Assessing lake ecological status across a trophic gradient through environmental and biological variables

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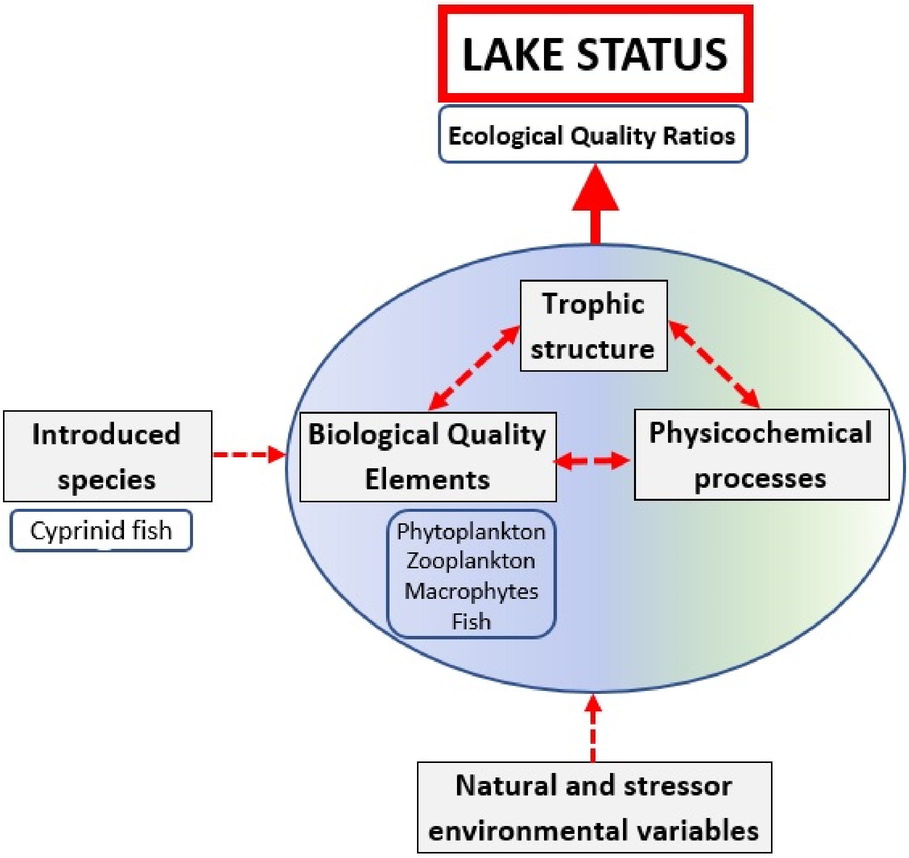
Graphical Abstract



Highlights

- Contributions of environmental and biotic variables were determined for 50 lakes
- Strong evidence for bottom-up rather than top-down trophic effects detected
- Lakes with introduced cyprinid fish had lower ecological quality
- Limited concordance in the responses of BQEs to environmental variables
- Chlorophyll *a*, TP, population density, water colour and elevation predict mean EQR

Abstract

The Water Framework Directive was widely welcomed because it sought to integrate chemical and biological elements of aquatic ecosystems to achieve 'good ecological status', reflecting at most slight anthropogenic impact. However, implementation has been criticised because of the failure to adequately integrate these elements and assess status of the whole ecosystem. In this study, a suite of environmental and biotic variables was measured to assess their relative importance as predictors of lake status for 50 lakes in the north of the island of Ireland. Total Phosphorus (TP) had a strong effect on taxon biomasses and ecological quality ratios (EQR) for most taxa, as expected, but other environmental variables, such as pH, water colour and spatial location, were also important. Most variance in mean EQR, the average of the taxon values, was predicted by five environmental variables (chlorophyll *a*, TP, population density, water colour and elevation) and whether (alien) cyprinid fish were present. Oligotrophic lakes with cyprinid fish had lower mean EQRs than cyprinid-free lakes, indicating the importance of recording species introductions when assessing lake status. Strong evidence for bottom-up effects was also detected, and cyprinids probably influenced trophic structure by increasing nutrient release from the sediment rather than by top-down effects. Phytoplankton biomass, fish biomasses, and the percentage of predatory fish, increased with TP. Our results further emphasize the need to adopt a more integrated approach when assessing lake status.

Keywords: Nutrient gradient; Lake quality metrics; Environmental and biotic variables; Species introductions; Trophic structure

1. Introduction

Lake trophic state is affected by natural intra-lacustrine and catchment-scale processes, and anthropogenic influences. Eutrophication typically changes ecosystem structure and functioning by increasing phytoplankton abundance and dominance by filamentous cyanophytes, with consequent reductions in photic depth, macrophyte loss and a shift in the fish community to benthic feeding species, which compound such impacts through increased internal nutrient re-circulation (Moss, 1998; Scheffer, 1998; Smith, 2003). In addition to these largely nutrient determined bottom-up processes, top-down predator-prey (consumption) effects are regarded as important in controlling ecosystem structure and function (Leibold et al., 1997). For example, fish selectively consume larger zooplankters, particularly the more efficient grazer cladocerans, with positive effects on phytoplankton abundance (Hall et al., 1976).

Phosphorus (P) is generally regarded as a major driver of eutrophication and hence trophic structure but other factors are important. For example, nitrogen (N) compounds can have significant acidification, eutrophication and toxicity effects on biota (Camargo & Alonso, 2006). While P and N concentrations are often correlated (Downing & McCauley, 1992) oligotrophic lakes are generally P-limited, whereas N-limitation is more likely in eutrophic water bodies (Sterner & Elser, 2002). Nutrient limitation can affect species composition: for example, cyanophytes are more likely to dominate in P-rich lakes as some species can fix atmospheric N, and thus are not limited by water-column N. Water colour also affects phytoplankton composition and biomass, and overall lake productivity (Nürnberg & Shaw, 1999; Carvalho et al., 2011). Furthermore, species introductions are known to alter lake trophic state. For example, cyprinids such as roach (*Rutilus rutilus*) and bream (*Abramis brama*), species characteristic of eutrophic waters, can reduce zooplankton abundance and release nutrients from lake sediments through bioturbation, with effects on the abundance of phytoplankton and consequently of macrophytes (Winfield & Townsend, 1991; Scheffer, 1998), while zebra mussels (*Dreissena polymorpha*) can have major impacts on lake plankton, benthos and fish and thus on ecosystem function (MacIsaac, 1996).

The European Union Water Framework Directive 2000/60/EC (WFD) seeks to assess anthropogenic impacts on water bodies by comparing observed lake conditions with those expected for unimpacted lakes. The WFD defines ecological status as an expression of the quality of aquatic ecosystem structure and functioning, using physical, chemical and biological quality elements. To simplify the establishment of reference conditions a set of habitat types is defined using features that are regarded as insensitive to human impact. For example, lake area, mean depth and alkalinity are used to classify lake types in the Republic of Ireland (Free et al., 2016). Considerable effort has been expended in establishing procedures for calculating ecological quality ratios (EQR, the ratio of observed/unimpacted (reference) lake conditions) (Poikane et al., 2015). Implementation of the WFD has, however, been heavily criticised because of the failure to integrate the biological quality elements (BQE) to provide a holistic view of lake quality (Moss, 2008; Voulvoulis, Arpon & Giakoumis, 2017), instead focussing more on ecosystem structure (abundances) than function (the interactions between BQEs) (Caroni & Irvine, 2010). Not all potentially important elements (such as zooplankton, Jeppesen et al., 2011) are measured or, if measured, taken into account (in the 'one-out, all-out' principle) when determining lake status. Furthermore, species introductions are not explicitly considered, notwithstanding their potentially significant effects on ecosystem function and interactions with other factors; for example, as outlined above, cyprinids are characteristic of eutrophic waters and can also increase nutrient concentrations by bioturbation. Both bottom-up and top-down processes are important in regulating lake trophic structure (Carpenter & Kitchell, 1993; Jeppesen et al., 1997; Vanni, 2002; Jeppesen et al., 2003). For example, Carpenter and Kitchell (1993) noted that nutrient supply accounted for only half the variance in primary production and concluded that there were strong trophic interaction effects in their three study lakes. However, the WFD considers nutrient effects but does not take account of top-down processes, i.e. consumptive interactions between the BQEs, with the danger of missing important key predictors of EQRs.

