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Eutrophication impacts littoral biota in Lake Ohrid while water phosphorus concentrations are low

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Running title

Eutrophication in Lake Ohrid

Keywords

Functional group, biomass, macrophytes, diatoms, macroinvertebrates, benthic algae, Cladophora, feeding type, Water Framework Directive, metric

Abstract

Eutrophication has traditionally been measured as increased phosphorus concentrations. In some lakes, however, such as transboundary Lake Ohrid situated between Macedonia and Albania, pelagic phosphorus concentrations are low, in spite of known sources of nutrient input. We assumed that littoral biota may be more responsive to phosphorus load than water chemistry, and studied nearshore water chemistry, macrophytes, diatoms and macroinvertebrates at 30 sites around the lake, analyzing functional groups as well as standard eutrophication metrics. We hypothesized that the incorporation of nutrients into benthic biomass will conceal correlations between water phosphorus concentrations and biological eutrophication metrics, but that analysis of functional groups in addition to eutrophication metrics may help draw a plausible picture of how phosphorus is transferred through the food web.

Water total phosphorus concentrations in the Lake Ohrid littoral were generally low, while all three analyzed organism groups indicated at least some degree of eutrophication. This shows that littoral biota are more sensitive indicators of nutrient input than hydrochemistry. The abundance of the benthic alga *Cladophora* sp. correlated positively with water total phosphorus concentrations, indicating that P-loading at local scales may be an important driver of *Cladophora* biomass. In contrast, none of the biotic metrics (macrophyte index, diatom index, and macroinvertebrate ICM) correlated with ambient water P-concentrations. We argue that this is not a sign of poorly working biological metrics, but a consequence of ecosystem processes in the lake littoral. Analysis of macrophyte and benthic algae abundance, and macroinvertebrate feeding types together with the biotic metrics suggests a meso- to slightly eutrophic littoral ecosystem where nutrient supply is incorporated into macrophyte and benthic algae biomass, and transferred through the food web from benthic algae to grazers, and from macrophytes to shredders and gatherers. Macroinvertebrate filter feeders correlate negatively with water total phosphorus concentrations, suggesting they remove phosphorus from the water. Our results indicate that the combined use of classical biological eutrophication metrics and functional groups may be a way to not only distinguish between oligotrophic and eutrophic ecosystems, but in addition give information as to whether or not nutrient input and nutrient removal in an ecosystem are balanced. This may eventually also give information about ecosystem functioning and ecosystem stability, and thus provide a basis for the development of “second generation” metrics for ecosystem assessment.

1. Introduction

Eutrophication has traditionally been measured as increased phosphorus concentrations (OECD, 1982). However, in large deep lakes with long residence times, pelagic phosphorus concentrations are often low, in spite of known sources of nutrient input (Matzinger et al. 2007). To detect eutrophication in spite of low pelagic phosphorus concentrations, nutrient inputs can be monitored. However, accurate assessment of these is costly and may still be unreliable (Moosmann et al., 2005).

The European Water Framework Directive (WFD), which was adopted in 2000, changed water management in member states of the European Union fundamentally by putting aquatic ecology rather than hydrochemistry at the base of management decisions (Moss, 2007; Hering et al., 2010). The objective of the WFD is for all surface water bodies to achieve “good ecological status”, which is defined not by chemical values, but by having a biota showing only slight alterations from that expected in the absence of human impacts. The use of biota for ecological status assessment requires standardized procedures for field work, sample processing and species identification. The collected biotic information on species composition and abundance occurring at a site is usually summarized in one or several biological metrics. Literally hundreds of such metrics have been developed in response to the WFD, all over Europe (Birk et al., 2012). The development of these metrics typically involved their correlation with a certain stressor or stressor combination, typically nutrient enrichment or organic pollution, via a dose-response curve (Birk et al., 2012). In many cases, total phosphorus concentration was the only pressure against which a metric was tested (e.g. Penning et al., 2008; Donohue et al., 2009; Schneider and Lindstrøm, 2011). While establishing a dose-response curve is an important part of metric quality assurance, the focus on strong correlations between stressors and metrics clearly also creates a conundrum: if ecological metrics are expected to closely correlate with a chemical stressor such as phosphorus concentration, what has been gained by putting ecology rather than chemistry at the base of management decisions? In practice, nutrient chemistry will continue to be monitored in ecosystems, so it is important to know what added value biological metrics give to water managers. Despite the achievements of the WFD, practical experience often shows that it still is difficult to convince water managers that altered biota might be a serious warning signal even if water phosphorus concentrations are low.

Transboundary Lake Ohrid, situated at the Balkan peninsula between Macedonia and Albania, is an example of a large, deep lake which despite known sources of nutrient input still has low pelagic phosphorus concentrations (Matzinger et al., 2007). In large lakes, however, the nearshore zone is often chemically and biologically different from the offshore zone (Makarewicz et al., 2012). In our study, we tried to detect if the phosphorus input to Lake Ohrid, in spite of low pelagic phosphorus concentrations, has led to altered biota and hydrochemistry in the lake littoral. We hypothesized that littoral biota may be more responsive to phosphorus load than water chemistry measurements, but also that the incorporation of nutrients into benthic biomass will conceal correlations between water phosphorus concentrations and biological eutrophication metrics. We analyzed the interrelationships between primary producers and macroinvertebrates based on functional groups as well as standard eutrophication metrics because we hypothesized that this may help us to draw a plausible picture of how phosphorus is transferred through the food web.

