with problems associated with adapting indices developed in one geographic region (e.g., Austria or France) to other parts of Europe (Spain or Hungary). The UK metric used in this study has strong correlations with both the river metric developed for the same region (Bennion et al., 2014) and the TI (Table 2), suggesting that the problems encountered are more likely to reflect differences in the taxon list of the index rather than the fundamental performance of the index.

Response of diatoms in low alkalinity lakes

The relatively weak relationships in low alkalinity lakes stand in contrast to the situation for moderate and high alkalinity lakes. Low alkalinity lakes in this study were restricted to Scandinavia,

335 UK and Ireland and, generally, are situated in remote regions suited only to forestry or low intensity
336 pastoral agriculture. Consequently, it is hard to capture “eutrophication” gradients that are as long as
337 for moderate and high alkalinity lakes. However, this artefact of the dataset is further complicated by
338 the presence, in many cases, of a confounding acidity gradient, itself composed of both “natural” and
339 “anthropogenic” components (Fig. 1). Schneider et al. (2013) demonstrate the problems of
340 evaluating nutrient status in the presence of a strong acid pressure and although their paper deals
341 with rivers rather than lakes, the principles should be transferable. Furthermore, Schoenfelder et al.
342 (2002) revealed a statistically significant influence of high concentrations of dissoved organic carbon
343 on benthic diatom assemblages in low, moderate and high alkalinity lakes in northern Germany. High
344 DOC worked contrary to the eutrophication impact of enhanced dissolved and total phosphorus
345 concentrations. Juggins (2013) points out other issues associated with interpretation of univariate
346 responses in the presence of confounding variables. Although we lack the supporting data necessary
347 to evaluate the extent to which the effects observed in Fig. 1 are due to anthropogenic acidification
348 or dissolved organic carbon, we suspect a mix of factors.

349 Interactions between benthic algae and low level nutrient enrichment will be complicated: such lakes
350 may be N-limited (Maberley et al., 2003), and the N load may be derived, at least in part, from
351 atmospheric deposition, even as S deposition is decreasing (Flower et al., 2010). Moreover, one
352 effect of acidification will be to reduce phytoplankton densities (Battarbee et al., 1999) and colouring
353 by humic substances (Monteith et al., 2007), potentially increasing transparency and encouraging
354 benthic productivity. Humic substances may also influence the availability of P to benthic algae
355 (Broberg & Persson, 1988; Ekholm & Krogerus, 2003) Conversely additional nutrients in the absence
356 of acidification may be manifest first in phytoplankton productivity and assemblage changes, rather
357 than in changes to the benthic assemblage (Bennion et al., 2004). There is, in other words, no a priori
358 case for benthic algae in low alkalinity lakes necessarily being the most sensitive indicator of nutrient
359 changes.

360 *Implications for biogeography and diatom ecology*

361 The consistent pan-European response might appear surprising, bearing in mind the scale of cryptic
362 diversity and endemism discovered within diatoms in recent years (Mann et al., 2007; Trobajo et al.,
363 2009). This finding is, however, consistent with Kelly et al. (2012) and suggests that this type of status
364 assessment is robust. In broad terms, the scenario presented in the introduction, that biologists from
365 Ireland and Slovenia could look down a microscope and arrive at similar judgements about the
366 ecological status of a sample originating from Finland would appear to be correct. This, in turn, lends
367 weight to the use of diatoms as part of status assessment toolkits. However, we recognise that this
368 approach glosses over many real albeit often subtle differences amongst diatom species.

369 Until recently, many believed that most diatom species were widespread, or even cosmopolitan (e.g.
370 Round, 1981). This, in turn, led to the use of diatom Floras outside the regions for which they were
371 originally written and, in particular, the de facto adoption of the *Susswässerflora von Mitteleuropa*
372 (Hustedt, 1930, Krammer and Lange-Bertalot, 1986, 1988, 1991a, b) throughout Europe and beyond.
373 Many continue to use these volumes because the more recent taxonomic literature is often
374 scattered between many monographs and journal articles. However, even if many of the names in
375 the standard floras are, in effect, "operational taxonomic units" rather than true biological species,
376 they do provide a measure of consistency when considering pan-European datasets such as these.