In this study, the relationships between a number of natural and stressor environmental variables and phytoplankton, macrophyte, zooplankton and fish assemblages were explored to identify the relative importance of factors driving taxa abundances and composition and their interactions, and hence ecosystem structure and function, across a lake trophic gradient. We take an integrated view of the effects of multiple metrics on lake ecosystems by addressing the following questions:

1) Which environmental variables best predict phytoplankton, macrophyte, zooplankton and fish biomasses and are these BQE responses concordant across taxa?

2) Which environmental and biotic variables best predict EQRs?

3) Do trophic interactions have measurable effects on taxon biomasses and EQRs? Specifically, is there evidence of (a) bottom-up and/or top-down effects (positive and negative correlations between BQE biomasses, respectively), (b) selective predation on zooplankton by fish (decreases in zooplankton:phytoplankton ratio, *Daphnia* abundance, and zooplankton size with increasing fish predation), and (c) interactions between and within BQEs varying with eutrophication?

2. Methods

2.1. Study lakes and measures

Fifty, mainly small and shallow, lakes (**Table S1a**) in the north of the island of Ireland (**Fig. 1**) were selected to incorporate a trophic gradient, ranging from oligotrophic in the west to eutrophic conditions in the east. Lake chemistry, phytoplankton and zooplankton were sampled seasonally, in spring (March-May), summer (June-August) and autumn (September-November) in 2012 or 2013, while macrophytes and fish were sampled once, in the summers of those years. Total phosphorus (TP), total oxidised nitrogen (TON), ammoniacal nitrogen (NH_4N), chloride (Cl), soluble reactive silica (SiO_2), pH, alkalinity, water depth, water colour were measured as direct, and lake area and elevation as indirect, potential influences on taxon biomasses. Human population density within the catchment (PopDen) and % of the catchment used for agriculture were recorded as possible indicators of anthropogenic impact. The presence or absence of zebra mussels was noted. Hydrological condition was not assessed but, as far as we know, water level is not regulated in any of the lakes.

2.2. Sampling and laboratory analysis procedures

2.2.1 Water chemistry

A composite mixed layer water sample was collected at the deepest point of each lake using a 500 ml Ruttner water sampler. Water temperature, and dissolved oxygen concentration, conductivity and pH were measured at 1 m depth intervals using a YSI 556 MPS multi-probe. Chlorophyll *a* (Chla) was determined by spectrometry using the procedure of Marker, Crowther and Gunn (1980) after cold extraction in the dark at 4 °C. Water colour (Hazen) was determined by spectrometry using potassium chloroplatinate and cobaltous chloride hexahydrate (Standing Committee of Analysts, 1984). A Mettler DL25 autotitrator, was used to measure pH and determine alkalinity by acidometric titration to pH 4.5 (Standing Committee of Analysts, 1979, 1982). Composite water sub-samples were preserved by freezing for the determination of TP, TON and SiO_2. TP was analysed following the method of Eisenreich, Bannerman and Armstrong (1975). TON and NH_4N were determined using the methodologies of Chapman, Cokke & Whitehead (1967) and Scheiner (1976). SiO_2 was measured spectrophotometrically according to Golterman, Clymo & Ohnstad (1978).

2.2.2 Phytoplankton

An unfiltered sub-surface (approximately 0.3 m depth) phytoplankton sample was collected using a 250 ml brown polypropylene bottle at the lake deepest point and preserved using acidified Lugol's iodine. Sample counts were performed using the Ütermohl technique on an inverted microscope (CEN, 2006). Taxa were identified using John, Whitton & Brook (2011), Kelly (2000), Cox (1996), Komarek and Anagnostidis (1999, 2007), Bellinger and Sigee (2010), Canter-Lund and Lund (1995) and Komárek (1999). Biovolumes were calculated from recorded cell counts and measured cell dimensions which were then approximated to simple geometric shapes (Mischke et al., 2012).

2.2.3. Macrophytes

Sampling followed the UKTAG (2008) procedure. A shoreline investigation of littoral macrophytes was conducted 10 m either side of transect starting locations. Transects ran perpendicular to the shore and samples were taken at 0, 2.5, 5, 7.5, 10, 25, 50, 75, and 100 m along transects. The number of transects was determined by lake size and shoreline complexity: at least four transects were taken per lake. For each transect the maximum depth of colonisation was recorded. When plant presence was recorded at one position but not at the next, the previous position was returned to and sampled at every depth change of 0.5 m until the maximum depth of colonisation was located. If plants were found to occur at the 100 m distance, the survey was continued for every 0.5 m change in depth, until the maximum depth and distance of colonisation was found. A double-headed rake was used to take four macrophyte samples at each sampling position. Macrophytes were identified to species using standard works (Rose, 1981; Moore, 1986; Hill, Preston & Smith, 1991-4; Preston, 1995; Preston & Croft, 1997).

2.2.4. Zooplankton

Five vertical zooplankton net hauls were collected per lake, using a net and filter of mesh 250 μm: nets with this mesh size do not sample small zooplankters such as rotifers. Haul depths were selected relative to lake internal shape. Each lake was divided into five vertical sections, of equal volume, based on the hypsometric curve of that lake. A vertical net haul was then taken from the mean depth of each section. Aliquots of each of the five net hauls were combined, relative to haul volume, to form a composite sample for each lake. While the water depth of each haul was predetermined, sampling position in the lake was selected at random. Samples were preserved in 70 % industrial methylated spirits. A sub-sample was then enumerated using an Olympus SX15 binocular microscope, cladocerans and copepods were identified using keys (Scourfield & Harding, 1966; Harding & Smith, 1974), and zooplankton densities (numbers L^{-1}) calculated.

2.2.5. Fish

Fish were sampled in 46 of the 50 study lakes, using a standardised combination of benthic monofilament gill nets, floating monofilament gill nets and fyke nets (Kelly et al., 2008). The procedure was an adaptation of the CEN standard for sampling fish using multi-mesh gill nets (CEN, 2005). Lakes were allocated to six area classes (≤ 20, 21-50, 101-250, 251-1000, 1001-5000 ha), each with a prescribed number of nets to be deployed in each depth zone: the number of nets utilised increased with maximum depth and surface area. Nets were left overnight, for approximately 18 hours. Depending on bathymetry, lakes were randomly sampled at depth strata (0-2.9, 3-5.9, 6-11.9, 12-19.9, 20-34.9 and 35-49.9 m strata). The multi-panel gill nets were CEN compliant (length 30 m x depth 1.5 m, consisting of 12 x 2.5 m panels of different mesh sizes following a geometric series ranging from 5 to 55 mm, knot to knot). Floating pelagic gill nets with identical dimensions to the benthic gill nets were deployed over the deepest sections of the study lakes. Each survey was supplemented with a minimum of three fyke net units (three nets per unit: each net total length of 10 m and hoop diameters of 0.5, 0.45, 0.35 and 0.28 m, mesh 22 mm knot to knot). Fish biomass was estimated as g m^{-1} net: following the FIL2 (Kelly et al., 2012) protocol fyke net catches were calculated, like gill nets, as m^{-1} net. Fish were identified to species and measured (fork length ±1 mm, blotted wet mass ±0.1 g). A sub-sample of fish were aged: scales were taken from cyprinids, opercular bones from perch and pike, and sagittal otoliths from eels.