In principle, allochthonous phosphorus input to a lake may either be deposited on the sediment, or remains in the water where it is measured as water total phosphorus (TP) concentration (Hecky et al., 2004). Phosphorus may be taken up or ingested by different organism groups, and transferred through the food web. In our study, we focused on the current standard quality elements used for assessment in a lake littoral, and analyzed water chemistry, benthic diatoms, submerged macrophytes, and benthic invertebrates at 30 sites in Lake Ohrid.

A priori, the following hypotheses for how phosphorus is transferred through the benthic food web in a lake littoral, apply (Fig. 1): from the water, phosphorus may be taken up by macrophytes or benthic algae (we here disregard planktonic organisms because we have no such data in our study). Dying macrophyte biomass may be ingested by shredders (which degrade coarse organic material), and the resulting smaller particles may in turn be used by gatherers. Living benthic algae may be ingested by grazers, whose activity at the same time reduces benthic algae biomass. We hypothesize that increased phosphorus supply will primarily cause increased macrophyte and benthic algae growth. Species specific differences in plant growth may then lead to altered macrophyte and benthic algae assemblages. This means that we expect no direct relation between water phosphorus concentrations and macrophyte or benthic algae assemblages, since this relation goes via plant growth (Fig. 1). Macrophyte and benthic algae growth reduce water nutrient concentrations due to incorporation of nutrients into benthic biomass, but macrophytes can also take up sediment nutrients. Filter feeding invertebrates may profit from nutrient input but at the same time reduce water nutrient concentrations, while excretion of faeces by invertebrates may increase nutrient concentrations.

These hypotheses, summarized in Fig. 1, are a simplification. For example, only part of water TP is available to macrophytes and benthic algae. Likewise, we neglect planktonic organisms, since we have not analyzed these groups in our study. Thus, we do not attempt to construct budgets of P-turnover in Lake Ohrid. Our aim was for the first time to consistently analyze ecological and chemical status for the whole lake across political borders, and to detect interrelationships among chemical and biological parameters, the later including functional groups as well as standard eutrophication metrics, in an attempt to understand how phosphorus is transferred through the food web of the Lake Ohrid littoral, and how this is reflected in ecological status assessment.

2. Material and Methods

Lake Ohrid is situated in the Balkan peninsula at the Macedonian/Albanian border and is one of few ancient (2-5 million years), long-lived lakes in the world (Albrecht and Wilke, 2008). The lake is about 30 km long, 15 km wide, and covers an area of 360 km^2. Its maximum depth is 286 m. Although the general status of the lake is still assumed to be oligotrophic because average pelagic total phosphorus concentrations are around 4.6 µg/l, Lake Ohrid has been found to be impacted by eutrophication, with global warming expected to amplify the effects of increased nutrient input (Matzinger et al., 2007).

A total of 30 sampling sites were established in Lake Ohrid (Fig. 2), 10 of which lie in Albania and 20 in Macedonia. Half of the sites were sampled in 2009/2010, the other half in 2010/2011. Care was taken that the 15 sites investigated in each year were distributed evenly around the whole lake, in order to avoid any bias which might result from an uneven exposition of sites between sampling years. Overall values for the investigated chemical and biological parameters did not differ between the two sampling years, with the single exception of NO_3-N (average log(NO_3-N) = 1.63 for the sites investigated in 2009/2010, compared to 1.43 for the sites investigated in 2010/2011; $p = 0.009$). Water chemistry, diatoms, macrophytes and macroinvertebrates from each site were analyzed within one year.

Water chemistry

At each site, a water sample was collected at a few meters distance from the shoreline at approximately 0.5 m depth in January, May, July and October. Chemical parameters were measured according to the following standard procedures: dissolved oxygen (DO): ISO 5813:1983; nitrate (NO3): Standard Methods for the Examination of Water and Wastewater - 4500-NO3– B; total phosphorus (TP): ISO 6878:2004; biochemical oxygen demand after 5 days (bod): EN 1899-2:1998; pH: ISO 10523:1994; electrical conductivity (cond): ISO 7888:1985. Site specific averages of water chemical parameters were calculated for the samples taken in January, May, July, and October and were used for further analysis.

Diatoms

At each site, diatoms were collected in July (half of the sites in 2010, the other half in 2011) at a water depth of approximately 0.5 m from ten cobbles with diameters of roughly 10 cm. The upper side of each stone was brushed with a toothbrush, and the algae were transferred into a beaker. All samples were preserved with a few drops of formaldehyde to a final concentration of approximately 3.5%.

The samples were treated with concentrated HCl, concentrated H_2SO_4, and KNO_3 (Krammer and Lange-Bertalot, 1986-91). Permanent slides were prepared from the cleaned suspensions using Naphrax as a mountant, and approximately 400 undamaged valves of non-planktonic taxa were identified and counted using 1000 × magnification. The primary floras and identification guides used were Krammer and Lange-Bertalot (1986-91).

The Trophieindex (TI) was calculated according to Rott et al. (1999). The TI was chosen as a metric because it reflects eutrophication, and has been shown to be generally applicable in Europe both in rivers (Kelly et al., 2009a) and lakes (Poikane, 2013). The TI ranges from 1 to 4, with high values indicating nutrient-rich conditions.

