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Development of a fish-based index to assess the eutrophication status of European lakes

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Abstract

The use of the CEN (European Committee for Standardization) standard method for sampling fish in lakes using multi-mesh gillnets allowed the collection of fish assemblages of 445 European lakes in 12 countries. The lakes were additionally characterised by environmental drivers and eutrophication proxies. Following a site-specific approach including a validation procedure, a fish index including two abundance metrics (catch per unit effort expressed as fish number and biomass) and one functional metric of composition (abundance of omnivorous fish) was developed. Correlated with the proxy of eutrophication, this index discriminates between heavily and moderately impacted lakes. Additional analyses on a subset of data from Nordic lakes revealed a stronger correlation between the new fish index and the pressure data. Despite an uneven geographical distribution of the lakes and certain shortcomings in the environmental and pressure data, the fish index proved to be useful for ecological status assessment of lakes applying standardised protocols and thus supports the development of national lake fish assessment tools in line with the European Water Framework Directive.

Keywords: lakes, Europe, fish index, metrics, eutrophication

Introduction

Fish are widely considered as relevant for detecting and quantifying impacts of human activities on lakes and reservoirs. Ten years after the publication of the original Index of Biotic Integrity (IBI) for streams in Illinois, U.S.A. (IBI, Karr, 1981), the first article describing the development of a fish index for lentic systems (reservoirs in the Tennessee Valley) was published (Karr & Dionne, 1991). Since then, several studies have been conducted to develop IBIs in a wide array of lentic water bodies, most of which are adaptations of Karr's concept (1981) to local environments: Central and North America (e.g. Mexico (Lyons et al., 2000), Colorado (Harig & Bain, 1998), New England (McDonough & Hickman, 1999; Whittier, 1999), the Great Lakes Area (Minns et al., 1994), Wisconsin (Jennings et al., 1999; Lyons et al., 2000), Florida (Schulz et al., 1999), and Minnesota (Drake & Pereira, 2002; Drake & Valley, 2005)). All these indices are combinations of taxonomic and/or functional metrics for the ecosystems' health assessment.

In Europe, numerous ecological studies have demonstrated relationships between abiotic environmental characteristics and fish assemblages in lakes (Jeppesen et al., 2000; Olin et al., 2002; Gassner et al., 2005; Mehner et al., 2005; Jeppesen et al., 2006; Mehner et al., 2007), reservoirs (Godinho et al., 1998; Kubečka et al., 1998; Irz et al., 2002; Lara et al., 2009) or both (Irz et al., 2004). However, limited effort has been dedicated to applying this knowledge to the development of biological monitoring tools. The use of fish for assessing the environmental state of lakes and reservoirs became an important aspect for the implementation of the Water Framework Directive (WFD) (European Commission, 2000). However, 12 years after the WFD's ratification, fish-based assessment systems for lakes are still under construction in most EU countries.

Nevertheless, a number of national fish indices have been developed. Thus, a multimetric fish index has been proposed to evaluate the ecological quality of Flemish standing waters (Belpaire et al., 2000), and another simple index based on the abundance of a single species and importance of fish diseases has been suggested for assessing of the ecological quality of Catalan reservoirs (Catalan & Ventura, 2003). More recently, in Austria, Italy, France, Ireland and Estonia, scientists have proposed metrics to be used in assessment methods based on lake fish assemblages (Gassner et al., 2003; Gassner et al., 2005; Launois et al., 2011a; Launois et al., 2011b; Volta et al., 2011; Kelly et al., 2012; Palm et al., 2012). However, a common feature of all these indices or metrics is their restricted applicability due to the limited number of lakes available for estimating pressure–response relationships, as required by the WFD. Furthermore, a sufficiently large gradient in the physical and biological characteristics of the lakes has often not been achieved (Jackson et al., 2001). Moreover, the diversity of sampling gears and strategies used prevents inter-comparisons at a greater spatial scale. The most robust of these analyses relate to the natural lakes of Northern Europe, for which a fish sampling method for lakes has been developed in cooperation between Nordic countries (Kurkilahti et al., 2002) and standardised following analysis by Swedish experts in the European Committee for Standardization (CEN, 2005). This favoured the development of Nordic Fish Indices based on harmonised datasets (Appelberg et al., 1995; Appelberg et al., 2000; Tammi et al., 2001; Holmgren et al., 2007; Rask et al., 2010). Additionally, multi-mesh gillnets (CEN, 2005) have more recently become a standard method to assess fish assemblages in lakes and reservoirs throughout Europe according to the requirements of the WFD. This standardisation has made it possible to compile a large database on fish assemblages and environmental descriptors from nearly 2000 natural lakes and reservoirs within Europe. These data have been used in an inter-calibration exercise (European Commission, 2009) and in the EU 7th Framework WISER project (http://www.wiser.eu/). Considering that in many European countries the number of natural lakes (or the amount of collected data) is too low to trace clear response patterns of fish assemblages to pressures, this common database offers a unique opportunity to explore these relationships and to develop a fish index aimed at assessing the ecological status of natural European lakes. Such a pan-European lake-fish assessment tool would be of paramount importance as a yardstick for assessing the comparability and feasibility of national indices.

Two main questions will be addressed: (1) Do fish metrics exist that respond significantly to eutrophication at the European scale? (2) Can an index based on fish distinguish between the least, moderately and most heavily impacted European lakes? One of the difficulties with this type of large-scale approach (12 countries covering most of the European regions), was to determine the true effect of pressures on fish assemblage structure when a large part of its variability was due to the natural environment (Irz et al., 2007a). We hypothesised that, to some extent, undertaking the analyses on a more homogeneous environmental dataset would yield stronger relationships between metrics and pressures. Therefore, the same analysis was conducted on a subset of Finnish and Swedish lakes from the extensively surveyed Nordic area of Europe. The method implemented followed a site-specific approach involving (1) searching for metrics that respond to pressures, (2) defining site-specific reference conditions, and (3) aggregating metrics into an index. All the models were validated with an external dataset, as recommended by Borja & Dauer (2008).

Material and methods

The analyses were conducted on a subset of 445 natural lakes out of the 1922 lakes included in the European database (Caussé et al., 2011). The 445 lakes are located in Denmark (49), Estonia (21), Finland (89), France (40), Germany (69), Ireland (33), Italy (3), Norway (1), Romania (1), Slovenia (2), Sweden (143) and the United Kingdom (3) (Figure 1). These lakes were selected because fish data, environmental parameters and descriptors of anthropogenic eutrophication were available in a comparable format. Moreover, because the efficiency of an index based on fish assemblage structure with low species richness is obviously poor, lakes with fewer than three species were removed. Acidified lakes (pH<6) were also excluded from the analyses.