2.3. Data treatment

The macrophyte category included bryophytes and macroalgae as well as angiosperms. The zooplankters *Daphnia galeata* and *D. hyalina* form a species complex and were grouped (Caroni & Irvine, 2010). All roach, rudd, bream hybrids were allocated to a Hybrid category. Phytoplankton abundance was estimated as Chla concentration and total cell biovolume. Macrophytes were assigned a DAFOR (Dominant (5); Abundant (4); Frequent (3); Occasional (2) and Rare (1)) numeric score based on estimated percentage occurrence in rake samples. Relative abundances in each lake were estimated as the sum of the DAFOR scores, scaled by the mean depth of species presence as a proportion of maximum lake depth. Zooplankton biomass was estimated as μg DW $litre^{-1}$, from length-weight regression equations in Downing and Rigler (1984). To simplify presentation all these (density) measures are referred to as biomasses.

Area, mean depth and alkalinity are used to identify WFD lake types in the Republic of Ireland (Free et al., 2016). Alkalinity and conductivity were strongly collinear and were replaced by the morphoedaphic index (MEI, calculated as alkalinity/mean lake depth) as an indicator of nutrient availability (Chow-Fraser, 1991). While many of the environmental variables were correlated with the % of the catchment used for agriculture (**Table S1b**) inclusion of this variable did not improve prediction of taxon biomass or EQR and is not reported.

There was some seasonal variation in taxon and environmental variables but ‘annual’ means were calculated to simplify the analysis and because overall lake status measures should be insensitive to seasonality.

2.4. Analysis

To normalise the data, most variables, including trophic level biomass ratios, were $\log_{10}$ transformed but elevation was square-root transformed and pH was normally distributed: unless otherwise stated the results are based on transformed values. The number of observations per BQE varied (phytoplankton 50, macrophytes 48, zooplankton 50, fish 46 lakes).

EQRs were calculated for phytoplankton biovolumes using the associated software (UKTAG, 2014), macrophyte scores using the Free Index (UKTAG, 2008) and fish using FIL2 (Kelly et al., 2012). Phytoplankton EQRs require information on Chla and taxon biovolumes, macrophyte EQRs information on depth of occurrence, and the relative occurrence of *Chara* species and elodeids; both

EQRs use information on taxon-specific sensitivity to TP concentrations. Fish EQRs use information on 13 variables covering species composition, catch and biomass per unit effort. Mean EQR was the average of these scores across BQEs. BQEs were weighted equally, but boundary values for the quality classes differed across BQEs e.g. high/good quality 0.8, 0.9, 0.76 for phytoplankton, macrophytes, fish, respectively. However, rescaling to the same boundary values had no effect on predicted mean EQR values. The missing macrophyte and fish EQR values were estimated from observed EQR–environmental variable regressions (macrophyte EQR=1.251-0.318*TP-0.148*Colour-0.095*MEI, R^2=0.63; fish EQR=0.219-0.260*MEI, R^2=0.30).

Phytoplankton genus/species optimal responses to TP (from Appendix A in UKTAG, 2014) were weighted by their corresponding biovolumes to calculate mean lake phytoplankton response to TP. For each lake, the upper and lower quartile optima, unweighted by biovolume, were also calculated; these quartile optima correspond to species tolerant and intolerant of TP, respectively.

Relations between environmental variables were summarised using principal components analysis (PCA), relations between environmental variables, BQE biomasses and EQRs investigated using pairwise Pearson (*r*) correlation coefficients while environmental variable differences between lakes with and without cyprinids and with and without zebra mussels were examined by linear discriminant analysis. Boosted regression trees (BRT) were used to estimate the relative contributions of the environmental predictors to taxon biomasses and EQRs. Biomass-environmental variable correlation coefficient values (**Table S2**) were correlated with the BRT relative influence values (coefficients ranged from 0.72 to 0.86 across the four BQEs). Means are shown with standard errors. Most statistical calculations were conducted using Systat v13.

BRT is a machine-learning method which can handle continuous and categorical variables, and avoids many of the problems of more traditional regression approaches (Elith, Leathwick & Hastie, 2008). Code provided by Elith et al. (2008), modified from the 'dismo' (Hijmans et al., 2016) and 'gbm' (Ridgeway, 2015) packages, was used in R 3.1.2 (R Development Core Team, 2014). Optimal models were fitted by altering the number of regression trees generated and the learning rate (a parameter that determines the contribution each tree makes to the model) to minimize predictive deviance. Final models used a learning rate of 0.005, tree complexity (the number of splits in a tree) of two and bag fraction (proportion of data used when selecting optimal tree number) of 0.75. Variable importance was assessed by relative influence, the proportion of times that a variable is used to split a tree, weighted by the improvement in model fit by adding that tree.

3. Results

3.1. Environmental trends

The first three factors of the PCA accounted for 66% of the variance in environmental variables (**Table 1**). The first axis corresponds to a nutrient gradient, from clear water, nutrient-rich, lakes to coloured water, low nutrient, lakes, and the second to a lake area/elevation gradient, lowland lakes being larger, with higher N concentrations and greater catchment population densities than upland lakes. There is a strong spatial component to this variation in environmental variables, which potentially reflects anthropogenic activity. TP, pH, TON, SiO_2, PopDen and MEI were greater in the east (and south), while colour and lake size were greater in the west (**Table 1**). Chloride was greater in lakes close to the coast.

3.2. Biomass- environment relations

TP had the largest relative influence value for phytoplankton and fish biomasses (**Table 2**). In contrast, PopDen, MEI and elevation were most influential for macrophytes, while water colour and SiO_2 were influential for zooplankton.

Some within-BQE compositional differences were correlated with environmental variables. Most correlations (19/28; 68%) between phytoplankton phyla biomasses were significant and positive, and phyla biomasses increased with TP ($F_{1,346}$=54.22, P<0.001). However, there was little evidence of a systematic change in phyla dominance with enrichment; % biomass-TP slopes did not differ significantly across phyla ($F_{7,384}$=1.90, P>0.05). *Daphnia* spp. biomass increased with NH_4N and PopDen, whereas the biomass of the other herbivorous cladocerans increased with water colour and declined with pH, MEI, PopDen and SiO_2 (**Table S2**). Discriminant analysis showed that pH alone correctly identified 76% of lakes with or without cyprinids (mean pH 7.23±0.14, 6.37±0.15, respectively). Fish biomass in cyprinid-free lakes was predicted by area (r=0.55, P<0.01), whereas MEI and NH_4N predicted fish biomass in lakes with cyprinids (r=0.84, P<0.001). The biomass of the potential bioturbators, roach, bream and tench (*Tinca tinca*) comprised 13.4% (range 0-84%) of total fish biomass, and increased with TP (**Table S2**).

3.4. EQR scores

The environmental variables examined had predominantly negative effects on EQR scores (sum of negative relative influence values varied from 74 to 98% across BQEs). TP had the largest relative influence value on EQR scores for phytoplankton and macrophytes, whereas MEI and PopDen were most influential for fish EQRs (**Table 2**). EQR relative influence values for phytoplankton and macrophytes were correlated, but neither was correlated with that for fish (r = 0.73, -0.25, 0.08, respectively). Low EQR scores were associated with large biomasses for phytoplankton and fish but not for macrophytes (**Table 3**). EQR scores also varied spatially (**Table 3**, **Fig. 1**), suggesting that the spatial variation is at least partly due to anthropogenic impact.

EQR scores for phytoplankton, macrophytes and fish were positively, albeit weakly, correlated across lakes (r=0.36, 0.45, 0.53, all P<0.01). Eighty-two % of variation in mean EQR, the average of the three EQR scores, was predicted by cyprinid presence/absence and five environmental variables, namely TP, Chla:TP ratio, PopDen, water colour and elevation (**Table 4**; mean EQR cyprinids present 0.501±0.026, n=24; cyprinids absent 0.752±0.028, n=22). This model did not show collinearity amongst predictors (all variance inflation factors <1.9). Observed mean EQR was strongly correlated with predicted scores (**Fig. 2**) and, for example, 65% of lakes were correctly assigned to 0.2, 0.4, 0.6 and 0.8 EQR boundary classes and this rose to 87% when including values within 0.02 of a boundary class.