Macrophytes

Submerged macrophytes, that is monocotyledonous and dicotyledonous plants, and charophytes were surveyed in July (half of the sites in 2010, the other half in 2011) in belt transects of approximately 10 m width - perpendicularly to the shoreline - from the upper littoral to the lower vegetation limit. In addition, the abundance of the macroscopic filamentous alga *Cladophora* sp. Kützing was noted, because it is the by far most conspicuous benthic algal taxon in Lake Ohrid. The taxon was tentatively identified as *Cladophora glomerata* (L.) Kütz., but since we did not check species identity at each site we will use the genus name hereafter. Primary floras and identification guides were Casper and Krausch (1980, 1981) and Krause (1997). Each transect was divided into depth zones: 0-2 m, 2-4 m, 4-10 m, and >10 m depth. Species occurrence was registered in each transect and each depth zone, and the abundance of each species was estimated according to a five degree scale (1 = very rare, 2 = infrequent, 3 = common, 4 = frequent, 5 = abundant, predominant). In order to ensure comparability with the hydrochemistry, diatom and macroinvertebrate results, only the macrophyte data from shallow water, i.e. depth zone 0-2 m, was used for further analysis.

As an approximation for the readily degradable biomass of macrophytes, we calculated the sum of the cubed abundances for non-charophyte macrophytes. We did so because non-charophytes are annual plants in Lake Ohrid, while most charophytes are perennial (own observations). In addition, are charophytes more slowly decomposed as e.g. *Potamogeton* species (Lan et al., 2012). The cubed abundance estimates were used as an approximation for the biomass of non-charophyte macrophytes since they better reflect relative values than the five-degree scale used for estimation in the field (Melzer, 1999). The macrophyte index (MI) was calculated as described in Melzer (1999), but with updated indicator values and class boundaries as described in Melzer and Schneider (2001). The macrophyte index was chosen as a metric because it reflects phosphorus supply, is applicable to calcareous lakes, and most macrophyte species observed in Lake Ohrid are included in the list of indicators. The MI ranges from 1 to 5, with high values indicating nutrient pollution.

Macroinvertebrates

At each site, macroinvertebrates were sampled in approximately 0.5 m water depth in late April/early May, thus covering the late-stage larval forms from the past year. We used the kick-and-sweep method with a standard D-shaped net with a metal frame holding a mesh bag of 400-µm size and sampled for 5 minutes (ISO:EN 27828:1994). Samples were preserved in 70% ethanol, and species were later identified using the following primary identification guides: Snegarova (1954), Sapkare (1966), Hubendick (1970), Brinkhurst and Jamieson (1978), Radoman (1983), Kerovec (1986), Sket and Šapkarev (1992), Bodon et al. (2001).

Data on relative abundance of feeding types were calculated by means of the computer programme ASTERICS (version 3.1.1), developed in the EU projects AQEM (www.aqem.de) and STAR (www.eu-star.at). We differentiated shredders (organisms that feed on coarse particulate organic material such as small sections of leaves), grazers (organisms that feed on periphyton that accumulates on larger structures such as stones), filter feeders (organisms that consume organic matter suspended in the water column), and gatherers (organisms that consume fine particulate organic matter found on the sediment). Since we intend to focus on the interrelationships between primary producers and macroinvertebrates we chose to not analyze invertebrate predators in this study.

The lake macroinvertebrate intercalibration metric for the Central-Baltic ecoregion (ICM) was calculated according to Pilotto et al. (2011). The ICM is a multimetric index including species composition and abundances as well as functional indicators. It was specifically developed for lakes, is applicable in large parts of Europe and is correlated to shoreline alterations and landuse in the lake surroundings, as well as lake total phosphorus concentrations. The ICM ranges from 0 to 1, with high values indicating undisturbed conditions.

Data treatment and statistics

Diatom and water chemistry samples were analyzed at all 30 sites. However, no macrophytes were present at one of the sites such that no macrophyte index could be calculated, and no macroinvertebrate samples were taken at three other sites. Thus, the complete dataset comprising all quality elements included 26 sites.

After exploratory analysis, data were log- or (log+1)-transformed where necessary to improve normality and homoscedasticity before calculating average values. Nevertheless, Spearman correlation was used to test for correlations among indices, functional groups and water chemical parameters, because we expected the correlations to be monotonic, but not necessarily linear. Because each analysis represented a separate hypothesis, there was no need to adjust α for multiple testing (Perneger, 1998). These tests were performed with STATISTICA 10.

To explore the structure in our data, we computed a NMDS on the square root transformed biological data (to reduce the contribution of the most abundant species to the dissimilarity). NMDS was used because it in contrast to other ordination methods also can handle non-linear responses. The NMDS was computed using the metaMDS function in R version 2.14.2 (R Development Core Team, 2012), extended with the “vegan” package 2.0-4 (Oksanen et al., 2012). Bray-Curtis was used as dissimilarity measure because it is less dominated by single large differences than many other dissimilarity measures, and it is generally assumed to be well suited for species abundance data (Quinn and Keough, 2002). Hydrochemistry vectors were fitted using the “envfit” command in vegan, a function that fits environmental vectors onto an ordination (Oksanen et al., 2012).

3. Results

The shallow littoral of Lake Ohrid had on average a pH above 8, and a conductivity slightly above 200 µS/cm (Table 1). Total phosphorus concentrations were mostly below 10 µg/l. Maximum values, however, were measured close to the inflow of the river Grasnica (50 µg/l), and close to the city of Pogradec (23 µg/l). NO_3-N-concentrations were around 40 mg/l, with a maximum of 124 mg/l close to the inflow of the river Grasnica. BOD5 concentrations were on average below 2 mg/l O_2, but with a maximum of 3.6 mg/l O_2 close to the city of Pogradec.