377 At a practical level, cryptic diversity creates problems in ensuring consistent identification of the
378 myriad newly-described taxa even by specialist diatomists (Kahlert et al., 2012). The onus, therefore,
379 lies with individual member states to enact rigorous quality control to ensure consistency and to
380 liaise with neighbouring states to ensure that identification is not a source of systematic error when
381 water bodies that span national boundaries are assessed. Consistent use of fine-scale taxonomy is
382 possible but requires ongoing effort to ensure harmonisation (Kahlert et al., 2009) and, as such, is
383 better suited to national programs. The role that cryptic species may play in ecological status
384 assessment is still an open question as very few studies have gone beyond documenting taxonomic

variability. It is possible, as Kelly and Ector (2012) suggest, that many cryptic species are biogeographical and typological forms which all play similar roles in the functioning of aquatic ecosystems. It is also possible that cryptic forms may have different preferences for pressure variables such as pH and TP but still have little effect on ecological processes within littoral ecosystems. Yet the possibility that a shift between two subtly different forms within a complex either precipitates or indicates a significant shift in functioning must not be overlooked either. Without more detailed studies on the autecology of individual taxa within complexes it will not be possible to answer these questions.

Conclusions

This study has demonstrated broad-scale agreement of approach between assessment methods used around Europe for assessing ecological status using diatoms. Whilst recognising that diatoms are only one part of the phytobenthos, and taxonomic composition forms only part of the normative definition for the BQE macrophytes and phytobenthos, this is an encouraging start.

This exercise is part of a much broader process by which all BQEs across all water body types should have been intercalibrated following a standard procedure (Birk et al., 2013). This means not only that we have harmonised status class boundaries between participating countries for lake phytobenthos but that boundaries set for lake phytobenthos should be compatible with those of other BQEs in lakes and comparable with boundaries set for other BQEs in other water body types, all of which have been subject to the same procedures (Bennett et al., 2011; Birk et al., 2012; Poikane et al., 2010). The good/moderate boundary for a lake in the west of Ireland should, in theory at least, represent a similar level of ambition to the good/moderate boundary set for marine invertebrates in benthic habitats off the coast of Cyprus. Limitations encountered here such as a lack of reference sites and (in many cases) short gradients in some countries are common to many exercises (Birk et al., 2012, 2013). The outcome of this, and other completed intercalibration exercises is a « Decision » (EU, 2013) which makes the boundaries legally binding on those member states involved. Even those

410 countries which could not be included in the Decision were able to learn from the process: Italy, for
411 example, has a larger dataset on which it has demonstrated that several existing diatom metrics have
412 low or no relationship with TP in their deep lakes and has developed a new national index
413 (Marchetto et al., 2013) which will, in due course, be included in national legislation.

414 The wide geographical extent of this exercise was, however, unusual. As discussed above, this
415 reflects a measure of pragmatism in how many complexes of closely-related taxa were handled yet
416 also the remarkably unified approaches adopted for the collection and analysis of samples (CEN
417 2003, 2004). In practice, biogeographical differences in some other groups (e.g. benthic invertebrates
418 in rivers) are entangled with methodological differences, both in sampling and analysis (Bennett et
419 al., 2011) which create greater problems in IC than encountered here.

420 What are the next steps? Only 11 out of 28 member states of the European Union were involved in
421 this exercise, which means that over half the EU is not formally compliant. Several states argued that
422 their macrophyte assessment systems were adequate to fulfill their obligations. For such an
423 assumption to be valid, a strong correlation between macrophytes and phytobenthos EQRs and
424 identical pressure responses would need to be demonstrated. Our belief is that macrophytes and
425 phytobenthos provide complementary information and, more importantly, there will be situations
426 where macrophytes cannot be used or where the faster response times of diatoms will provide
427 information that macrophytes cannot offer (DeNicola & Kelly, 2014). There should now be fewer
428 impediments to countries adopting phytobenthos methods, with a standard metric, reference values
429 and boundaries now available for most European lake types as a result of this study. The applicability
430 of this approach will need to be checked for each new country, particularly to ascertain whether
431 there are any major constituents of the benthic flora that are overlooked by the TI. This provides an
432 “off the shelf” method, which will enable the collection of robust assessment data which, in turn, will
433 provide a foundation from which more locally-specific methods can be developed.

More generally, having established relationships between metrics and pressures, and harmonised boundaries, the focus should now shift to how metrics should be used to ensure that sites can be classified with high confidence. Assessment is, after all, just the first stage in the process of identifying water bodies in need of « programmes of measures » and variability, particularly around the good/moderate boundary, needs to be minimised if failing water bodies are to be correctly identified and prioritised. This study – and, indeed, the implementation of the WFD as a whole – takes place at a time of economic uncertainty for much of Europe which adds a greater incentive to ensure that public money is spent wisely.

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Table 1. Countries/regions participating in the lake phytobenthos intercalibration exercise, and their national methods. Further details of national methods can be obtained from http://www.wiser.eu/results/method-database/ LA: Low alkalinity (< 0.2 meq L^{-1}); MA, moderate alkalinity (≥ 0.2, < 1 meq L^{-1}); HA: high alkalinity (≥ 1 meq L^{-1}).