Mean EQR was consistently greater in cyprinid-free lakes for all environmental variables. For example, cyprinid-free lakes had higher mean EQR scores at low TP concentrations, but this difference declined in eutrophic lakes (**Fig. 3a**; ANOVA TP^2 $F_{1,42}$=23.51, P<0.001, TP-cyprinid occurrence interaction $F_{1,42}$=10.19, P<0.01, TP^2-cyprinid occurrence interaction $F_{1,42}$=5.66, P<0.05, R^2=0.68). Similarly, mean EQR scores declined more steeply with Chla and were lower in lakes with cyprinids, except in the most Chla-rich lakes (**Fig. 3b**; for Chla <10µg L^{-1} slopes $F_{1,31}$=46.14, P<0.001, R^2=0.81; mean EQR $F_{1,32}$=35.70, P<0.001). Note that whereas mean EQR declined with increasing TP it appeared to increase with Chla in eutrophic (TP>40 µg L^{-1}, Chla >10µg L^{-1}) lakes with cyprinids, suggesting a change in phytoplankton composition in these lakes.

3.3. Trophic structure

Across lakes, there were no correlations between phytoplankton and macrophyte biomasses (r=-0.17, P>0.2) or between adjacent trophic level biomasses. The Macro:Phyto and Zoo:Phyto ratios were greater in acidic, low nutrient lakes (**Table 5**) but the Zoo:Phyto ratio–TP slope (-0.81±0.24) was not different from -1.0, consistent with no change in zooplankton grazing impact across the nutrient gradient. However, the Zoo:Phyto biomass ratio declined with increasing cyprinid biomass (r=-0.36, P<0.05) but this occurred because phytoplankton biomass increased and not because zooplankton biomass decreased (r=0.35, P<0.05, r=-0.04, P>0.7).

Phytoplankton composition changed with eutrophication, with TP-tolerant taxa more abundant in cyprinid lakes (**Fig. S1a**). Both the upper and lower quartile TP optima were significantly greater in lakes with cyprinids (**Fig. S1b**) but the sensitive taxa (lower quartile) optima varied with Chla only in cyprinid-free lakes.

Total zooplankton biomass and mean size were not correlated with any phytoplankton or fish biomass measures (**Table 6**). Across lakes, copepods comprised 43% (range <1-99%) of zooplankton biomass and calanoid biomass declined with increasing cyprinid biomass. Neither cladoceran and copepod biomasses nor *Daphnia* and other cladoceran biomasses were correlated (r=0.25, P>0.05; r=0.19, P>0.2, respectively). However, *Daphnia* spp. biomass, and the % contribution of *Daphnia* to total cladoceran biomass, increased with fish biomass while that of the other grazer cladocerans declined. The biomass and mean size of small cladocerans (mean species mass <3.0 µg DW), which comprised an average of 13% (range 0-86%) of grazer cladocerans, declined as cyprinid biomass increased but no fish effect was detected on *Daphnia* or copepod sizes. Only two of 23 zooplankton species differed in mean size with cyprinid presence/absence and these small species (*Bosmina longirostris*, *Diaphanosoma*) were larger in cyprinid free lakes i.e. at the species level there is no evidence for a fish size-selective predation effect.

Cyprinid, like total fish, biomass increased with TP (**Table S2**). Total fish biomass was greater in lakes with cyprinids than in cyprinid-free lakes (ANOVA $F_{1.44}$=24.02; biomass 92.06±8.26, 33.51±8.63 g m^{-1} net respectively). Trout (*Salmo trutta*) were found in unproductive lakes (Chla $F_{1.44}$=26.52, P<0.001) and rarely with cyprinids (χ^2 = 19.60, P<0.001). Pike (*Esox lucius*) biomass increased with that of cyprinids, but trout biomass declined (r=0.59, P<0.001, -0.56, P=0.01). Pike biomass as a % of total fish biomass was greater in more productive lakes (**Table 5**; Chla r=0.41, P<0.01). Zebra mussels occurred in seven lakes and these lakes had significantly greater cyprinid biomass than mussel-free lakes ($F_{1,44}$=24.79; 75.00±11.37, n=7; 13.55±4.82, n=39 g m^{-1} net, respectively); discriminant analysis also showed that Chla was lower, zooplankton biomass and pH greater in lakes with zebra mussels.

4. Discussion

This study, in general, confirms previously established taxon-nutrient relationships (see section 4.1) but is novel in examining the importance of other key environmental variables, ecological interactions, and species introductions, when assessing lake status. Across the 50 study lakes, TP had a strong effect on biomasses and EQRs for most taxa, but other environmental variables were also important; for example, TP, pH, NH_4N, SiO_2 and MEI all had correlation coefficients >0.5 for at least some BQEs. However, only one of six biomass - environment correlation coefficients (for phyto--zooplankton), and none of the BRT relative influence - environment correlation coefficients, were significantly correlated across BQEs, i.e., there was little concordance in BQE responses to environmental variables. We show that trophic interactions affected taxon biomasses and EQRs, with mean EQR being up to 0.2 less in cyprinid lakes. Surprisingly, the majority of work to-date on the implementation of WFD procedures has focussed on single BQEs (Lyche-Solheim et al., 2013), whereas Jeppesen and colleagues (for example, Jeppesen et al., 2000; 2003) have taken a more integrated view of lake condition along TP gradients, but did not assess EQR status.

4.1 Environmental variables and taxon abundance and composition

There is a broad agreement between the effect of environmental variables on taxon composition in this study and previous findings. Cyanophytes, chlorophytes and diatoms comprised 83% of phytoplankton biomass, but, we found little evidence of a systematic change in phyla dominance across the enrichment gradient. Järvinen et al. (2012) showed that pristine northern European lakes tend to be dominated by chrysophytes, with chlorophytes also being important in clearwater lakes and dinophytes in humic lakes, while cyanophytes increased with declining lake status.

Macrophytes in our study lakes, which tended to be to the west and south of those examined by Heegaard et al. (2001), were most abundant at low elevations in high MEI catchments. Heegaard et al. (2001) noted a strong altitudinal influence on macrophyte composition in Northern Ireland, with nutrient-rich lakes being restricted to lowland areas. In our lakes, the macrophyte:phytoplankton ratio was greater in oligotrophic lakes, consistent with a negative interaction between these groups driven by changes in light regime and nutrient supply (Scheffer & van Nes, 2007).

Jeppesen et al. (2003) showed that the % *Daphnia* increased in shallow lakes up to 150 $\mu g\ L^{-1}$ TP: in our lakes % *Daphnia* did not change with TP but did increase with Chla. Our fish composition results are consistent with previous findings (Persson et al., 1991; Olin et al., 2002), with salmonids characteristic of oligotrophic lakes and cyprinids of eutrophic waters.

4.2 Determining lake status

We did not sample dissolved organic matter, bacteria, small zooplankters, benthic algae or invertebrates, all of which can be important to understanding ecosystem structure and function (Arndt, 1993; Nürnberg & Shaw, 1999; Jones & Sayer, 2003). The extent to which these biological elements are concordant with those required by the WFD needs to be established to determine the optimal number of variables necessary for adequate monitoring of overall lake quality (Moss, 2007).

The best way to combine EQRs across BQEs and determine overall lake status is not straightforward (Caroni et al., 2013). However, averaging is close to the spirit of the WFD as an "expression of the quality of the structure and functioning of aquatic ecosystems" because mean EQR integrates quality ratios, whereas application of the current 'one-out, all-out' principle can result in a lake's status being determined by just a single BQE. The mean EQR equation had a lower standard error of estimate than predictive EQR regressions for macrophytes and fish and performed as well as the phytoplankton EQR regressions i.e. did not increase predictive variance. In our lakes, mean EQR was predicted by cyprinid presence/absence, TP, Chla:TP ratio, PopDen, water colour and elevation, with the first three variables having the strongest effects. While the last two variables are clearly not direct indicators of anthropogenic impact they are weakly correlated with PopDen (**Table S1b**). The Chla:TP ratio was included as a potential predictor because Chla cell concentrations vary considerably across taxa (Reynolds, 2006) and because Chla-nutrient relationships vary with lake type (Phillips et al., 2008).