We registered a total of 28 macrophyte taxa in Lake Ohrid (in addition to *Cladophora* sp.), with an average number of 7 taxa per site (Table 1). In addition, we found 144 diatom taxa (on average 31 per site), and 65 macroinvertebrate taxa (on average 6 per site; see Appendix 1 for complete taxa lists). The most conspicuous taxon across all studied organism groups was *Cladophora* sp., which was both more frequent and more abundant than any other macrophyte or macroscopic benthic algae taxon in shallow water of Lake Ohrid (data not shown). The species was generally common in the shallow littoral of the whole lake (Fig. 2), and was very abundant at five sites around the lake. These sites were all located close to inflows or villages, but not all inflows or villages at Lake Ohrid gave rise to high *Cladophora* biomasses (Fig. 2).

The macrophyte index was on average 3.2, thus indicating mesotrophic to slightly eutrophic conditions in the lake littoral (see Melzer and Schneider (2001) for description of scale). Correspondingly, diatoms also indicated on average meso-eutrophic conditions (the average TI was 2.11; see Rott et al. (1999) for description of scale). The macroinvertebrate ICM was on average 0.22 and thus indicated generally “poor” conditions in the lake littoral (corresponding to major alterations in macroinvertebrate communities compared to undisturbed conditions; see Pilotto et al., 2011 for boundaries between status classes). Thus, while TP concentrations were generally low, three biotic metrics from three different organism groups indicated at least some degree of eutrophication in the lake littoral.

None of these three biotic metrics, however, correlated with measured water TP concentrations (Tab. 2). Instead, TP was significantly positively correlated with the abundance of *Cladophora* sp., and significantly negatively correlated with the relative abundance of macroinvertebrate filter feeders (Tab. 2). The abundance of *Cladophora* sp. was in turn positively correlated with the biomass of easily biodegradable macrophytes (estimated as the quantity of non-charophyte macrophytes), and negatively with the macroinvertebrate ICM, indicating that the higher the biomass of *Cladophora*, the worse was ecological status. Finally, the quantity of non-charophyte macrophytes was positively correlated with the relative abundance of macroinvertebrate shredders, and with the macrophyte index, indicating that an enhanced biomass of easily degradable macrophytes is a sign of enhanced trophic status (Tab. 2).

The results from the multivariate NMDS analysis generally supported the univariate Spearman rank correlations (Tab. 2), but refined the picture. The NMDS plot constructed from the biological data represented an acceptable solution (stress = 0.18). Nitrate, conductivity and TP co-varied, and the relative abundance of macroinvertebrate grazers was positively related to increased concentrations of these nutrients, while the abundance of filter feeders was negatively related to them (Fig. 3). The abundance of *Cladophora* sp. was positively related to both high nutrient and dissolved oxygen concentrations. The relative abundance of shredders was related to enhanced BOD concentrations. Macrophyte (MI) and diatom (TI) trophic indices were close to the center of the NMDS plot, indicating no close correlation to any of the fitted chemistry gradients.

4. Discussion

Total phosphorus concentrations in the Lake Ohrid littoral were on average 7.2 µg/l, and thus somewhat higher than average offshore concentrations, which are around 4.6 µg/l (Matzinger et al., 2007). Enhanced nearshore compared to offshore phosphorus concentrations are a common phenomenon in large lakes that are subject to enhanced nutrient loading (Makarewicz et al., 2012b). However, TP concentrations below 10 µg/l, such as we measured at most sites in Lake Ohrid, are usually considered to be consistent with oligotrophic conditions (OECD, 1982). In contrast, both the macrophyte and the trophic diatom index denote mostly meso- to slightly eutrophic conditions in the lake littoral, and the macroinvertebrate ICM indicates “poor” status. Thus, it might seem that chemical and biological assessment systems disagree with each other. Such a “discrepancy” seems also to arise from the absence of any correlation between these indices and water TP-concentration. After all, each of these indices was indeed calibrated on TP-concentrations (Melzer, 1999; Rott et al., 1999; Pilotto et al., 2011). We argue that this is neither a discrepancy nor a sign of “poorly working biological indices”, but a consequence of ecosystem processes in the lake littoral.

One might argue that the absence of a correlation between any of the three indices and water chemistry was due to the “wrong” indices being used, and that we should have tested others, which might work “better” in Lake Ohrid. However, notwithstanding existing minor differences, macrophyte indicator values of different eutrophication assessment systems across Europe correlate with each other (Schneider, 2007). Likewise, although there are differences in trophic scores of diatom taxa for different indices (Besse-Lototskaya et al., 2011), different diatom indices in Europe generally correlate with each other (Schneider et al., 2013a). Also, the macroinvertebrate ICM has been shown to correlate with most national assessment methods in Central Europe (Pilotto et al., 2011). Thus, there is no reason to expect a major difference in outcome if other indices had been used. Apart from that, we calculated indices which a priory were likely to be applicable in Lake Ohrid (see Material and Methods).