Member State and abbreviation		Method	Samples (lakes)			Reference samples (lakes)		
			LA	MA	HA	LA	MA	HA
Belgium (Flanders)	BE-FL	Proportions of Impact-Sensitive and Impact-Associated Diatoms (PISIAD)	-	79 (18)	68 (14)	-	0(0)	0(0)
Germany	DE	PHYLIB: Multi metric index for Macrophytes and Phytobenthos	-	14 (3)	698 (119)	-	?	95(25)
Finland	FI	Indice de Pollusensibilité Spécifique (IPS)	25 (21)	25 (25)	-	5(4)	5(4)	-
France	FR	Indice Biologique Diatomées (IBD2007)	-	33 (5)	-	-	14 (2)	-
Hungary	HU	Multimetric Index for Lakes (MIL)	-	-	84 (#)	-	-	28(13)

Ireland	IE	Lake Trophic Diatom Index (LTDI mark 1)	45 (22)	34 (14)	120 (62)	?	?	28(13)
Italy	IT	EPI-L (not ready in time for intercalibration exercise)	-	7 (7)	17 (15)	-	2(2)	0(0)
Poland	PL	PL IOJ (multimetryczny Indeks Okrzemkowy dla Jezior = multimetric Diatom Index for Lakes)	-	-	156 (134)	-	-	11(10)
Sweden	SE	Indice de Pollusensibilité Spécifique (IPS)	32 (21)	21 (15)	28 (15)	?	2(2)	14(12)
Slovenia	SI	Trophie Index (TI)	-	-	36 (3)	-	-	19(1)
United Kingdom	UK	Diatoms for Assessing River and Lake Ecological Quality (DARLEQ mark 2)	438 (72)	201 (40)	320 (66)	75(13)	3(3)	15(8)

Table 2. Relationships between national metrics and log Total Phosphorus and the intercalibration metric, TI_EQR.

Country	Type	Relationship with TI_EQR			Relationship with log_{10} TP	
		intercept	slope	R^2	significance	R^2
BE-FL	HA	0.152	1.01	0.769	P < 0.001	0.829
	MA	0.007	1.190	0.800	P < 0.001	0.855
DE	HA	0.529	0.50	0.598	P < 0.001	0.198
	MA	0.825	0.090	0.064	P = 0.802	0.0055
FI	LA	0.488	1.507	0.868	P = 0.031	0.299
	MA	0.529	0.423	0.870	P < 0.001	0.719
FR	MA	0.601	1.593	0.826	P = 0.606 P = 0.091	0.0055 * 0.119**
HU	HA	-0.576	1.905	0.759	P = 0.014	0.137
IE	LA	0.51	0.439	0.098	P < 0.0001	0.3519
	HA	0.303	0.748	0.786	P < 0.001	0.476
	MA	0.302	0.628	0.589	P = 0.023	0.2929
IT	MA	0.008	0.948	0.852	P = 0.602	0.0583

	HA	??				
PL	HA	-0.008	0.985	0.644	P < 0.001	0.133
SE	LA	0.380	0.634	0.164	P = 0.184	0.058
	HA	-0.187	1.25	0.402	P < 0.001	0.145
	MA	-0.409	1.349	0.550	P = 0.045	0.55195
SI	HA	0.320	0.858	0.884	P = 0.002	0.311
UK	LA	1.210	-0.275	0.051	P = 0.022	0.059
	HA	0.320	0.717	0.877	P < 0.001	0.375
	MA	-0.182	1.054	0.759	P < 0.001	0.2907

* includes Carcans-Hourtin; ** excludes Carcans-Hourtin

Table 3. Common view and member-state specific mean Trophic index EQR values (TI_EQR) for high and moderate alkalinity lakes using General linear models. Covariates appearing in the model are evaluated at log TP = 1.6665 μg L^{-1} for high alkalinity lakes and 1.3689 μg L^{-1} for moderate alkalinity lakes. n.a. = not applicable.