The mean EQR-environmental variable relationship accounted for 82% of the variance in our lakes but its predictive utility needs to be tested by comparing observed and predicted values for other lakes within this ecoregion. Lyche-Solheim et al. (2013) compared many of the metrics used when assessing BQE responses but did not include cross-taxon comparisons. Although the need, central to the WFD, for an integrated approach when assessing lake status is recognised (Moss, 2008; Kelly et al., 2016; Voulvoulis et al., 2017), the only other cross-taxon analysis of which we aware is that of Free et al. (2016). They found that TP explained 84% of the variation in the mean of EQRs for phytoplankton, phytobenthos and macrophytes in Ireland: the contribution of other environmental variables was not examined. Phytoplankton, macrophyte and benthic macroinvertebrate EQRs declined with increasing TP for 32 large lakes in Finland but there was no trend for fish (Rask et al., 2011) and a mean EQR was not calculated.

Many of the WFD assessment methods were established using BQE responses along a TP gradient, so strong TP responses are to be expected. However, BQEs respond to different parts of the ecosystem (pelagic, benthic) at different time scales (rapid for phytoplankton, slow for macrophytes and fish) so some differences in response are likely. In our study, TP was the most influential predictor for phytoplankton and fish biomasses but not for macrophytes or zooplankton. The different macrophyte response may be due to rooted species obtaining their nutrients from the sediment rather than from the water column. Free et al. (2016) did observe similar macrophyte and phytoplankton responses to TP using the same macrophyte index, but a different phytoplankton one to this study. Ireland has an impoverished fish fauna (Kelly et al., 2012) and in other ecoregions with more fish species fish effects might be less marked.

4.3 The influence of trophic interactions

While the importance of nutrients, particularly TP, in predicting Chla (for example, Phillips et al., 2008) suggests that bottom-up processes are the main determinants of lake status, there is also strong evidence for top-down influences on ecosystem structure in many lakes (for example, Jeppesen et al., 2003; Jones & Sayer, 2003). Our data provide much evidence of bottom-up but little for top-down effects, a pattern found in cross-lake, correlational, studies such as this one (Leibold et al., 1997). For example, phytoplankton and fish biomasses, and the percentage of predatory fish, increased with TP. Further, contrary to a size-selective predation effect (Hall et al., 1976), the biomass of the larger, *Daphnia* spp., cladocerans increased with fish and cyprinid biomasses, while small cladocerans declined. Moreover, neither overall zooplankton size nor that of *Daphnia* varied with fish biomass. The Zoo:Phyto biomass ratio declined with increasing cyprinid biomass, a trend usually interpreted as due to increased zooplanktivory (Jeppesen et al., 2003), but in our lakes this was because of increasing phytoplankton, not declining zooplankton, biomass. The weak evidence for top-down effects in our study is probably not a consequence of missing other predators: few invertebrate zooplanktivores were recorded (*Chaoborus* occurred in only eight lakes) and no effects were found on the biomasses or mean sizes of the zooplankton components. Other cross-lake comparisons indicate that planktivorous fish do reduce zooplankton size, by size-selective predation on large cladocerans (references in Hall et al., 1976; Taylor & Carter, 1997; Jeppesen et al., 2003) but the strongest evidence for top-down control comes from within-lake studies (McQueen, Post & Mills, 1986; Leibold et al., 1997) which we did not conduct.

Cyprinids have important effects on lake ecosystems (Winfield & Townsend, 1991). Cyprinid zooplanktivory potentially increases phytoplankton biomass by reducing zooplankton grazing pressure and cyprinids can release sediment nutrients when foraging on benthic invertebrates (Vanni, 2002). In our study trophic structure and lake status did vary with cyprinid occurrence. However, the change outlined above in cladoceran sizes with increasing cyprinid biomass is contrary to the usual fish size-selective predation effect. Bioturbation by cyprinids releases nutrients to the water column, thereby affecting phytoplankton biomass and composition: the loss of the more TP sensitive taxa with increasing Chla supports this second hypothesis. TP increased with the biomass of sediment-feeding roach, bream and tench, consistent with a bioturbation effect. The negative correlations between cyprinid and trout occurrence, and pike and trout biomasses, might indicate competition and predation effects, but equally could result from different environmental preferences for these species. Cyprinids were introduced into Ireland (Champ, Kelly & King, 2009) so their effect, in part, reflects where they were introduced: the association of cyprinid biomass and zebra mussel occurrence also reflects introduction of the latter. The major effects that both taxa can have on ecosystem structure and function (Winfield & Townsend, 1991; MacIsaac, 1996; Salgado et al., 2018) strongly argues for recording whether or not such influential introduced species are present when assessing lake status.

While several metrics distinguish between native and introduced species (Kelly et al., 2012; Petriki, Lazaridou & Bobori, 2017) we demonstrate for the first time that fish have a quantifiable effect on EQRs. Mean EQR scores in lakes with cyprinids were lower than in cyprinid-free lakes below about 10 μg L^{-1} Chla and 40 μg L^{-1} TP, consistent with cyprinids contributing to eutrophication. Cyprinids in more productive lakes appeared to increase EQRs (**Fig. 3b**); however, the number of observations for these eutrophic lakes in our study is small. Increased EQRs could also occur from a change in phytoplankton composition due to increased zooplankton grazing on phytoplankton, and/or an increase in zooplanktivory. Phytoplankton composition did change with eutrophication and TP-tolerant taxa were more abundant in cyprinid lakes, largely because of the loss of the most TP-intolerant taxa (**Fig. S1**).

5. Conclusions

EQRs for phytoplankton, macrophytes and fish were positively correlated with each other and much of the variance in mean EQR was accounted for by six, easily measured variables: cyprinid occurrence, Chla, TP, water colour, population density and elevation. Introduced, cyprinid, fish reduced mean EQRs in oligotrophic lakes but this effect decreased in eutrophic lakes, probably from nutrients released from the sediment during benthic feeding. The limited concordance in the responses of BQEs to environmental variables, coupled with evidence showing a cyprinid eutrophication effect on EQRs when using multiple BQEs, strongly supports the importance of adopting a more integrated approach to the assessment of lake quality status. In the spirit of Moss (2007), it should be possible to develop simple, yet robust, relationships to easily assess lake ecological quality that can inform compliance with environmental management objectives by using multiple biotic elements.

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Table 1. PCA of environmental variables for the study lakes showing varimax rotated component loadings for factors with eigenvalues >1. Correlations between the environmental variables and West-East (*r*WE) and South-North (*r*SN) lake spatial position are also shown.

Variable	Factor 1	Factor 2	Factor 3	*r*WE	*r*SN
Morphoedaphic index (MEI)	**0.892*****	-0.007	0.109	**0.409*****	**-0.484*****
pH	**0.861*****	0.207	0.178	**0.457*****	**-0.373****
Silicate (SiO_2)	**0.790*****	0.295	0.074	**0.386****	**-0.308***
Total phosphorus (TP)	**0.759*****	0.042	-0.269	**0.389****	**-0.455*****
Population density	**0.542*****	**0.402****	**0.447*****	**0.279***	-0.135
Ammoniacal nitrogen (NH_4N)	**0.315***	**0.613*****	-0.263	0.057	-0.215
Total oxidised nitrogen (TON)	0.212	**0.493*****	0.230	**0.433****	-0.242
Chloride	0.047	-0.199	**0.820*****	0.252	**0.429****
Elevation	-0.107	**-0.383****	**-0.713*****	0.074	-0.153
Area	-0.211	**0.872*****	0.016	**-0.352***	0.093
Water colour	**-0.616*****	0.137	**-0.312***	**-0.560*****	0.102
% variance explained	32.8	17.1	15.7		

*** P<0.001, ** P<0.01, * P<0.05

Table 2. Boosted regression tree relative influence values (%) across taxa. Signs have been added to these values, to indicate whether biomass increases or decreases (-) with the environmental variable.