Fish data

Fish data were collected between 2003 and 2010 using benthic gillnets following the Norden gillnet standardised protocol (CEN, 2005). Random samplings were performed in different depth strata during the summer period. The number of nets set in each stratum depended on lake depth and area. A standard test fishing period involved setting gillnets around 7 p.m. and lifting them at 7 a.m. the following morning. The benthic multi-mesh gillnets were 30 m long and 1.5 m high, and composed of 12 different panels with mesh sizes ranging between 5 mm to 55 mm knot to knot in a geometric row.

The captured fish were identified to species level, counted and weighed in grams. This method provides a whole-lake estimate of the occurrence and relative abundance and biomass of catchable species (CEN, 2005).

Based on gillnet catches, fish assemblages were described by metrics previously developed to monitor lakes and rivers (Simon, 1999; Oberdorff et al., 2001). Fish structure was first described by species richness (SR), diversity, i.e. the Shannon and Simpson indices (Shannon, 1948; Simpson, 1949), equitability (Pielou, 1969) and total abundance expressed as catch (CPUE) and biomass (BPUE) per net and per hour.

Furthermore, the abundance of the Cyprinidae, Percidae and Salmonidae fish families and the ratio of Cyprinidae to Percidae were calculated. These metrics were expressed as the number of species, individuals and biomass caught per unit effort and as proportions of individuals and biomass in the total gillnet catches. The ratio of roach (*Rutilus rutilus* (L.)) to perch (*Perca fluviatilis* L.) was also calculated, expressing abundances in both number and biomass. Accordingly, 24 taxonomic metrics, traditionally used in the literature as proxies of trophic structure, were calculated.

Additionally, tolerance, trophic, reproductive and habitat preferences (Table 1) were used to calculate guild metrics of the lake fish assemblages (Pont et al., 2006). When related to ecological processes, these characteristics make the diagnosis easier to interpret. Moreover, being independent of taxonomic positions, the functional characteristics of the species assemblages facilitate comparisons across different biogeographical areas. Twelve traits, defined according to a literature survey elaborated from national experts' judgment, were used to describe species attributes. Species were assigned to trophic guilds as follows: invertivorous (INVE) species whose adult diet consists of more than 75% insects; planktivorous (PLAN) species whose adult diet consists of more than 75% zooplankton and/or phytoplankton; piscivorous species (PISC) feeding on fish, at least partly, as adults; and carnivorous (CARN) species that are both invertivorous and piscivorous. If plant and animal material both contributed at least 25% to the diet, the species was considered omnivorous (OMNI) (Schlosser, 1982). Only one benthivorous species (BEN, adult diet containing more than 75% benthic organisms), *Cobitis taenia* L., and one invertivorous/planktivorous species, *Coregonus autumnalis* P., were included in the dataset. These traits were not used to calculate metrics.

Species were also classified according to their pelagic (WCOL) or benthic (BENT) living and feeding habitats. The reproductive guilds considered were phytophilic (PHYT) species spawning on different parts of living or dead vegetation and lithophilic (LITH) species spawning on clean mineral substrate. Species showing indifferent spawning preferences were considered to be both phytophilic and lithophilic (PHLI). Reproductive traits represented by only two species (ariadnophilic, i.e. species exhibiting some form of parental care, and ostracophilic, i.e. species spawning in shells) or shared by a few low-occuring species (pelagophilic species spawning in the pelagic zone) were not taken into account for the metric calculation. Species were also classified as being either tolerant (TOL) or intolerant (INT) to any stressor related to lake morphology (habitat), hydrology or water chemistry according to Karr et al. (1986).

These guild-based metrics were given either as numbers (of species, individuals or biomass of individuals) or percentages (of fish or biomass of fish sharing a trait). In summary, a total of 60 guild-based metrics was used to characterise the fish assemblages and as many as 90 metrics were calculated.

Environmental variables

The lakes were characterised by seven environmental parameters that are important factors in influencing the structure of fish assemblages in lakes (Eadie et al., 1986; Magnuson et al., 1998; Irz et al., 2004; Mehner et al., 2005; Irz et al., 2007b; Mehner et al., 2007) (Table 2).

Maximum depth (m), lake area (km^2), catchment area (km^2) and altitude (m) were measured in the field or extracted from topographical maps or estimated using GIS. Mean monthly air temperatures were obtained from a climate model (New et al., 2002) and were used to derive two temperature variables:

(i) Average temperature = (TJanuary+TFebruary+…+TDecember)/12
(ii) Amplitude of temperature = TJuly – TJanuary

Additionally, geology was defined as either calcareous or siliceous (derived from geological maps).

To meet the requirements of linear models (normality, linearity), maximum depth, lake area and catchment area were log-transformed.

Two datasets were considered: one including the 445 lakes (EUdataset) and a subset of 155 Swedish and Finnish lakes (Nordic lakes) exhibiting more homogeneous environmental conditions (Ndataset) (Table 2).

Pressure gradient

For each lake, the extent of eutrophication was assessed at the catchment scale by the percentage of non-natural land cover (NNLC) obtained from the Corine Land Cover database (Union européenne – SOeS 2006). Additionally, the total phosphorus (TP) concentration ($\mu g\ L^{-1}$) of the lake was calculated as the mean of four samples taken throughout the year (one for each season for most of the lakes) matching the fish sampling campaign as close as possible. The TP concentration was log-transformed and NNLC was arcsine square-root transformed in order to meet a normal distribution assumption.

In the two datasets, the lakes were split into three classes containing approximately the same number of sites, separately based on NNLC and TP gradients. For each pressure, the least, moderately and most eutrophic lakes were identified.

Metric selection

The screening procedure was performed independently on the EUdataset and the Ndataset. The different steps of the procedure are presented in Figure 2.

First, numerically unsuitable metrics, i.e. metrics with a small range of values or many outliers and extreme values, were excluded following the recommendations of Hering et al. (2006).

For the remaining metrics, classic monotonic transformations were performed in order to meet the requirements of linear models (normality, linearity): count (abundance, richness) and biomass metrics were log-transformed and proportion metrics were arcsine square-root transformed, whereas diversity indices were not transformed. The datasets were divided into training subsets (randomly choosing two-thirds of the lakes in dataset) composed of 300 and 102 lakes for the EUdataset and Ndataset, respectively, and validation subsets (the remaining one-third of the data), including 145 lakes from the EUdataset and 53 lakes from the Ndataset to predict the selected metric. These training subsets were used to select the metrics and the remaining lakes were then used to validate the models.

With the training subsets, each metric was first regressed with a multiple linear regression (MLR) using environmental variables and stressors as predictors, followed by a stepwise procedure based on the Akaike information criterion (AIC) in order to select the best fitting model for each metric containing a reduced number of explanatory variables (Oberdorff et al. 2002). The quality of the remaining models was checked by quantile-comparison plots (qq-plot), regression leverage plots and the value of the adjusted R^2. For a metric to be retained, at least one pressure had to be significantly included as explanatory variable in the model of the metric, and the model had to fulfil the following requirements: $R^2>0.3$, normal distribution of residuals and no leverage effect.