Lake type	High alkalinity		Moderate alkalinity	
Member state	Mean TI_EQR	Std. Error	Mean TI_EQR	Std. Error
BE-FL	0.719	0.042	0.900	0.037
DE	0.775	0.014	0.914	0.038
FI	n.a.	n.a.	0.662	0.028
FR	n.a.	n.a.	1.076	0.025
HU	0.862	0.026	n.a.	n.a.
IE	0.708	0.021	0.911	0.024
IT	n.a.	n.a.	0.774	0.054
PL	0.826	0.013	n.a.	n.a.
SE	0.691	0.029	0.859	0.032
SI	1.085	0.029	n.a.	n.a.
UK	0.860	0.018	0.834	0.024
Common view	0.816	0.009	0.866	0.012

n.a. - not applicable

Table 4. Average values for status class boundaries, expressed as TI-EQR, for high and moderate alkalinity European lakes

Lake type	Boundary			
	High/Good	**Good/Moderate**	**Moderate/Poor**	**Poor/Bad**
High alkalinity	0.965	0.790	0.604	0.416
Moderate alkalinity	0.849	0.588	0.309	0.025

Table 5. Indicator species for high, good and moderate status in moderate alkalinity lakes. Only taxa found in ≥ 10% of sites and with a maximum relative abundance ≥5% are included. Status classes are defined from the mean location of the national boundaries, calculated using the ICM, adjusted using national offsets. Based on analysis of 343 taxa in 195 H, 73 G and 20 M samples. Number of occurrences (obs), average (avg) and maximum (max) abundance (%), indicated status class (status), indicator value (IV), significance level (p) and values for fidelity and specificity.

Taxon	obs	avg	max	status	IV	p	fidelity	specificity
Achnanthidium minutissimum (Kützing) Czarnecki	286	34.7	95.7	H	53.0	0.0002	100	53
Tabellaria flocculosa (Roth) Kützing	194	4.2	51.5	H	42.2	0.0222	72	58
Gomphonema angustum Agardh	164	3.8	85.1	H	39.2	0.0284	62	64
Encyonopsis microcephala (Grunow) Krammer	119	2.3	58.3	H	38.7	0.0034	49	79
Brachysira vitrea (Grunow) Ross	142	2.7	55.4	H	33.5	0.025	56	59
Rossithidium pusillum (Grunow) Round & Bukhtiyarova	88	0.5	12.4	H	25.0	0.0318	36	69
Encyonema neogracile Krammer	74	0.3	13.5	H	23.8	0.0326	31	77
Denticula tenuis Kützing	62	0.6	20.3	H	23.6	0.0172	27	89
Cymbella affinis Kützing	53	0.4	17.8	H	18.5	0.0418	24	77
Eunotia implicata Nörpel, Lange-Bertalot & Alles	36	0.1	9.8	H	15.5	0.038	17	92
Ulnaria delicatissima var. angustissima (Grunow) Aboal & Silva	29	0.2	7.2	H	13.9	0.0312	14	97

Taxon	obs	avg	max	status	IV	p	fidelity	specificity
Amphora pediculus (Kützing) Grunow s.l.	107	0.6	11.7	G	31.1	0.014	58	54
Navicula cryptocephala Kützing	95	0.5	14.3	G	30.1	0.0166	52	58
Nitzschia fonticola Grunow in Cleve et Möller	91	0.8	37.3	G	28.5	0.0244	45	63
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	90	0.6	22.4	G	26.9	0.0322	47	58
Nitzschia lacuum Lange-Bertalot	31	0.3	15.3	G	22.5	0.0046	25	91
Achnanthes clevei Grunow	56	0.2	6.4	G	21.3	0.0218	33	65
Epithemia sorex Kützing	34	0.3	18.3	G	18.7	0.0158	22	85
Encyonopsis minuta Krammer & Reichardt	22	0.2	14.4	G	13.4	0.0238	19	70
Encyonema reichardtii (Krammer) D.G. Mann	7	0.1	12.1	G	6.8	0.0262	7	99
Navicula gregaria Donkin	69	0.4	23.2	M	65.9	0.0002	75	88
Gomphonema parvulum (Kützing) Kützing	128	1.0	34.9	M	61.4	0.0002	80	77
Cocconeis placentula Ehrenberg	173	1.4	31.2	M	55.4	0.0002	90	62
Nitzschia palea (Kützing) W. Smith	144	0.8	15.1	M	47.8	0.0004	80	60
Synedra ulna (Nitzsch) Ehrenberg	88	0.3	7.7	M	37.2	0.0014	60	62