	Biomass				EQR			
	Phyto	Macro	Zoo	Fish	Phyto	Macro	Fish	Mean
TP	37.8	-5.8	5.2	20.2	-35.2	-56.7	-5.7	-31.5
pH	19.0	1.7	-3.8	10.4	-11.7	-4.2	-3.8	-12.2
Colour	-9.6	-2.9	-44.9	-3.0	5.6	-8.1	-1.0	-3.2
TON	2.2	-3.0	0.5	2.7	-4.4	1.2	-2.7	-3.6
NH_4N	-3.1	1.1	0.8	18.1	-2.7	-1.4	-2.2	-2.5
Cl	5.5	3.3	2.6	-2.8	-9.3	1.3	3.1	-4.5
SiO_2	-6.6	-0.7	-27.6	13.5	8.4	-5.3	-1.0	1.8
Area	2.0	-3.0	-3.4	10.2	2.4	-2.1	2.6	4.9
Elevation	6.2	-18.3	3.7	-5.1	-11.0	-3.5	-4.0	-6.4
Population density	-4.6	33.8	6.8	2.0	7.7	-7.5	-34.9	-13.4
MEI	3.4	26.5	0.8	12.0	1.6	-8.7	-39.1	-16.1
Pseudo R^2 (%)	88	32	99	58	80	82	60	91

Table 3. Correlations between EQR scores and taxon biomass and spatial position.

	EQR Phyto	EQR Macro	EQR Fish	Mean EQR
Chla	**-0.825*** **	**-0.649*** **	**-0.434** **	**-0.767*** **
Phytoplankton	**-0.819*** **	**-0.602*** **	**-0.322* **	**-0.688*** **
Cyanophytes	**-0.699*** **	**-0.514*** **	**-0.279* **	**-0.589*** **
Macrophytes	**0.362** **	0.087	-0.231	0.038
Zooplankton	-0.142	-0.152	-0.028	-0.121
Fish	-0.153	**-0.414** **	**-0.648*** **	**-0.557*** **
WE position	**-0.651*** **	**-0.360** **	**-0.418** **	**-0.593*** **
SN position	**0.353* **	**0.545*** **	**0.458*** **	**0.569*** **

*** *P*<0.001, ** *P*<0.01, * *P*<0.05

699 **Table 4.** Multiple linear regression environmental predictors of mean EQR.

700

	Regression coefficient	Standardised coefficient	*t*
Intercept	1.535±0.129		11.88***
Cyprinid presence(1)/absence(0)	-0.141±0.028	-0.395	4.96***
TP	-0.326±0.051	-0.588	6.44***
Chla:TP ratio	-0.233±0.046	-0.408	5.04***
PopDen	-0.043±0.016	-0.222	2.74**
Water colour	-0.151±0.055	-0.232	2.74**
Elevation	-0.008±0.004	-0.155	2.04*

701 *** P<0.001, ** P<0.01, * P<0.05

Table 5. Correlations between trophic level biomass ratios, % piscivorous fish biomass and the environmental variables.

	Phyto:TP	Macro:Phyto	Zoo:Phyto	Fish:Zoo	% piscivorous fish
TP	0.115	**-0.510*** **	**-0.436** **	0.155	**0.390** **
pH	0.223	**-0.315***	**-0.481*** **	**0.403** **	**0.379** **
Colour	**-0.366** **	0.269	0.270	0.041	**-0.335***
TON	0.150	-0.200	-0.204	0.284	0.193
NH_4N	-0.181	0.048	0.159	0.219	0.146
Cl	0.192	0.052	-0.056	-0.207	0.163
SiO_2	0.057	-0.131	-0.240	**0.353***	**0.335***
Area	-0.253	0.169	**0.286***	0.097	-0.087
Elevation	0.127	-0.258	-0.077	-0.267	0.047
Population density	-0.134	0.131	0.025	0.168	**0.305***
MEI	0.172	-0.179	**-0.379** **	**0.325***	**0.291***

*** $P<0.001$, ** $P<0.01$, * $P<0.05$

Table 6. Correlations between zooplankton biomass and mean body mass and Chla, phytoplankton and cyanophyte, total fish and cyprinid biomasses.

	Chla	Phytoplankton	Cyanophyte	Fish	Cyprinid
Biomass					
All species	0.202	0.256	0.270	0.122	-0.042
Daphnia	0.153	0.166	0.238	**0.457***	0.214
Other grazer cladocerans†	-0.275	-0.174	-0.270	**-0.337***	**-0.420***
Predatory cladocerans	-0.159	0.115	-0.002	-0.020	-0.137
Small cladocerans‡	-0.202	-0.090	-0.196	-0.281	**-0.418***
Cyclopoid copepods	0.124	0.059	0.088	0.224	0.092
Calanoid copepods	-0.147	-0.115	0.060	-0.267	**-0.410***
% *Daphnia*	0.261	0.217	0.188	**0.440***	**0.353***
Mean mass					
All species	0.050	0.080	0.047	0.067	0.091
Daphnia	-0.050	0.002	0.078	-0.007	0.043
Other grazer cladocerans†	**-0.341***	**-0.295***	**-0.308***	-0.120	-0.293
Predatory cladocerans	-0.010	0.103	0.100	-0.288	-0.312
Small cladocerans‡	-0.253	-0.193	-0.250	0.082	**-0.342***
Cyclopoid copepods	-0.064	-0.072	0.034	0.053	0.040
Calanoid copepods	0.147	0.196	-0.002	-0.095	-0.112

*** $P<0.001$, ** $P<0.01$, *$P\leq0.05$

† Other grazer cladocerans: *Bosmina*, *Ceriodaphnia*, *Diaphanosoma*, *Eurycercus*, *Holopedium*, *Sida*

‡ Small cladocerans: *Bosmina*, *Ceriodaphnia*, *Diaphanosoma*

Figures

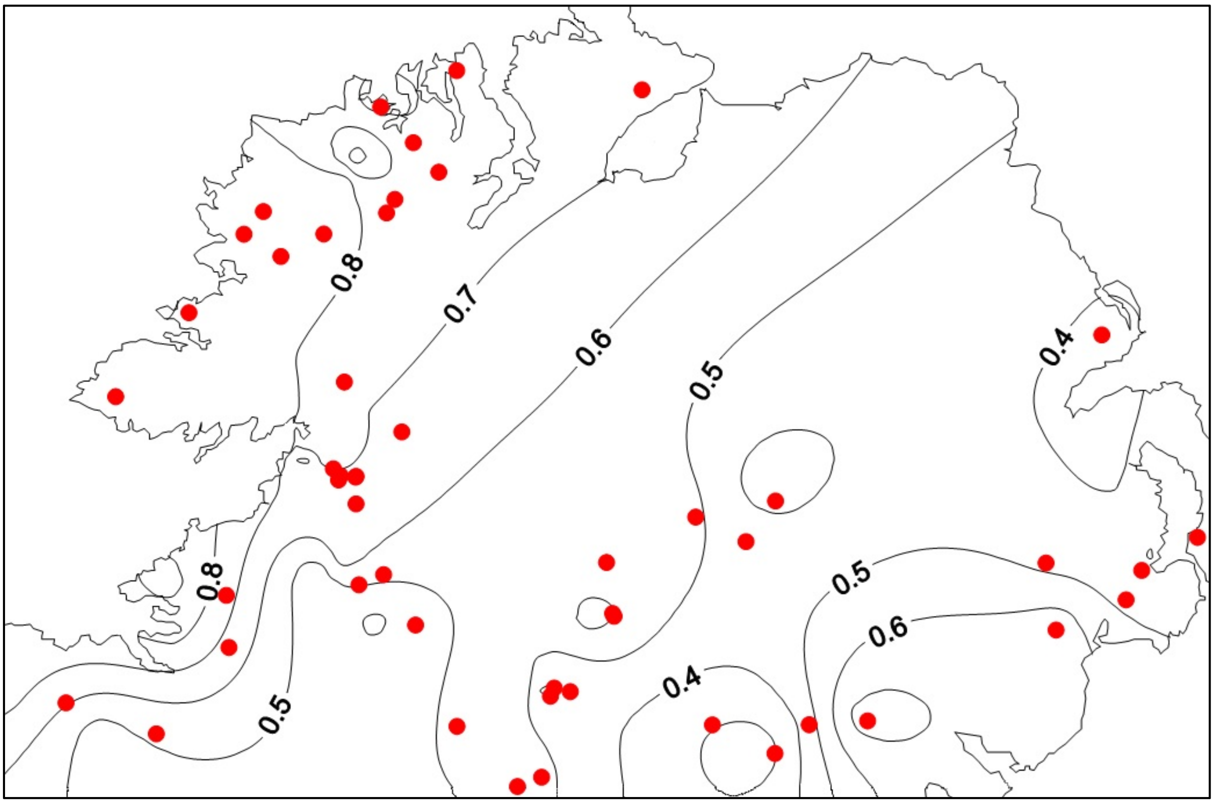

Fig. 1. Lake locations and trends in mean ecological quality ratio (EQR) scores. Contours were drawn using Surfer® 8 (Golden Software, LLC).