A large number of studies have been published in recent years, testing different metrics based on correlations with measured TP-concentrations (e.g. Timm and Moels, 2012; del Pozo et al., 2010; Penning et al., 2008). These studies are usually based on the underlying assumption that the metric having the closest correlation to measured TP-concentration is “best”, and consequently this is the one which is recommended for future monitoring of eutrophication. However, if it was crucial for ecological metrics to always correlate closely with a measured chemical variable such as phosphorus concentration, then little would have been gained by putting ecology rather than chemistry at the base of management decisions. The problem with the “correlation approach” is that it ignores the difference between “cause” and “effect”. On the one hand, enhanced P-concentrations cause enhanced plant growth leading to different plant assemblages which are expressed in a biological metric. But on the other hand, plant growth also reduces water P-concentrations to such an extent that correlations between plant assemblages and measured P-concentrations will be concealed.

Consequently, for a better understanding and assessment of eutrophication processes it is necessary to go beyond the simple search for metric-water chemistry correlations, and rather look into how nutrients might be taken up and turned over by different functional groups in the ecosystem. We do this by coding the strength of the pathways phosphorus might take in a lake littoral (that is, the a priory pathways which we graphically presented in Fig. 1) according to our results presented in Table 2: thin line weights represent pathways which are not supported by our data, intermediate line weights represent pathways which are supported but not statistically significant, and bold lines represent significant correlations (Table 2, Fig. 4). We use the macrophyte index as an approximation for the macrophyte assemblage (because the index is based on species composition and abundance), and the diatom trophic index as an expression for the benthic algae assemblage. We further assume that macrophyte growth is approximated by the quantity of non-charophyte macrophytes (because they are annual), while benthic algae growth is approximated by *Cladophora* abundance (because this species is annual and the by far most abundant benthic algae taxon in Lake Ohrid).

Altogether, the following picture of phosphorus turnover in the littoral of Lake Ohrid arises (Fig. 4): enhanced water TP caused enhanced *Cladophora* biomass, and partly also enhanced non-charophyte macrophyte biomass. This is consistent with data from Lake Ontario, where *Cladophora* growth rates were strongly P-limited (Higgins et al., 2012). At the same time, TP was reduced by filter feeders. Again, this is consistent with data from the North American Great Lakes, where filtering activity of benthic invertebrates diverted nutrients from the water column to the nearshore benthos (Hecky et al., 2004). Shredders and gatherers rather enhanced water TP concentrations, likely due to the excretion of faeces. Grazers were unrelated to water TP, probably because they generally were present in lower abundances than shredders and gatherers (Table 1). *Cladophora* abundance, and not TP, was related to the diatom index. While the absence of a correlation between diatom index and TP may be explained by the incorporation of phosphorus into plant and algal biomass and the according removal of P from the water column, is the relation between *Cladophora* abundance and the diatom index consistent with an earlier suggestion that *Cladophora* may exhibit a cascading effect on other benthic algae (Kelly et al., 2009b). *Cladophora* is a firmly attached taxon which forms a canopy, and thus may favor more mobile or facultative heterotrophic taxa instead of taxa which attach directly to the substratum (Kelly et al., 2009b), an effect which in turn may convey to the diatom index. Likewise, plant quantity of non-charophyte macrophytes (as an approximation for the biomass of annual macrophytes), and not TP, was related to the macrophyte index. Again, the absence of a correlation between indices and TP is not surprising, since water TP interacts with many functional groups, while both macrophyte and diatom index are more directly related to macrophyte and benthic algae growth than to TP (Fig. 4). Enhanced macrophyte and benthic algae growth usually is a first consequence of enhanced nutrient supply. Plant growth, together with macroinvertebrate filter feeders, reduces water TP-concentration by incorporation of nutrients into benthic biomass. This feedback can explain the seeming “paradox” of high plant and algal biomasses and meso-eutrophic biological metrics observed in the nutrient poor water of Lake Ohrid. Such a phenomenon, that is enhanced plant biomass in nutrient poor sites, has been described before (Schneider et al., 2013b) and may be a rather common first signal of eutrophication in ecosystems which still can buffer nutrient input.

Cladophora sp. was the most conspicuous taxon in the shallow littoral of Lake Ohrid, and enhanced biomass was associated with enhanced phosphorus concentrations. Similar results have been obtained by Higgins et al. (2012) in Lake Ontario, and they indicate that P-loading at local scales may be an important driver of *Cladophora* biomass. This biomass was, however, consumed by grazers, while macrophyte biomass was consumed by shredders, whose excretion products again were consumed by gatherers (Fig. 4). This indicates that the ecosystem turned over enhanced phosphorus input. Although not all of these relations were significant in a unidirectional analysis (Table 2), there was not a single disagreement to our hypothetical pathways outlined in Fig. 1. The absence of the “significance” criterion for some unidirectional analyses is not surprising, since there are several interactions among most of the biological and chemical parameters we analyzed in the lake littoral (Figs. 1 and 4).

In addition to the pathways outlined in Fig. 4, there was a positive correlation between *Cladophora* biomass, and the quantity of non-charophyte macrophytes. This is likely due to both being influenced by water TP. While the pathway from TP to *Cladophora* was significant, the pathway from TP to macrophytes was not. This is likely due to P-deposition on the sediment in the trophogenic zone of Lake Ohrid (Matzinger et al., 2007), from where it is available to macrophytes (Carignan and Kalff, 1980), such that macrophyte growth, in contrast to *Cladophora* growth, is influenced not only by water but also by sediment P. This further blurs the correlation between water TP and macrophyte growth. In addition, was *Cladophora* biomass significantly related to the macroinvertebrate ICM, likely because *Cladophora* is annual and builds large biomasses (Fig. 2), and decomposition of this biomass interacts with benthic macroinvertebrates and thus the ICM. A similar relationship emerged between non-charophyte macrophytes and ICM, only it was not significant in a unidirectional correlation analysis (Table 2).