The models retained after these steps were then applied to the validation subsets. The metrics for which the correlation coefficients between expected and observed values in the validation step were lower than 0.7 were not retained.

Index calculation

For each metric, three steps were necessary before their aggregation in a multimetric index: the definition of reference conditions, the measurement of the deviation from the reference and the standardisation of this deviation from the reference.

Reference conditions

The lake-specific reference values of each selected metric were determined using a hindcasting procedure following Baker et al. (2005) and Kilgour et al. (2006). The models obtained for the selected metrics were applied to the whole dataset after artificially setting the pressures to null values. This arithmetic procedure provides site-specific expectation values of the metrics in the absence of anthropogenic pressures.

Deviance measurement

The difference between the observed metric value (obs_metric) and the metric value predicted by the hindcasting procedure (hind_metric) corresponds to the deviation between the observed value and the predicted estimated value at reference conditions. It therefore described the expected response range of each metric, whatever the natural environmental variability.

All deviation scores (obs_metric-hind_metric) were calculated for the metrics passing the previous steps.

Metric scoring

Given that a common scale of ecological quality is required, this deviation score has to be standardised. Therefore, ecological quality ratio (EQR) was calculated following Hering et al. (2006) recommendations, with the upper anchor and lower anchor defined by the 95th (95perc) and 5th percentiles (5perc) of the non normalised deviation scores as:

$$\mathrm{EQR} = \frac{(\mathrm{obs\_metric - hind\_metric}) - \mathrm{5perc(obs\_metric - hind\_metric)}}{\mathrm{95perc(obs\_metric - hind\_metric)} - \mathrm{5perc(obs\_metric - hind\_metric)}}$$

for metrics that decrease with increasing stress and

$$\mathrm{EQR} = 1 - \frac{(\mathrm{obs\_metric - hind\_metric}) - \mathrm{5perc(obs\_metric - hind\_metric)}}{\mathrm{95perc(obs\_metric - hind\_metric)} - \mathrm{5perc(obs\_metric - hind\_metric)}}$$

for metrics that increase with increasing stress.

A value of EQR close to 1 corresponds to the least impacted sites. Conversely, a value close to 0 corresponds to the most degraded site.

EQR values were then related to the pressure variables. The Pearson correlation between the EQR values and the pressure gradient should be significant with a reduced dispersion of the EQR values along the stressor gradient ($r>0.5$ and $p<0.05$). Moreover, the observed trend of the selected metric on the pressure gradient should be ecologically interpretable. When two metrics showed strong redundancy (Spearman $r>0.8$), only the one with the highest correlation with the stressor was selected (McCormick et al., 2001; Oberdorff et al., 2002; Hughes et al., 2004).

Multi-metric index development and classification

After transformation into EQR, the different combinations of metrics having passed the previous selection steps were aggregated by averaging the different metric EQRs and subsequently transformed into a final EQR using the same transformation. For each dataset, the combinations of composition and abundance metrics showing the strongest relationship with the pressure gradient were retained.

The indices were normalised with the algorithm of Yeo and Johnson (2000) following the recommendations of Fox and Weisberg (2010). Their ability to discriminate between least, moderate and most disturbed lakes was assessed for each dataset. The distribution of the index values in the three categories of lakes was compared using ANOVAs (Chambers et al., 1992). When ANOVAs revealed significant differences, Tukey multi-comparison tests were performed to identify differing classes (Miller, 1981).

All statistical analyses were performed using R statistical software (R2.9.1) (Ihaka & Gentleman, 1996; R Development Core Team, 2009).

Results

Characterisation of fish assemblages

Totals of 57 and 31 freshwater species were identified in the EUdataset and the Ndataset, respectively (Table 1).

Roach and perch were caught in more than 90% of the lakes (Table 1). The third most frequently occurring species was pike (*Esox lucius* L.) with more than 70% occurrence in the EUdataset lakes and close to 90% occurrence in those of the Ndataset. Only five species, the three previously mentioned plus ruffe (*Gymnocephalus cernuus* (L.)) and bream (*Abramis brama* (L.)), were present in more than 50% of the lakes. In contrast, 47% of the collected species were caught in less than 2% of the 445 lakes (EUdataset), and 55% of the species occurred in less than 5% of the 155 lakes of the Ndataset. Roach and perch were also the most abundant taxa among all fish (Table 3) whereas the proportion of individuals and biomass of pike, ruffe and bream were lower than 10% in the two datasets (Table 3).

Among the reproductive traits characterising the species, the lithophilic was the most frequent (25 and 15 species of the EUdataset and the Ndataset). Nonetheless, these lithophilic trait was represented by few individuals and biomass in the datasets (Table 3). Ariadnophilic and ostracophilous traits characterised two species of the EUdataset and were not represented in the Ndataset. Moreover, the pelagophilic trait was not represented in the Ndataset. The intermediate frequency of occurrence of phytophilic and phytolithophilic species was comparable between the two datasets (13 and 10 species in the EUdataset, 7 and 9 in the Ndataset).

The dominant trophic trait in number of species (15 and 9 species in the EUdataset and the Ndataset, respectively), number of fish and biomass of fish (Table 3) was omnivorous feeding. Trophic traits in benthivores and invertivores/planktivores were observed for less than two species in both datasets. The frequencies of the other traits had intermediate values.

The WCOL and BENT traits characterising the living and feeding habitat were common for 36 and 21 species in the EUdataset and 20 and 11 species in the Ndataset, respectively. In both datasets, more than 80% of fish caught and more than 80 % of the biomass were representing the WCOL trait.

Selection of metrics

In the two datasets, about 60% of the 90 tested metrics were eliminated as they did not meet the criteria of distribution range and adjusted R^2 >0.3 in the stepwise multiple linear regression (Figure 2). The highest adjusted R^2 value (0.57) was obtained for the metric “OMNI species richness” in the EUdataset. In the Ndataset, the highest values (0.71 and 0.69) were obtained for metrics related to the abundance of cyprinids in biomass and number of individuals, respectively.

In the EUdataset, the models of only six metrics passed the cross-validation procedure with a goodness of fit value higher than 0.7. Finally, the two eutrophication proxies were retain by the models predicting BPUE, CPUE and number of omnivorous fish caught per unit effort (CPUE_OMNI). NNLC was significant in the three metric models; TP was only significant for the CPUE and CPUE_OMNI metric models (Table 4). These metrics could be considered as non-redundant with a Spearman rank correlation coefficient lower than 0.8. Consequently, the three selected metrics were retained to compose the index of the EUdataset (EUindex) (Table 4).