Taxon	obs	avg	max	status	IV	p	fidelity	specificity
Planothidium delicatulum (Kützing)	17	0.4	58.4	M	34.4	0.0002	35	98
Eolimna minima (Grunow) Lange-Bertalot	97	1.1	45.1	M	33.5	0.0096	60	56
Nitzschia amphibia Grunow	27	0.1	8.4	M	33.2	0.0002	40	83
Melosira varians Agardh	17	0.1	18.2	M	31.3	0.0002	35	89
Nitzschia inconspicua Grunow s.l.	22	0.3	48.1	M	28.1	0.0002	30	94
Navicula lanceolata (Agardh) Ehrenberg	28	0.1	7.0	M	26.3	0.0012	30	88
Nitzschia gracilis Hantzsch	55	0.2	10.8	M	23.0	0.0136	45	51
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	44	0.7	52.1	M	20.6	0.0222	35	59
Nitzschia linearis (Agardh) W.M. Smith	34	0.2	13.0	M	18.4	0.0208	25	74
Gomphonema angustatum (Kützing) Rabenhorst	21	0.2	12.4	M	17.0	0.0074	20	85
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	31	0.1	6.5	M	14.2	0.0282	20	71
Stephanodiscus sp.	17	0.1	8.4	M	12.7	0.0182	15	85
Staurosira berolinensis (Lem.) Lange-Bertalot	18	0.4	27.1	M	11.4	0.0316	15	76
Fragilaria bidens Heiberg	19	0.1	5.7	M	11.0	0.0366	20	55

Taxon	obs	avg	max	status	IV	p	fidelity	specificity
Navicula submuralis Hustedt	15	0.1	6.3	M	9.7	0.0308	15	64
Diatoma elongatum (Lyngbye) Agardh	3	0.0	6.3	M	6.1	0.0142	10	61

Table 6. Indicator species for high, good and moderate status in high alkalinity lakes. Only taxa found in ≥ 10% of sites and with a maximum relative abundance ≥5% are included. Status classes are defined from the mean location of the national boundaries, calculated using the ICM, adjusted using national offsets. Number of occurrences (obs), average (avg) and maximum (max) abundance (%), indicated status class (status), indicator value (IV), significance level (p) and values for fidelity and specificity. Based on analysis of 394 taxa in 363 H, 360 G and 328 M samples.

	obs	avg (%)	max (%)	status	IV	p	fidelity	specificity
Achnanthidium minutissimum (Kützing) Czarnecki	973	17.8	96.9	H	61.1	0.0002	98	62
Brachysira vitrea (Grunow) Ross in Hartley	104	0.2	31.6	H	17.0	0.0002	19	89
Cymbella affinis Kützing	272	1.0	50.0	H	38.7	0.0002	47	83
Cymbella helvetica Kützing	122	0.2	13.5	H	20.0	0.0002	25	82
Denticula tenuis Kützing	181	0.7	35.5	H	34.9	0.0002	39	90
Encyonopsis cesatii (Rabenhorst) Krammer	109	0.1	10.2	H	21.8	0.0002	23	93
Encyonopsis microcephala (Grunow) Krammer	436	5.8	81.4	H	64.5	0.0002	75	86
Gomphonema angustum Agardh	687	3.1	88.1	H	45.4	0.0002	71	64
Nitzschia lacuum Lange-Bertalot	181	0.2	9.8	H	16.2	0.0002	27	60
Navicula subalpina Reichardt	190	0.2	6.0	H	13.4	0.0002	21	62

Achnanthes clevei Grunow	389	0.9	29.8	G	26.3	0.0002	48	55
Achnanthidium exiguum (Grunow) Czarnecki	173	0.2	8.8	G	20.7	0.0002	30	68
Cymbella excisa Kützing	161	0.2	21.1	G	15.2	0.0002	26	60
Cymbella cymbiformis Agardh	266	0.3	21.6	G	14.4	0.0114	38	38
Cymbella hustedtii Krasske	192	0.2	27.7	G	12.9	0.0018	30	43
Cocconeis neothumensis Krammer	276	0.6	18.3	G	26.3	0.0002	41	64
Cavinula scutelloides (W.Smith) Lange-Bertalot	181	0.2	10.6	G	15.5	0.0002	29	53
Epithemia adnata (Kützing) Brébisson	440	2.3	72.8	G	36.4	0.0002	63	58
Encyonema caespitosum Kützing	413	0.4	14.4	G	22.5	0.0002	52	44
Encyonopsis minuta Krammer & Reichardt	175	0.6	27.8	G	17.1	0.0002	31	55
Epithemia sorex Kützing	413	1.9	49.1	G	27.6	0.0002	52	52
Encyonopsis subminuta Krammer & Reichardt	161	0.5	22.4	G	14.4	0.0002	27.8	G
Navicula menisculus Schumann	303	0.7	17.9	G	27.7	0.0002	45	62
Navicula oblonga Kützing	146	0.2	30.0	G	15.0	0.0002	26	59
Navicula radiosa Kützing	474	0.5	35.1	G	27.5	0.0002	60	46
Navicula subrotundata Hustedt	171	0.2	8.3	G	20.0	0.0002	29	69