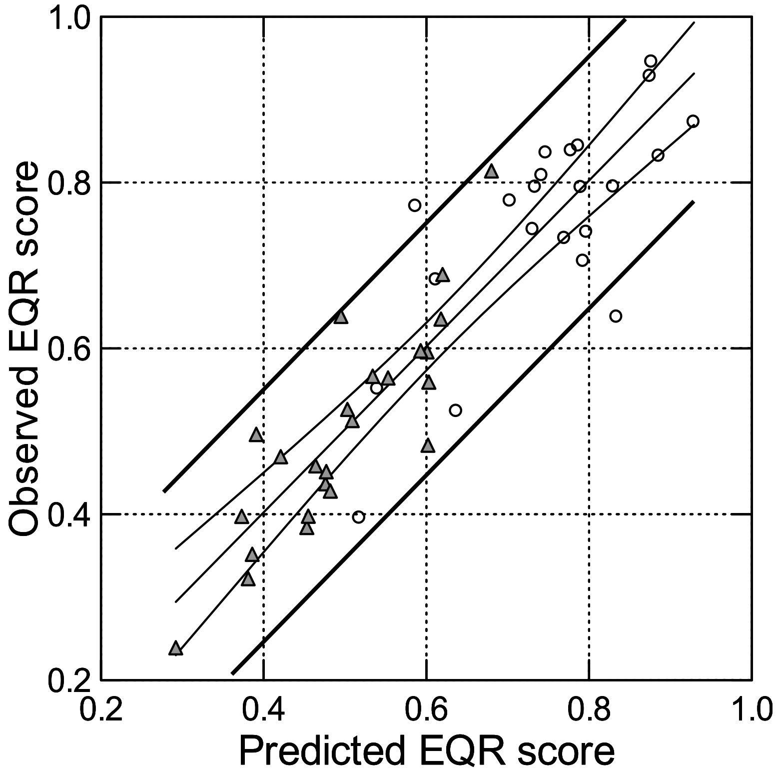

Fig. 2. Observed mean lake ecological quality ratio (EQR) as a function of predicted mean EQR in cyprinid (triangles) and cyprinid-free (circles) lakes. The 95% confidence and prediction limits are shown.

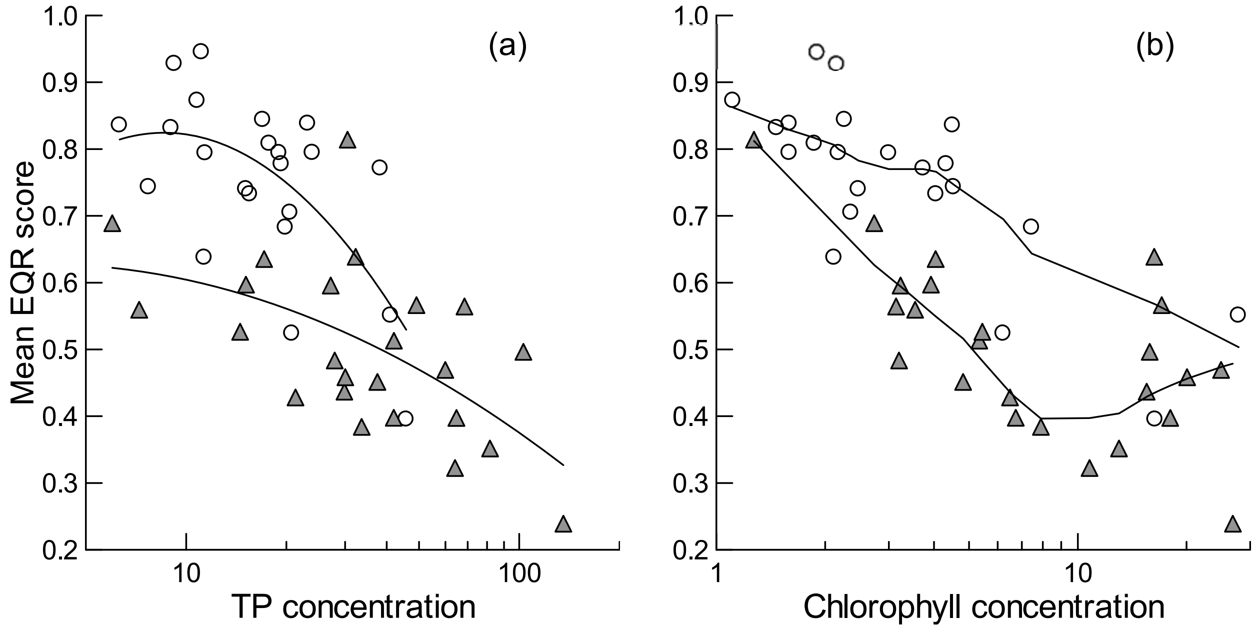

Fig. 3. Mean ecological quality ratio (EQR) scores as a function of (a) total phosphorus (TP) (µg L^{-1}) and (b) Chla concentrations in lakes with (triangles) and without (circles) cyprinid fish. Lowess smoothed lines (tension 0.5) are shown in (b).

Supplementary material

Table S1. (a) Descriptive statistics for the environmental variables, BQEs and EQRs and (b) Pearson correlation matrix between, the environmental variables.

(a)

	Median	Minimum	Maximum
Chla (µg L^{-1})	4.6	0.8	46.3
TP (µg L^{-1})	22.2	5.6	149.6
pH	7.08	5.10	8.53
Colour (mg L^{-1} Pt)	52.1	10.6	195.9
TON (mg L^{-1})	0.049	0.016	0.498
NH_4N (µg L^{-1})	10.96	0.54	48.75
Chloride (mg L^{-1})	14.76	5.75	44.77
SiO_2 (mg L^{-1})	0.84	0.08	7.19
Area (ha)	39.3	4.4	1399.6
Elevation (masl)	73	5	280
Population density (individuals ha^{-1})	0.142	0.002	3.690
MEI (mg L^{-1} m^{-1})	0.16	0.01	3.93
Mean depth (m)	4.0	1.0	11.9
Secchi depth (m)	1.8	0.9	4.4
N:P ratio (mass)	2.9	0.2	21.5
% agricultural land	36	0	100
Phytoplankton biomass (mm^3 L^{-1})	0.56	0.05	12.59
Cyanophyte biomass (mm^3 L^{-1})	0.11	0.01	3.78
Zooplankton biomass (µg DW L^{-1})	77.0	10.9	654.5
Fish biomass (g m^{-1} net)	47.6	2.3	224.8
Phytoplankton species	29	13	42
Macrophyte species	14	3	40
Zooplankton species	8	3	11
Fish species	4	1	7
Phytoplankton EQR	0.83	0.43	1.18
Macrophytes EQR	0.67	0.21	0.96
Fish EQR	0.39	0.01	1.00

(b)