When the same procedure of cross-validation was applied to the Ndataset, 15 metrics were retained according to the goodness of fit criteria, but only six of them exhibited a significant pressure in their models, always including TP. However, all metrics except CPUE_BENT ($r<0.8$, $p<0.001$ for all the combinations) were highly correlated with each other. Thus, it was decided to include only two metrics in the index of the Ndataset (Nindex): CPUE, showing the highest correlation with stressors (Table 4), and CPUE_BENT exhibiting a weaker correlation.

In both datasets, composition and abundance characteristics of the assemblages were represented. The final regression models included different subsets of significantly contributing environmental factors for each of the five selected metrics (Table 5). With the exception of the variable “geology”, all the natural environmental parameters included in the analyses were selected by at least one of the models retained for the selection of metrics irrespective of the dataset used.

Index development

EUdataset

The EUindex response to NNLC was tested for three combinations of the three metrics selected from the entire dataset: the average of BPUE and CPUE_OMNI, the average of CPUE and CPUE_OMNI or the average of all three metrics. Both combinations of two metrics correlated equally well with NNLC ($r = -0.48$, $p<0.001$). The average of the three metrics was strongly correlated with the NNLC stressor ($r = -0.5$, $p<0.001$) (Figure 3). The correlation of CPUE with BPUE ($r = 0.76$) was not further considered, as they are both metrics measuring abundance. The tree combinations were also significantly correlated with TP ($p<0.001$) but the correlations were weaker ($r = -0.42$ for the average of BPUE and CPUE_OMNI and $r = -0.35$ for the two other combinations).

EUindex scores among NNLC disturbance categories (least, moderately and most disturbed) of lakes (Figure 4a) differed significantly (ANOVA, F (2, 442) = 72.9, $p<0.001$). EUindex scores among TP disturbance categories of lakes (Figure 4b) were also significantly different (ANOVA, F (2, 442) = 28.8, $p<0.001$). For the two pressures, the differences were not significant between least and moderate classes (Tukey, adjusted $p=0.074$ for NNLC and $p=0.052$ for TP). Conversely, differences between most and least classes and between most and moderate classes were significant (Tukey, adjusted $p<0.001$) (Figure 4).

Ndataset

The CPUE and CPUE_BENT Nindex was significantly correlated with TP concentration ($r = -0.68$, $p<0.001$) (Figure 5) and NNLC but with weaker correlation ($r = 0.48$, $p<0.001$).

The Nindex scores differed significantly among the three disturbance categories for the two proxies of eutrophication (ANOVA, F (2, 152) = 34.46, p<0.001 for NNLC and ANOVA, F (2, 152) = 54.03, p<0.001 for TP) (Figure 6). Whatever pressure proxy was considered, the Nindex values differed significantly between least and moderate classes, between most and least classes and between most and moderate classes (Tukey, adjusted p<0.001) (Figure 6).

Discussion

We have developed a fish EUindex including composition and abundance metrics that reflects the eutrophication pressure in lakes at the European scale. A similar approach was used in the development of the European fish-based index for rivers (Pont et al., 2006; Pont et al., 2007). However, to our knowledge, our study is the first attempting to overcome the difficulties of developing national fish-based assessment systems for lakes, a task that is often precluded by the limitation of available data and/or few sites relative to the large environmental variability of the systems.

Our analyses of these large-scale European datasets highlighted the importance of abiotic factors in shaping fish assemblages, as also pointed out by Irz and co-authors (2007a). However, the contribution of catchment area to explaining the variability of the metrics was lower than the contribution of the other variables, suggesting greater importance of local versus regional factors in controlling fish assemblages (Irz et al., 2004). Obviously, the temperature descriptors seem less important in explaining the variability found in the Ndataset metrics than for the EUdataset, the latter being more homogeneous especially in terms of latitude, longitude and temperatures.

Selected metrics

The abundance metrics included in the final indices were fish density expressed as biomass or number per net and night, for which an increase with eutrophication is well documented (Minns et al., 1994; Appelberg et al., 2000; Belpaire et al., 2000; Gassner et al., 2003; Tammi et al., 2003; Garcia et al., 2006; Holmgren et al., 2007).

The two metrics selected to represent the composition of fish assemblages (CPUE_OMNI and CPUE_BENT) displayed significant responses to the eutrophication variables. Degraded conditions are probably beneficial to opportunistic species such as omnivorous feeders in contrast to specialist feeders because of their diet plasticity (McDonough & Hickman, 1999).

Use of benthic living and feeding fish in the development of lake indices is less common. However, in North America, an observed decrease in benthic fish reflected changes in catchment land use of inland lakes in Central Minnesota (Drake & Pereira, 2002) and Wisconsin (Jennings et al., 1999). These changes may reflect a general degradation of benthic habitats such as oxygen depletion and siltation.

No reproduction-based metrics were retained in the models, but response of these to anthropogenic factors has been demonstrated in similar studies on rivers (Oberdorff et al., 2002; Pont et al., 2006) and lakes (Irz et al., 2007a; Kelly et al., 2012). These traits might be more relevant to assess the hydromorphological alterations of lakes (Sutela et al., 2011), but this anthropogenic pressure was not considered here as comparable data on water level fluctuations and littoral habitat quality were not available. Similarly, the metrics using tolerance criteria did not exhibit a relationship with pressure. This re-enforces existing concerns on whether inclusion of these metrics is relevant in large-scale analyses of lake ecological status (Irz et al., 2007a), mainly due to the difficulty of determining species tolerance at such a large geographical scale. In this study, other reasons may be methodological. The catchability of many intolerant species (e.g. burbot *Lota lota* (L.) and whitefish *Coregonus lavaretus* (L.)) is relatively low in Norden gillnets (Prchalova et al., 2008), especially in benthic ones because of the pelagic life style or relatively low mobility of many intolerant species. Moreover, many of the lakes with intolerant fish species (such as Arctic charr *Salvelinus alpinus* (L.) and brown trout *Salmo trutta* L.) were removed from the dataset as species richness was too low.

Multimetric indices

The combination of two or three metrics in the multimetric EUindex resulted in similar but still weak correlations with the eutrophication variables (correlation coefficients between −0.48 and −0.5). The use of biomass rather than abundance was justified in assemblages with highly variable fish sizes and when information on energy flow was important (Minns et al., 1994; Lyons et al., 2000). As the correlation coefficient between BPUE and CPUE is less than 0.8, by including two metrics the fish assemblage is described with at least partly complementary characteristics. Moreover, the strongest correlation coefficient was observed with the three metrics in combination ($r=-0.5$).

The correlation between the Nindex combining the non-redundant BPUE and BPUE_BENT metrics and eutrophication gradient was higher than between the EUindex and eutrophication, likely reflecting a more homogeneous set of lakes in the Ndataset with respect to the environment.