Navicula seibigiana Lange-Bertalot	141	0.3	20.6	G	21.7	0.0002	28	77
Planothidium joursacense (Héribaud) Lange-Bertalot	144	0.2	7.4	G	17.7	0.0002	26	67
Planothidium rostratum (Oestrup) Lange-Bertalot	239	0.4	26.3	G	16.2	0.0002	39	42
Platessa conspicua (A. Mayer) Lange-Bertalot	300	0.3	10.3	G	17.9	0.0002	40	45
Rhopalodia gibba (Ehrenberg) O. Muller	292	0.5	31.0	G	21.6	0.0002	44	49
Staurosira dubia Grunow	157	0.2	15.5	G	13.6	0.0002	28	48
Tabellaria flocculosa (Roth) Kützing	195	0.4	36.4	G	13.0	0.0016	26	50
Amphora libyca Ehr. s.l.	383	0.3	13.1	M	32.8	0.0002	49	67
Amphora pediculus (Kützing) Grunow s.l.	835	6.0	56.5	M	44.0	0.0002	84	52
Cocconeis pediculus Ehrenberg	431	0.7	31.8	M	29.7	0.0002	52	57
Cocconeis placentula Ehrenberg	831	4.4	95.0	M	40.6	0.0002	85	48
Cymatopleura solea (Brebisson) W.Smith	122	0.0	6.1	M	7.7	0.0158	13	58
Diatoma vulgaris Bory	136	0.3	22.1	M	9.8	0.002	20	50
Eolimna minima (Grunow) Lange-Bertalot	290	0.6	51.8	M	34.0	0.0002	46	73
Fragilaria vaucheriae (Kützing) Petersen	502	1.3	40.7	M	27.1	0.0002	54	50
Gomphonema clavatum Ehrenberg	159	0.2	19.5	M	9.9	0.019	19	53

Gomphonema parvulum (Kützing) Kützing	410	0.7	22.0	M	28.5	0.0002	48	59
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	152	0.1	19.2	M	18.3	0.0002	24	76
Mayamaea atomus (Kützing) Lange-Bertalot	102	0.2	60.6	M	16.1	0.0002	18	88
Melosira varians Agardh	199	0.3	24.5	M	24.7	0.0002	34	74
Nitzschia amphibia Grunow	422	0.5	11.1	M	33.3	0.0002	55	60
Navicula antonii Lange-Bertalot	180	0.3	63.2	M	23.8	0.0002	30	80
Navicula capitatoradiata Germain	343	0.3	14.5	M	23.0	0.0002	43	53
Navicula cryptocephala Kützing	228	0.2	22.7	M	14.4	0.001	27	52
Nitzschia dissipata (Kützing) Grunow	458	1.1	53.4	M	34.6	0.0002	51	67
Nitzschia fonticola Grunow in Cleve et Möller	328	0.8	44.6	M	25.0	0.0002	43	58
Navicula gregaria Donkin	175	0.2	21.0	M	18.1	0.0002	26	70
Nitzschia frustulum (Kützing) Grunow	130	0.3	18.6	M	17.6	0.0002	23	78
Nitzschia inconspicua Grunow s.l.	140	0.3	23.0	M	22.8	0.0002	27	85
Navicula menisculus Schumann	146	0.2	13.1	M	14.9	0.0002	22	69
Nitzschia paleacea (Grunow) Grunow in Van Heurck	238	1.5	79.8	M	27.0	0.0002	38	71

Nitzschia palea (Kützing) W.Smith	251	0.4	17.4	M	24.5	0.0002	36	68
Navicula reichardtiana Lange-Bertalot	340	0.5	31.2	M	27.7	0.0002	43	64
Navicula tripunctata (O.F. Möller) Bory	429	0.7	34.5	M	37.1	0.0002	56	66
Navicula veneta Kützing	130	0.1	6.0	M	11.1	0.0004	19	60
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	292	0.3	14.9	M	16.5	0.001	31	53
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	250	0.3	12.7	M	27.1	0.0002	41	66
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	521	1.5	55.8	M	47.3	0.0002	71	66
Synedra ulna (Nitzsch) Ehrenberg	420	0.6	33.3	M	27.4	0.0002	49	56
Tabularia fasciculata (Agardh) Williams et Round	115	0.1	10.6	M	15.2	0.0002	20	75
Synedra acus Kützing	307	0.5	68.0	M	16.6	0.0254	33	50

List of figures

Figure 1. Relationship between TI, expressed as an ecological quality ratio (TI_EQR) and total phosphorus (TP) for low alkalinity lakes. The equation for the relationship (all data pooled) is TI_EQR = $0.090\log_{10}TP + 1.051$; $R^2 = 0.083$. See text for more details.