In total, our results draw the picture of a meso- to slightly eutrophic littoral ecosystem where nutrient supply is incorporated into plant and algal biomass and transferred through the food web, from benthic algae to grazers, and from macrophytes to shredders and gatherers. Species composition and abundance of all investigated organism groups was impacted, but water nutrient concentrations were low, likely as a result of ecosystem processes. We wish to point out that the results summarized in Fig. 4 are based on correlations between chemistry, metrics and functional groups. Whether or not they represent causal relationships needs to be ascertained using an experimental approach. They do, however, represent a plausible hypothesis for how the littoral ecosystem of Lake Ohrid functions, and they provide an explanation for why biological metrics can indicate eutrophication while water phosphorus concentrations are low.

All three biological metrics indicate on average impacted, meso- to slightly eutrophic conditions in the Lake Ohrid littoral. However, indicator values of these metrics were not correlated with each other (Table 2). Yet this is not surprising, since diatoms exclusively take up nutrients from the water, while macrophytes additionally can use sediment nutrients (Carignan and Kalff, 1980). Differences between macrophyte and diatom indices can therefore be interpreted as being indicative for the bioavailable pool of nutrients in the sediment. Macroinvertebrates are indicative of both the nutrients present in the water (via filter feeders), as well as the nutrients incorporated into benthic algae (via grazers) and macrophyte biomass (via shredders and gatherers)(Fig. 4). This likely explains the absence of unidirectional correlations between macrophyte or diatom index and the macroinvertebrate ICM, although we cannot exclude that other stressors which we have not analyzed in our study might also have played a role. Again, this must not be interpreted as a sign of poorly designed indices, but as a consequence of different pathways of nutrient turnover in the ecosystem.

Nevertheless, have these indices been designed and tested based on correlations with water TP-concentrations (Melzer, 1999; Rott et al., 1999; Pilotto et al., 2011), so how can it be that a biological metric correlates with TP in some instances (like e.g. the datasets used for developing the indices), but not in others (like e.g. Lake Ohrid)? Water nutrient concentrations will correlate with biological metrics when the part of total P-input which remains in dissolved or particulate form in the water is higher than what is incorporated into benthic biomass and deposited on the sediment. In large datasets, such as are commonly used for developing biological metrics, it is likely that at least part of the sites will receive more nutrients than are absorbed by the ecosystem, and therefore a correlation between metric and water TP will emerge. In other words: in ecosystems where the cause (nutrient input) is higher than the effect (nutrient removal by the ecosystem), a correlation between metric and nutrient concentration will emerge. In contrast, in ecosystems where the effect balances the cause, there will be no correlation, and biological metrics will indicate eutrophication while water nutrient concentrations are still low.

The objective of the WFD is not to double-check chemistry with biology or vice versa, but to detect the degree to which the biota of an ecosystem is altered from that expected in the absence of human impact. Thus, for a meaningful use of biological metrics in ecosystem management, we need to take better account of the difference between causation and correlation: a biological metric can be considered useful when there is a causal relation between stressor and metric. However, this does not necessarily mean that this metric always must correlate with measured chemical field data, because measured chemical field data are the result of both cause and effect. Thus, given that the causal relationship between metric and stressor is well-established, differences between chemical and biological assessments at any one site should be indicative for ecosystem processes such as nutrient removal. This may be further analyzed by studying plant and animal functional groups. For our data on Lake Ohrid, this also means that the biological assessment of the littoral as meso- to slightly eutrophic may be correct, in spite of low water phosphorus concentrations and in spite of the absence of correlations between indices and hydrochemistry.

In conclusion, our results indicate that the combined use of classical biological metrics and different functional groups gives a meaningful picture of ecosystem processes in a lake littoral. Nutrients in Lake Ohrid seem to be removed by filter feeding benthic invertebrates, as well as incorporated into plant and algal biomass from where they are transferred through the food web. These ecosystem processes may explain the removal of TP from the water in the nearshore zone, and why correlations between biological metrics and water TP concentrations are absent. Our study was not designed to quantify local nutrient loading or the nutrient turnover in the different trophic levels of the ecosystem. However, the results indicate that the combined use of chemistry, classical metrics and functional groups may be a way to not only distinguish between oligotrophic and eutrophic ecosystems, but also to give information on whether or not nutrient input and nutrient removal in an ecosystem are balanced. This may eventually also give information about ecosystem functioning and ecosystem stability, and thus provide a basis for the development of “second generation” metrics for ecosystem assessment.