In the EUdataset, significant differences between the EUindices of the least impacted lakes and the others were demonstrated. However, the difference between moderately and heavily impacted sites was not significant. This reflects the widespread distribution of the EUindex values around the regression line with the eutrophication proxies. In the Ndataset, the three lake categories were well differentiated, probably due to a higher proportion of oligotrophic lakes than in the EUdataset.

Compared to most of the existing indices developed for European lakes, the EU and N indices included only few metrics. However, our selection of metrics included a severe and robust validation phase which, to our knowledge, has never been implemented before although it is indispensible for any method's development. Moreover, pressure / impact relationships are clearly revealed whereas it is a serious flaw of most of the existing methods (see review by Birk et al. 2012).

In order to fully meet the normative requirements of the WFD, a new metric reflecting the age structure of communities/populations needs to be included in the indices. The response analysis of various size metrics to pressure is currently the subject of studies conducted to obtain this specific aim (Emmrich et al. 2011).

Considerations on the hindcasting method

Traditionally, biological reference conditions are determined using reference sites, i.e. sites unexposed or insignificantly exposed to pressures (Poikane et al., 2010; Pardo et al., 2012). Reference sites must be representative of the diversity of all lakes and consequently cover the whole environmental gradient. One of the difficulties in this study was to identify a sufficient number of reference sites, especially in continental Europe. This difficulty was overcome by modelling the reference conditions using the hindcasting method (Baker et al., 2005; Kilgour & Stanfield, 2006). A prerequisite for the application of hindcasting is the availability of a dataset covering a large range of pressures. This implies that when the index is calculated on a new lake *a posteriori*, this lake will most probably be within the range of pressures covered by the model. This requirement is fulfilled for the eutrophication variables of the EUdataset; the lowest value measured for NNLC being 1%, whereas the highest value exceeds 90%. This is similar for TP, with values varying between 1 and 330 µg L^{-1}, covering a wide eutrophication gradient from ultra-oligotrophic to hypertrophic lakes. The TP range of variation of 1–162 µg L^{-1} in the Nordic dataset is less pronounced. However, it covers the entire gradient when the predominant conditions in the northern countries (long winter, ice cover, etc.) are taken into account.

Considerations on the data

A way to better disentangle the effects of environmental and anthropogenic factors would be to increase the quality and quantity of lake descriptors, including those necessary to quantify the uncertainty related to applied methods (see Clarke, this issue). A challenge in the statistical development of such tools is to find a compromise between the minimum number of lakes required to meet the robustness of the analyses and the quality and quantity of data on the fish and the environment of the lakes to detect low signals of pressure. Despite the huge effort involved in compiling the European dataset, standardised fish data and comparable environmental descriptors and eutrophication proxies still cover only a few sites. Less than a quarter of the entire European database (445 out of 1992 lakes) included all data for the parameters considered in the models. Multiple environmental parameters known for their impact on fish assemblages were not considered because of a lack (or low occurrence) of appropriate data. Among these, lake area was considered as a surrogate of habitat diversity and modelled air temperature was used in place of water temperature. Information on the hydrological regime was not considered despite its impact on the overall ecosystem functioning (Leira & Cantonati, 2008), and pH, a driver of productivity, was roughly assessed by calcareous or siliceous categories of lakes. When considering human activities, better knowledge of usual direct manipulation of fish via recreational and commercial fishing, or stocking, would probably add significantly to our understanding of the variability of the fish assemblages considered (Welcomme et al., 1983; Cowx, 1998). Moreover, we focus here on the factors acting at the local scale. At the catchment scale, for example, connectivity can contribute to explain the structure of fish assemblages (Rahel, 1986; Robinson & Tonn, 1989) and probably also historical events (Banarescu, 1989).

Addition of new fish samples would also help to improve the results obtained, in particular from the entire European dataset. Indeed, many of the lakes studied are located in northeastern Europe, resulting in an unbalanced distribution of the sites, partly reflecting the natural distribution of European lakes. However, increasing and standardising the sampling efforts in currently underrepresented regions could improve this situation.

Finally, it is always difficult to obtain a comprehensive picture of the composition of fish communities including information on the abundance of each species in lacustrine environments. The present study is based on data from fish sampled with gear acknowledged to be selective, for example by under-sampling small individuals. Gillnets also underestimate the abundance of less mobile species, such as pike, or unusually shaped species, such as eel. Also, the protocol underestimates pelagic species (Lauridsen et al. 2008). Consequently, the future use of electrofishing in the littoral area of the lakes (Diekmann et al. 2005) or use of other passive gears would probably improve the picture of the assemblages obtained (Kubečka et al. 2009). Such developments would also allow calculation of other metrics

Concluding remarks

Despite an uneven geographical distribution of the study lakes within Europe, and certain shortcomings in the environmental and pressure data, metrics have been identified for assessing the responses of lake fish assemblages to eutrophication in European lakes. Our approach follows the ecological rationale for the implementation of the WFD highlighting ecosystem integrity. Because of the relatively long life span of fish, the fish fauna of any lake has an integrative nature. Consequently, our indices can account for long-term effects and provide essential additional information over that produced by chemical water quality and phytoplankton indices.

The present indices prove the usefulness of applying standardised lake fish data to assess the ecological status of lakes and support the development of national lake fish assessment tools. Future research should now focus on the setting of ecologically meaningful quality classes (e.g. looking for thresholds, if they exist, using pair-metrics,...). This is a difficult task and will be a major flaw of most assessment methods currently under development. Complementary studies are also required in order to determine the response of the fish fauna to the alteration of hydromorphology. This necessitates the collection of accurate and consistent information on hydrology (particularly the degree and periodicity of water level fluctuations) and habitat alterations. This data, however, was unavailable for the present study.

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Table 1. Categorisation of the species into guilds and the relative frequency of occurrence (% of lakes) of species in the two datasets. Rep: reproductive guild, Tol: tolerance guild and Fa: place of living and feeding activity.