	TP	pH	Colour	TON	NH_4N	Chloride	SiO_2	Area	Elevation	PopDen	MEI	Alkal	Mean Z	%agriculture
TP	1													
pH	0.600	1												
Colour	-0.243	-0.509	1											
TON	0.105	0.239	-0.137	1										
NH_4N	0.297	0.400	-0.065	0.112	1									
Chloride	-0.061	0.159	-0.250	0.040	-0.111	1								
SiO_2	0.435	0.692	-0.525	0.369	0.323	-0.018	1							
Area	-0.073	0.008	0.123	0.247	0.355	-0.157	0.091	1						
Elevation	0.053	-0.381	0.129	-0.209	-0.091	-0.382	-0.207	-0.276	1					
PopDen	0.331	0.512	-0.333	0.365	0.233	0.303	0.539	0.248	-0.392	1				
MEI	0.601	0.773	-0.429	0.204	0.172	0.051	0.670	-0.208	-0.245	0.564	1			
Alkalinity	0.558	0.854	-0.567	0.358	0.227	0.065	0.768	-0.040	-0.350	0.627	0.908	1		
Mean Z	-0.242	-0.029	-0.200	0.277	0.044	0.004	0.072	0.342	-0.136	-0.022	-0.437	-0.032	1	
% agriculture	0.550	0.551	-0.289	0.397	0.130	0.002	0.429	0.014	-0.080	0.441	0.541	0.591	-0.009	1

Underlined coefficients *P*<0.05

Table S2. Correlations (Pearson *r*) between phytoplankton, macrophytes, zooplankton and fish biomasses and the environmental variables.

	Phytoplankton				Macrophytes	Zooplankton				Fish			
	Chla	Total	Cyanophytes	Other phytoplankton		Total	*Daphnia*	Other Cladocera	Copepods	Total	Cyprinid	% predatory	% bioturbators
TP	**0.697*** **	**0.642*** **	**0.449*** **	**0.630*** **	-0.040	**0.328***	0.276	-0.129	0.018	**0.478*** **	**0.607*** **	**0.390** **	**0.617*** **
pH	**0.617*** **	**0.504** **	**0.396** **	**0.490*** **	0.139	0.019	0.131	**-0.535*** **	-0.106	**0.507*** **	**0.589*** **	**0.379** **	**0.546*** **
Colour	**-0.492*** **	**-0.417** **	**-0.352***	**-0.398** **	-0.133	-0.236	-0.161	**0.380***	**-0.280***	-0.219	-0.235	**-0.335***	-0.255
TON	0.191	0.174	0.148	0.158	-0.103	-0.058	0.092	-0.286	-0.059	**0.330***	**0.288***	0.193	0.197
NH_4N	0.076	0.024	0.094	0.039	0.150	**0.315***	**0.449** **	-0.136	0.186	**0.546*** **	**0.332***	0.146	**0.359** **
Chloride	0.227	0.115	-0.034	0.211	0.244	0.096	0.006	-0.015	-0.042	-0.152	-0.165	0.163	-0.243
SiO_2	**0.425** **	0.284	0.271	0.273	0.168	0.064	0.176	**-0.346***	0.001	**0.525*** **	**0.500*** **	**0.335***	**0.508*** **
Area	**-0.388** **	-0.236	-0.089	**-0.286***	-0.007	0.096	0.145	0.156	0.151	0.245	0.001	-0.087	0.099
Elevation	0.128	0.127	0.210	**-0.459*** **	**-0.311***	0.081	-0.032	0.240	0.118	-0.242	-0.097	0.047	-0.067
PopDens	0.238	0.079	0.018	0.100	**0.359***	0.176	**0.306***	**-0.384** **	0.020	**0.394** **	**0.334***	**0.305***	**0.344***
MEI	**0.615*** **	**0.465*** **	**0.312***	**0.452*** **	**0.352***	0.129	0.286	**-0.524*** **	-0.175	**0.502*** **	**0.631*** **	**0.291***	**0.601*** **
% agriculture	**0.504*** **	**0.517*** **	**0.296***	**0.508*** **	-0.055	0.009	-0.009	**-0.417** **	-0.099	**0.380** **	**0.396** **	**0.421** **	**0.386** **

*** *P*<0.001, ** *P*<0.01, * *P*<0.05

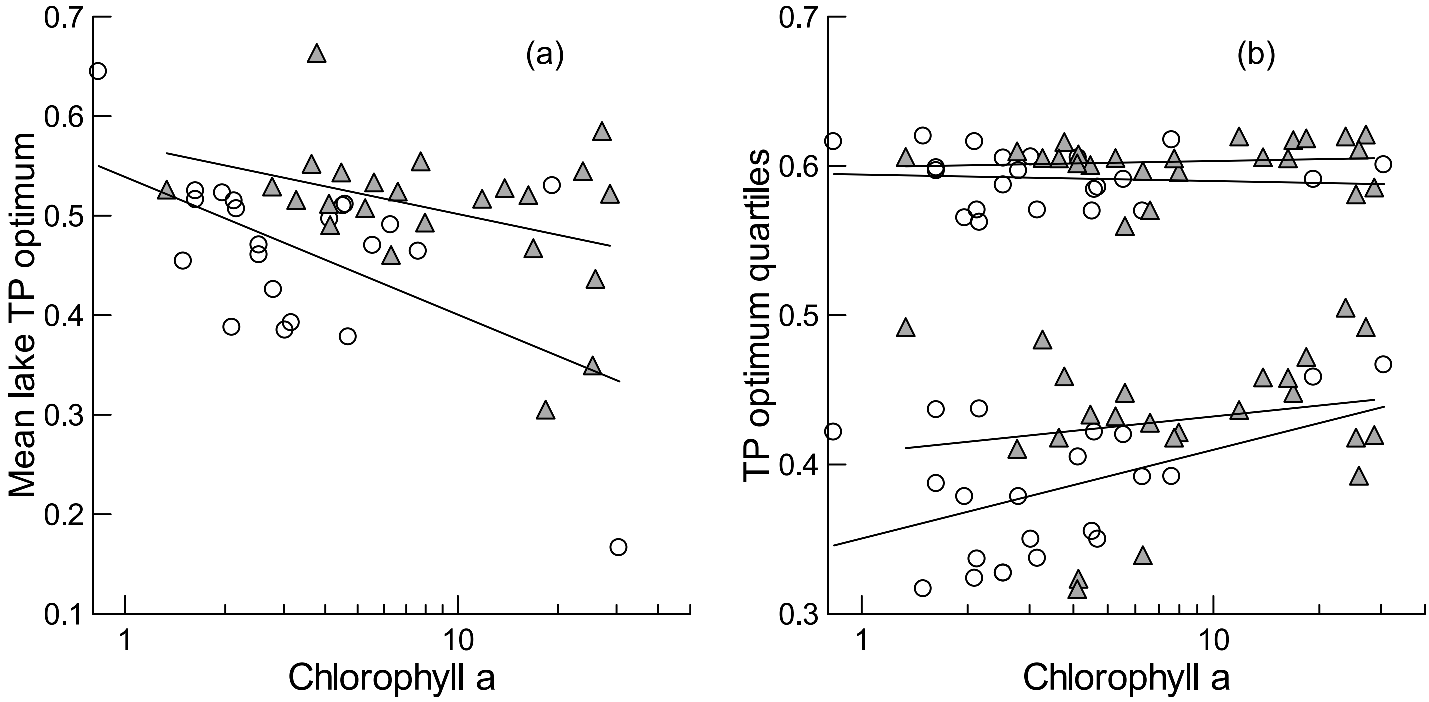

Fig. S1. (a) Mean lake TP optima and (b) upper and lower taxon TP optima quartiles as functions of chlorophyll *a* concentration in cyprinid (triangles) and cyprinid-free (circles) lakes. In (a) slopes were not significantly different (slopes $F_{1,42}$=3.00, P=0.09, common slope -0.120±0.033, P<0.01) but mean TP optima were significantly greater when cyprinids were present (intercepts $F_{1,43}$=11.09, P<0.01). In (b) both upper and lower quartiles differ in intercept ($F_{1,44}$=4.86, P<0.05; $F_{1,44}$=11.38, P<0.01 respectively) but only the lower (TP intolerant) quartile for cyprinid-free lakes varied with Chla (b=0.059±0.026, P<0.05).