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Figure captions

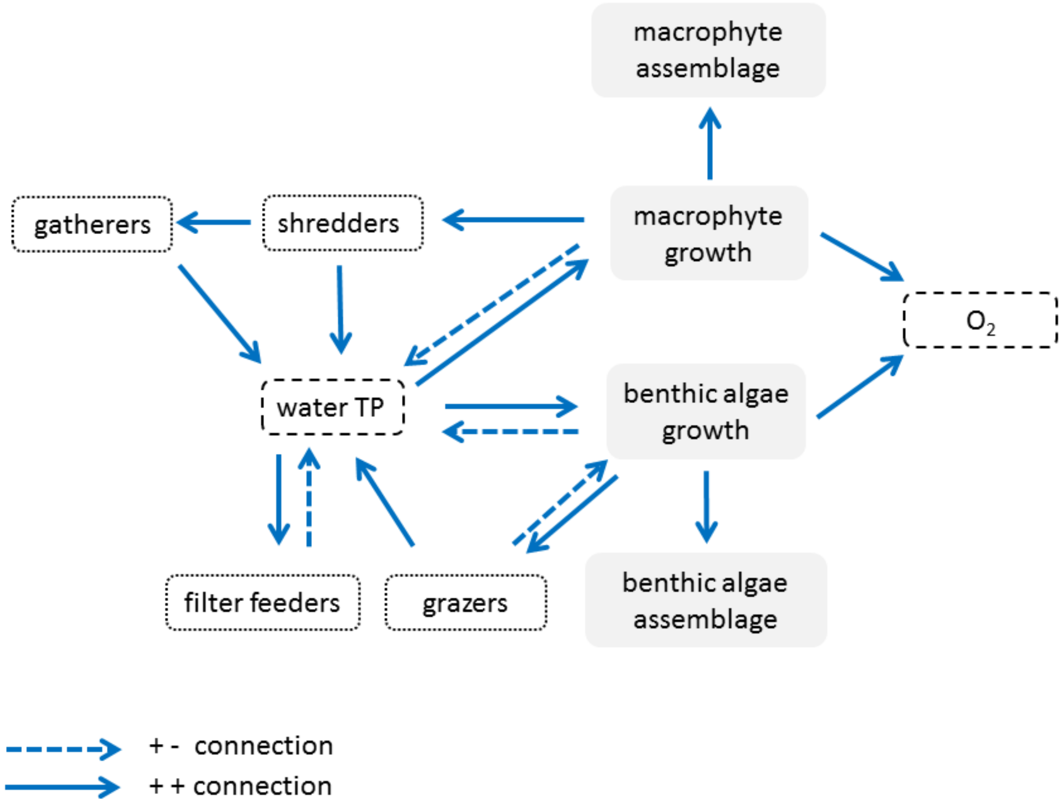

Fig. 1. Simplified hypothetical relations between chemistry, benthic algae, macrophytes and benthic invertebrates in a lake littoral. Note that we understand this figure as a graphical representation of a simplified hypothesis for the interrelations between elements we studied in Lake Ohrid. For example, we neglect planktonic organisms, since we have not analyzed plankton in our study.

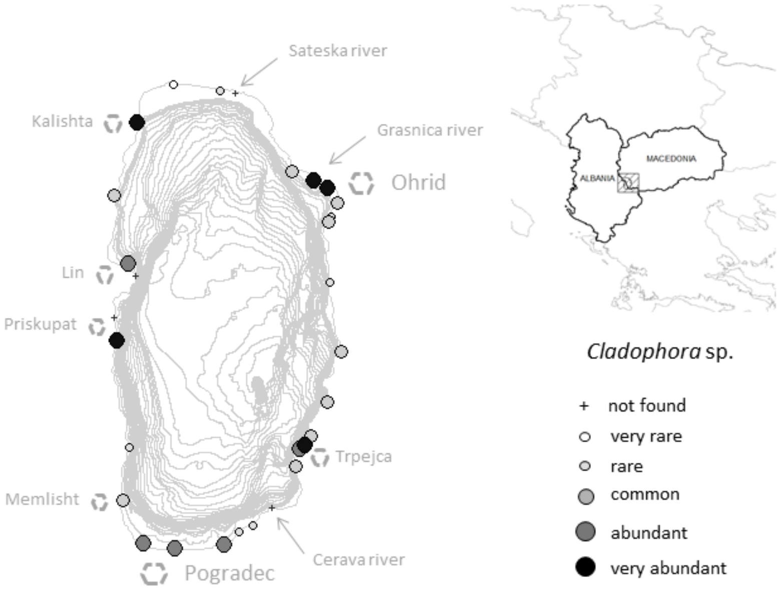

Fig. 2 Abundance of *Cladophora* sp. at 30 sampling sites between 0 and 2 m water depth in Lake Ohrid in 2009/2010.