Scientific name	*Rep*	*Trophic*	*Fa*	*Tol.*	*Occurrence EUdataset*	*Occurrence Ndataset*	*Scientific name*	*Rep.*	*Trophic*	*Fa.*	*Tol.*	*Occurrence EUdataset*	*Occurrence Ndataset*
Abramis brama	PHLI	PLAN	BENT	TOL	57.5	**49.7**	***Leuciscus idus***	PHLI	INV/PISC	WCOL		1.8	1.9
Alburnoides bipunctatus	LITH	INV	WCOL	INT	0.2		***Leuciscus leuciscus***	LITH	OMNI	WCOL		1.8	1.9
Alburnus alburnus	PHLI	PLAN	WCOL	TOL	40.0	40.0	***Lota lota***	LITH	PISC	WCOL		11.5	19.4
Alosa fallax	LITH	PLAN	BENT		0.2		*Micropterus salmoides*	ARIAD	PISC	WCOL	TOL	0.7	
Ameiurus melas	LITH	OMNI	BENT	TOL	2.5		***Oncorhynchus mykiss***	LITH	INV/PISC	WCOL		2.9	2.6
Anguilla anguilla	PELA	INV/PISC	WCOL	TOL	3.1		***Osmerus eperlanus***	LITH	INV/PISC	WCOL		16.6	19.4
Aspius aspius	LITH	PISC	BENT		2.0	**1.9**	***Perca fluviatilis***	PHLI	INV/PISC	WCOL	TOL	95.7	98.7
Ballerus ballerus	PHYT	PLAN	WCOL		2.0	1.9	***Phoxinus phoxinus***	LITH	INV	WCOL		3.8	4.5
Barbatula barbatula	PHLI	INV	BENT		0.4		*Platichthys flesus*	PELA	INV/PISC	BENT		0.9	
Blicca bjoerkna	PHYT	OMNI	BENT	TOL	26.5	**18.7**	*Pomatoschistus minutus*	OSTR	INV/PISC	BENT		0.2	
Carassius carassius	PHYT	OMNI	BENT	TOL	4.9	4.5	***Pungitius pungitius***	PHYT	INV	BENT	TOL	1.6	0.6
Carassius gibelio	PHYT	OMNI	BENT	TOL	0.2		*Rhodeus amarus*	OSTR	OMNI	WCOL		0.4	
Clupea sprattus	PELA	PLAN	WCOL		0.2		*Rutilus aula*	PHYT	OMNI	BENT		0.7	
Cobitis taenia	PHYT	BEN	BENT		5.4	1.3	***Rutilus rutilus***	PHLI	OMNI	WCOL	TOL	91.7	94.2
Coregonus albula	LITH	PLAN	WCOL	INT	19.5	20.6	*Salmo ferox*	LITH	PISC	WCOL		0.2	
Coregonus autumnalis	LITH	INV/PLAN	WCOL		0.2		*Salmo salar*	LITH	INV/PISC	WCOL	INT	2.0	
Coregonus lavaretus	LITH	INV	WCOL	INT	14.8	**25.8**	*Salmo trutta*	LITH	INV/PISC	WCOL		0.9	
Coregonus peled	LITH	PLAN	WCOL		0.4	0.6	***Salmo trutta fario***	LITH	INV/PISC	WCOL	INT	8.5	3.9
Cottus gobio	LITH	INV	BENT	INT	2.2	1.9	*Salmo trutta trutta*	LITH	INV/PISC	WCOL	INT	1.1	
Cottus poecilopus	LITH	OMNI	BENT	INT	1.1	**2.6**	*Salvelinus namaycush*	LITH	INV/PISC	WCOL	INT	0.4	
Cyprinus carpio	PHYT	OMNI	BENT	TOL	2.5		***Salvelinus umbla***	LITH	INV/PISC	WCOL		4.3	3.2
Esox lucius	PHYT	PISC	WCOL		71.7	**87.7**	***Sander lucioperca***	PHLI	INV/PISC	WCOL		28.1	23.9
Gasterosteus aculeatus	ARIAD	INV	BENT	TOL	4.7		***Scardinius erythrophthalmus***	PHYT	OMNI	WCOL		38.0	26.5
Gobio gobio	PHLI	INV	BENT	INT	8.3	1.3	*Silurus glanis*	PHYT	PISC	WCOL		1.3	
Gymnocephalus cernuus	PHLI	OMNI	BENT		64.0	61.9	*Squalius cephalus*	PHLI	OMNI	WCOL		4.0	
Hypophthalmichthys molitrix	PELA	PLAN	WCOL	TOL	0.2		*Telestes souffia*	LITH	INV	WCOL		0.4	
Hypophthalmichthys nobilis	PELA	PLAN	BENT		0.2		***Thymallus thymallus***	LITH	INV	WCOL	INT	0.7	1.3
Lepomis gibbosus	LITH	INV	WCOL	TOL	3.4		***Tinca tinca***	PHYT	OMNI	BENT	TOL	23.1	21.3
Leucaspius delineatus	PHYT	OMNI	WCOL		4.3	0.6							

Table 2. Environmental parameters of the lakes included in the European database (EUdataset) and in the subset of Nordic lakes (Ndataset).

Parameter	Unit	Mean		Range	
		EUdataset	Ndataset	EUdataset	Ndataset
Maximum depth	Metres (m)	17.6	16.4	0.6-145	2.1 - 64
Lake area	Square kilometres (km^2)	5.6	5.2	0.1-116.7	1.0 - 107.6
Catchment area	Square kilometres (km^2)	146.3	94.7	0.1 - 10630	0.5 - 3243
Altitude	Metres (m)	151	147	0 -1200	1 - 764
Average temperature	Degrees Celsius (°C)	6.1	4.6	-2.2 - 14	-2.2 - 7.7
Temperature amplitude	Degrees Celsius (°C)	19.5	21.1	8.5 - 30	17.2 - 28.9
Non-natural land cover (NNLC)*	% Of the catchment area	21.3	7.5	0 - 94.0	0 - 64.9
Total phosphorus (TP)*	µg/L	32	19	1 - 330	1 - 162

* Anthropogenic factors

Table 3. Representativeness of the most frequent species or groups of species included in the EUdataset and the Ndataset

	Abundance (% of the total number of fish caught)		Biomass (% of the total biomass caught)	
	EUdataset	Ndataset	EUdataset	Ndataset
Perca fluviatilis	44.3	42.9	34.4	41.3
Rutilus rutilus	31.9	33.2	28.3	25.5
Esox lucius	0.2	0.2	4.4	5.8
Gymnocephalus cernuus	8.8	8.3	1.9	2.1
Abramis brama	3.1	2.8	9.5	7.2
Lithophilic fish	2.5	2.7	4.6	4.1
Omnivorous fish	45.5	46.3	41.7	35.6
Pelagic living and feeding habitat	84	84.7	80.3	84

Table 4. Values of the statistical criteria used to select the metrics, R^2 is the adjusted value of the stepwise model, goodness of fit is the correlation value using a validation dataset, tendency corresponds to the way in which the metrics change depending on the pressure, Pearson NNLC and Pearson TP are the Pearson correlation coefficients between the metrics after EQR transformation and the stressors.