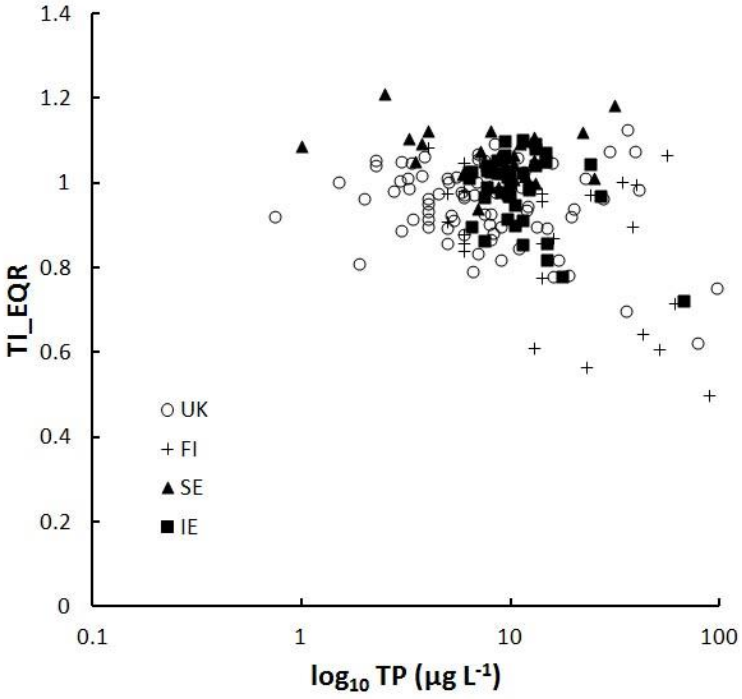
Figure 2. Relationship between TI, expressed as an ecological quality ratio (TI_EQR) and total phosphorus (TP) for moderate alkalinity lakes. a, shows the raw values (TI_EQR = $-0.243\log_{10}TP + 1.235$; R2 = 0.326), whilst b. shows the values after Lac Carcans-Hourtin had been removed from the dataset and national offsets (Table 3) had been subtracted (TI_EQR = $-0.286\log_{10}TP + 1.300$; $R^2 = 0.431$). See text for more details.

Figure 3. Relationship between TI, expressed as an ecological quality ratio (TI_EQR) and total phosphorus (TP) for high alkalinity lakes. a, shows the raw values (TI_EQR = - $-0.382\log_{10}TP + 1.431$; R2 = 0.555; HU and PL excluded), whilst b. national offsets (Table 3) had been subtracted (TI_EQR = $0.343\log_{10}TP + 1.361$; $R^2 = 0.621$; HU and PL excluded). See text for more details.

Figure 4. Variation in locations of boundaries between high and good status (a.) and good and moderate status (b.) in relation to the average boundary position for moderate alkalinity lakes. Dashed lines = $\pm$ 0.25 class widths.

Figure 5. Variation in locations of boundaries between high and good status (a.) and good and moderate status (b.) in relation to the average boundary position for high alkalinity lakes. Dashed lines = $\pm$ 0.25 class widths.

Fig. 1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1
0.8
0.6
0.4
0.2
0
TI_EQR
UK
FI
SE
IE
0.1
1
10
100
log10 TP (µg L-1)

Fig. 2

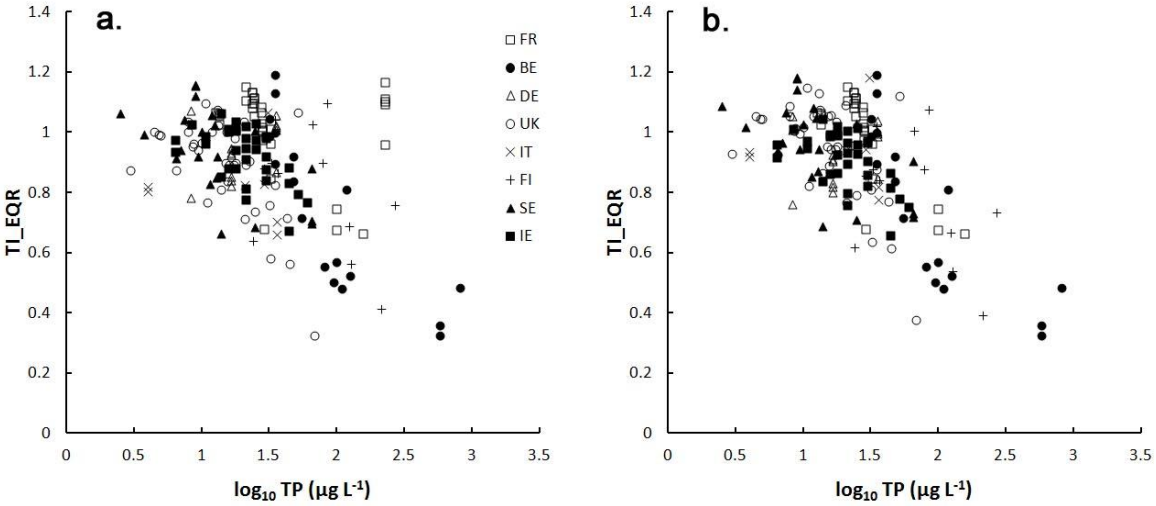
a.
b.
FR
BE
DE
UK
IT
FI
SE
IE
TI_EQR
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
0.5
1
1.5
2
2.5
3
3.5
log10 TP (µg L-1)