	R^2	*Goodness of fit*	*Tendency*	*Pearson NNLC*	*Pearson TP*
CPUE EUdataset	0.55	0.78	+	−0.40	−0.43
BPUE EUdataset	0.48	0.74	+	−0.44	−0.30
CPUE_OMNI EUdataset	0.54	0.78	+	−0.40	−0.43
CPUE Ndataset	0.67	0.80	+	−0.50	−0.61
CPUE_BENT Ndataset	0.61	0.83	-	−0.45	−0.55

Table 5. Results of the stepwise multiple linear regressions for the five metrics: values and significance of the models' equation coefficients.

	CPUE EUdataset	*BPUE EUdataset*	*CPUE_OMNI EUdataset*	*CPUE Ndataset*	*CPUE_BENT Ndataset*
Intercept	−6.129***	0.213	−2.189**	0.047***	−5.951*
Maximum depth				−1.23E-08**	−0.567**
(Maximum depth)^2	−0.460***	−0.361***	−0.504***	3.38E-09	
Lake area	0.193**	0.270***	0.135	2.25E-09*	0.386***
(Lake area)^2				−8.88E-10	
Altitude	−0.003***		−0.003***	−2.63E-11***	−0.005***
(Altitude)^2	2.77E-06***	2.21E-07	2.89E-06***	2.35E-14*	4.02E-06*
Catchment area		−0.113		−4.71E-09**	
(Catchment area)^2				1.26E-09**	
Average temperature		−0.081	0.100**		
(Average temperature)^2	0.003	0.006*		−5.48E-11**	
Temperature amplitude	0.296***	0.108*	0.325***		−0.6**
(Temperature amplitude)^2	−0.005***	−0.003	−0.005**		−0.012**
NNLC	1.469***	0.576***	1.990***		
NNLC^2	−0.542		−1.282**		
TP					0.694**
TP^2	0.019*	0.041***	0.049***	3.56E-10***	−0.101*

** Significant at 0.05 level, ** significant at 0.01 level, *** significant at 0.001 level.*

Figure captions

Figure 1. Distribution map of lakes included in the datasets.

Figure 2. Flow chart of the metric selection procedure with the results obtained from the EUdataset. MLR= multiple linear regression.

Figure 3. Relationship between the index developed from the EUdataset and the NNLC (arcsine squared-root scale).

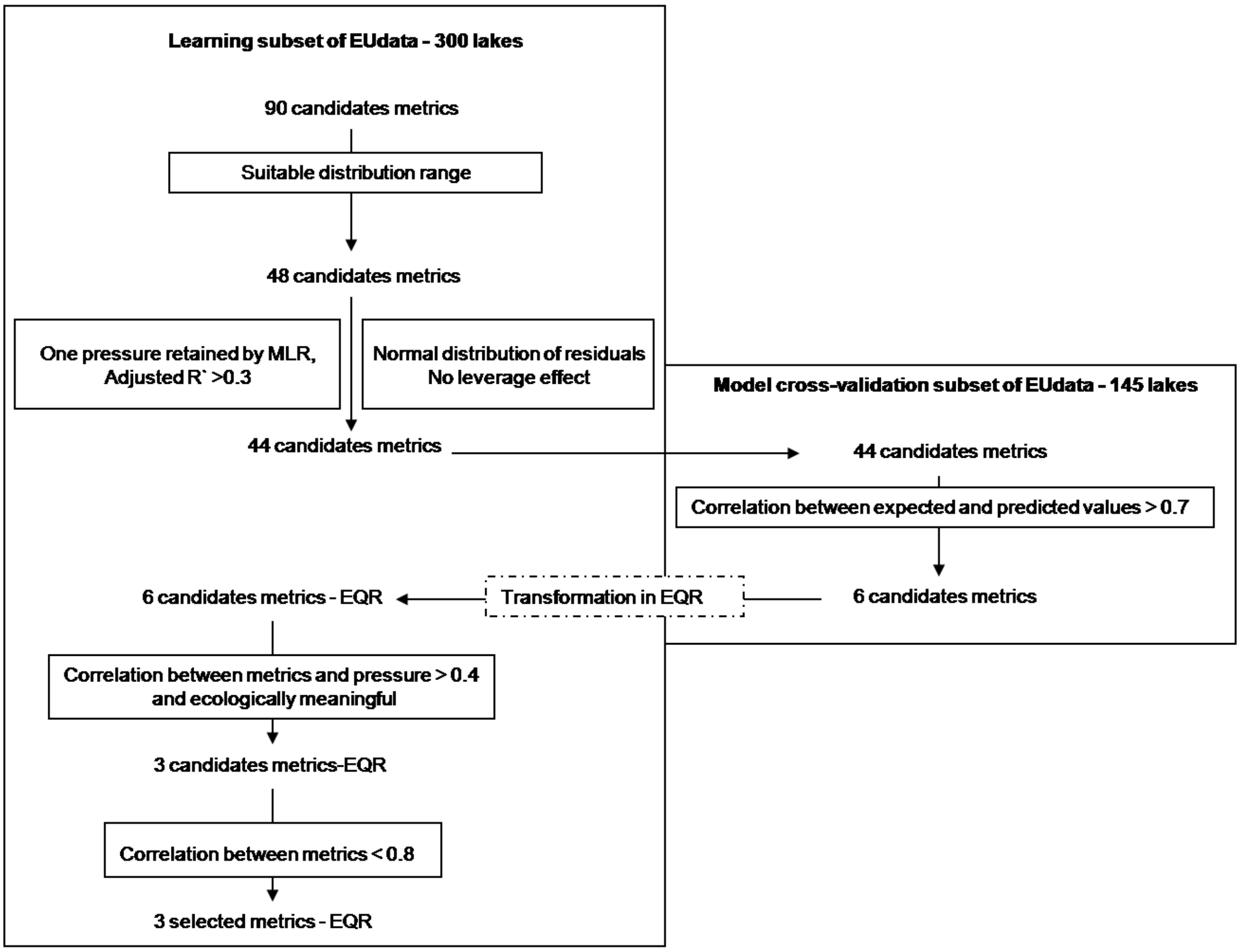
Figure 4. Distribution of the index values developed from the EUdataset for the least, moderate and most impacted lakes regarding NNLC (a) and TP (b).

Figure 5. Relationship between (a) the index developed from the Ndataset and the TP (logarithmic scale).

Figure 6. Distribution of the index values developed from the Ndataset for the least, moderate and most impacted lakes regarding NNLC (a) and TP (b).