Fig. 3

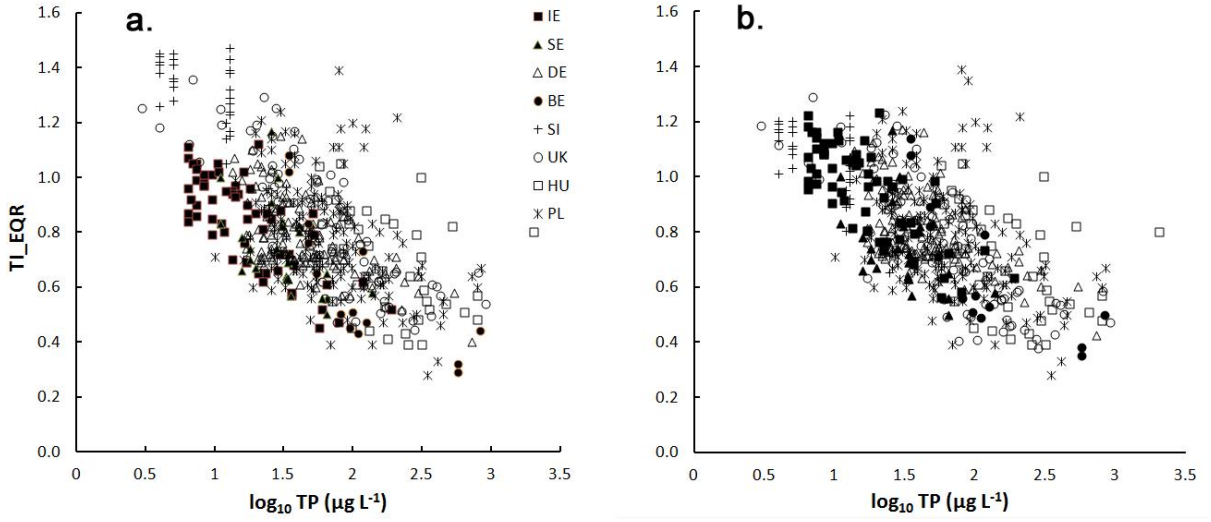

Fig. 4

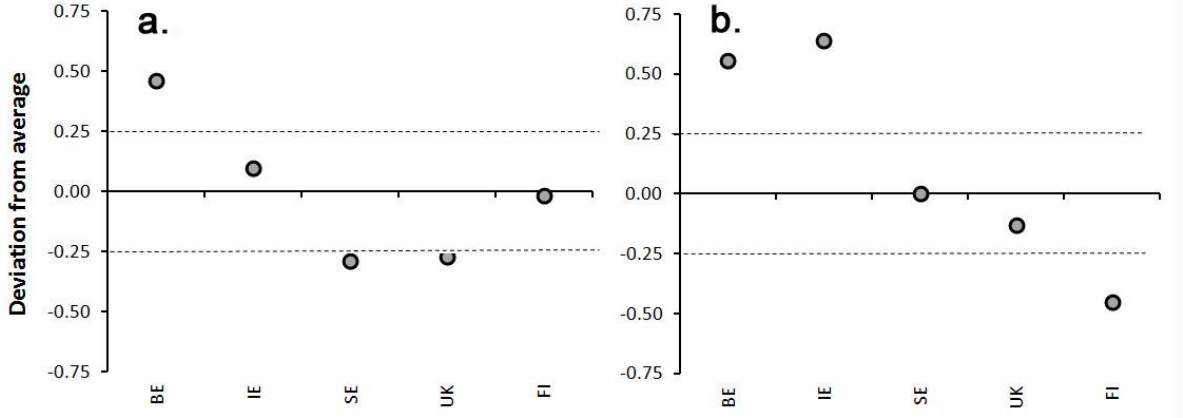

Fig. 5