Figure 1

Figure 2



Learning subset of EUdata - 300 lakes
90 candidates metrics
Suitable distribution range
48 candidates metrics
One pressure retained by MLR, Adjusted R` >0.3
Normal distribution of residuals No leverage effect
44 candidates metrics
Model cross-validation subset of EUdata - 145 lakes
44 candidates metrics
Correlation between expected and predicted values > 0.7
6 candidates metrics
Transformation in EQR
6 candidates metrics - EQR
Correlation between metrics and pressure > 0.4 and ecologically meaningful
3 candidates metrics-EQR
Correlation between metrics < 0.8
3 selected metrics - EQR

Figure 3

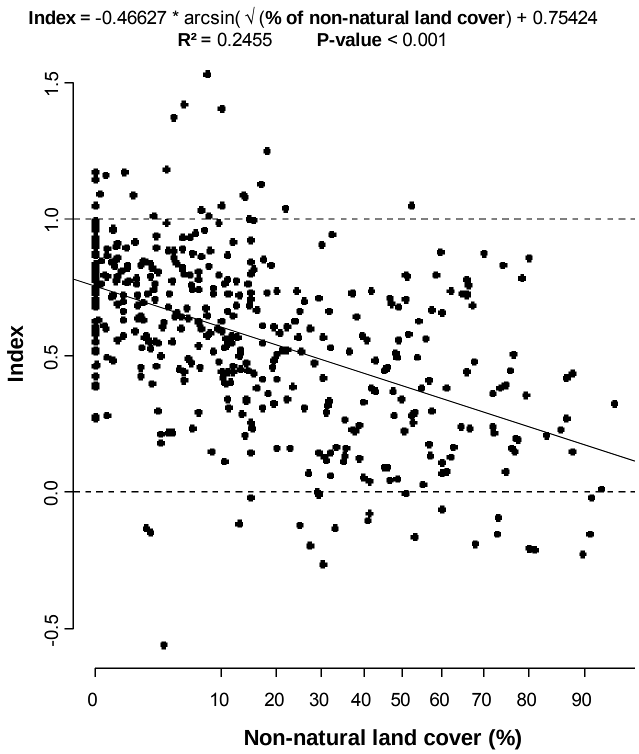

Index = -0.46627 * arcsin(√ (% of non-natural land cover) + 0.75424
R² = 0.2455 P-value < 0.001
Index
-0.5
0.0
0.5
1.0
1.5
0
10
20
30
40
50
60
70
80
90
Non-natural land cover (%)

Figure 4

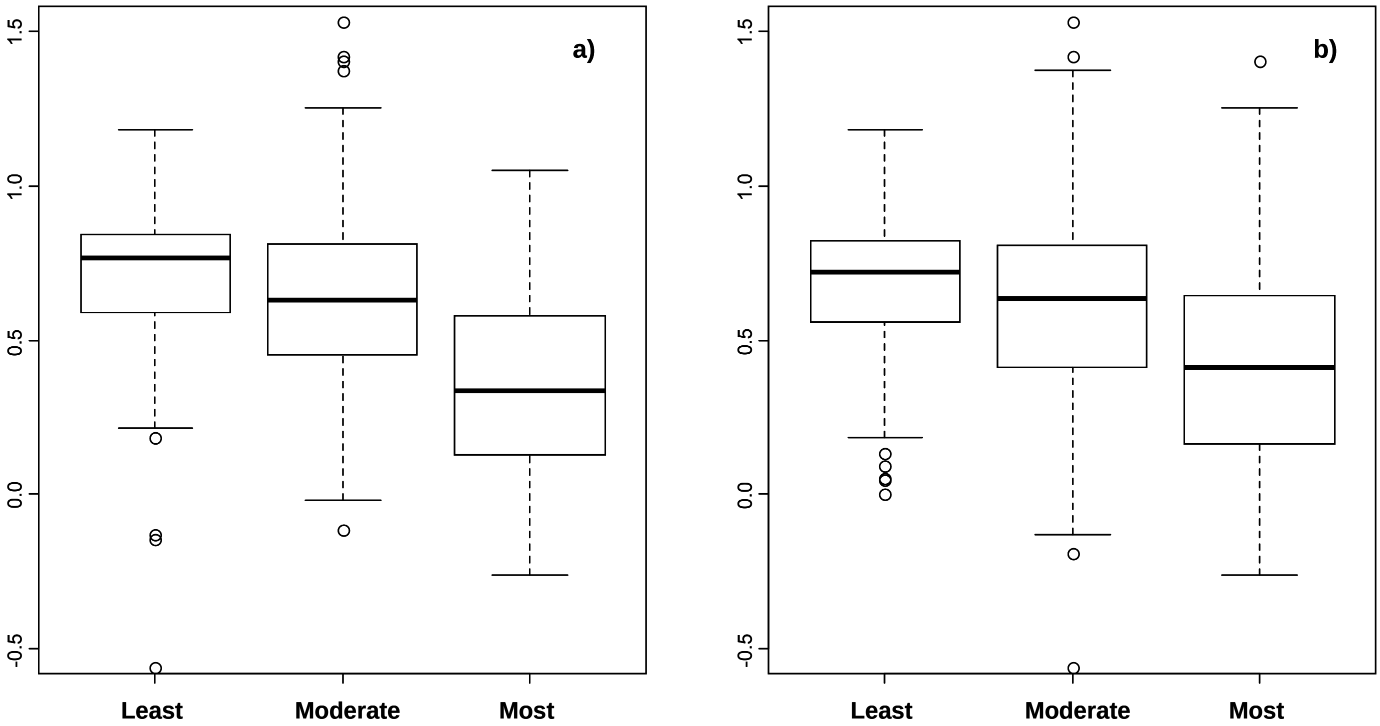


Figure 5

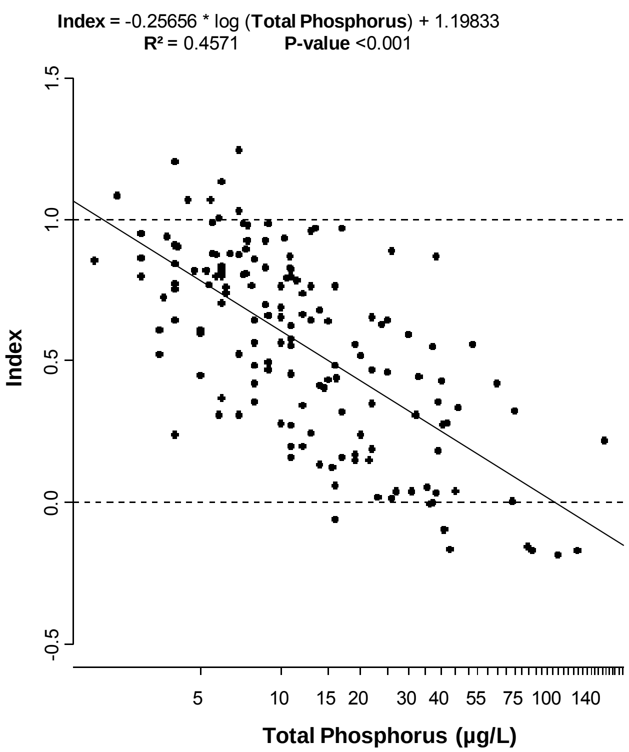


Figure 6