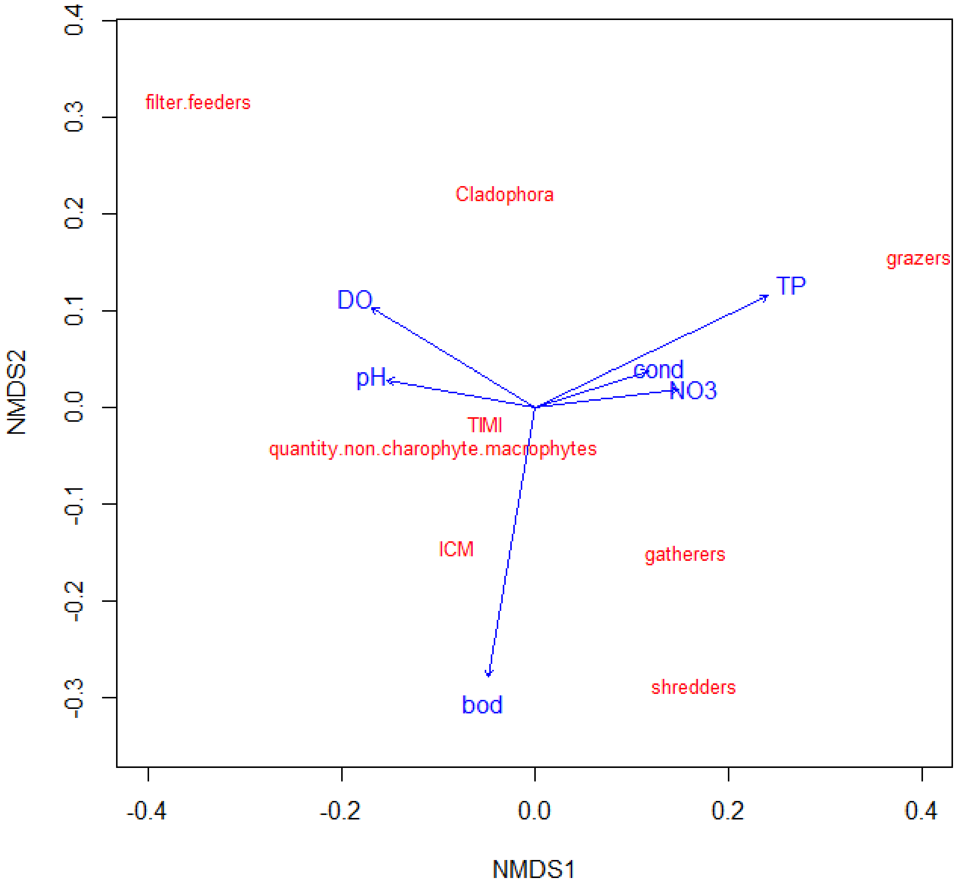

Fig. 3. Non-metric multidimensional scaling (NMDS) ordination plot of macrophyte, diatom and macroinvertebrate metrics and functional groups at 26 sites in Lake Ohrid. Centroids of biological metrics and functional groups are shown. Hydrochemistry vectors were fitted after NMDS ordination. TI = diatom trophic index, MI = macrophyte index, ICM = macroinvertebrate intercalibration metric.

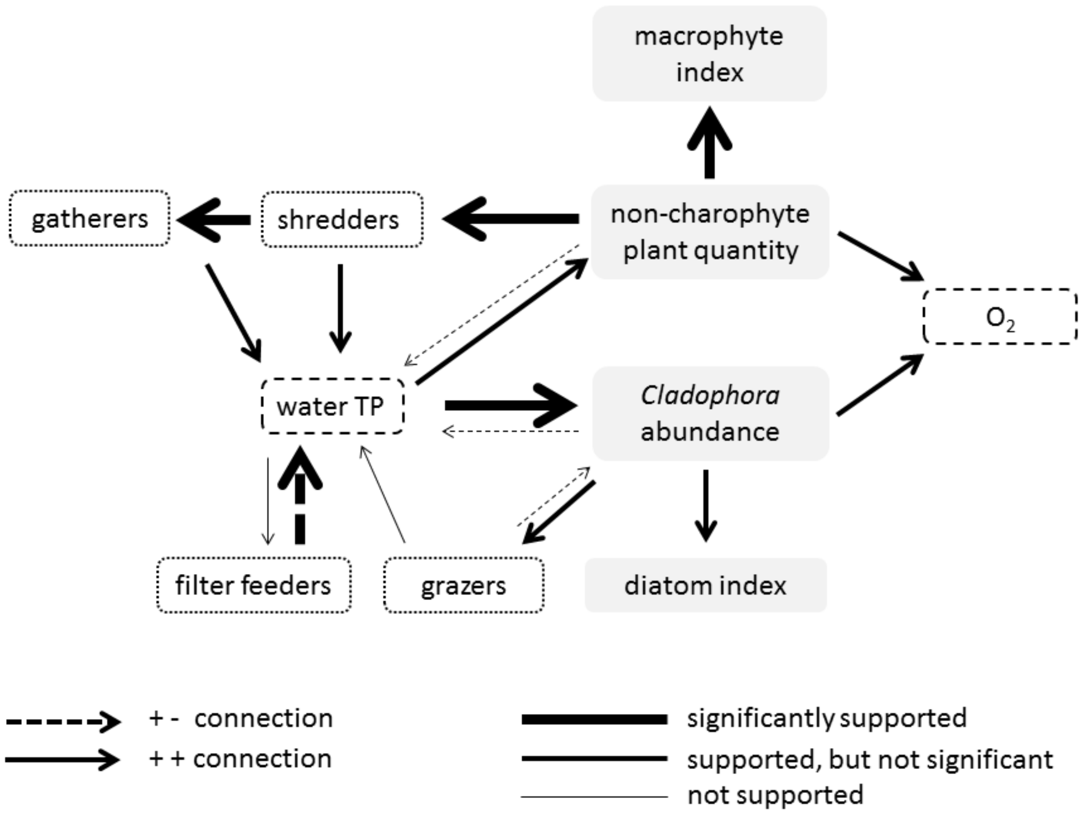

Fig. 4. Nutrient turnover in the shallow Lake Ohrid littoral (i.e. around 0.5 m depth); pathways are based on the hypotheses outlined in Fig. 1, with line weight of arrows coding the strength of the relationships according to Table 2: thin line weights represent pathways which are not supported by our data, intermediate line weights represent pathways which are supported but not statistically significant, and bold lines represent significant correlations.

Table headings

Table 1: summary statistics of abiotic variables and macrophyte, diatom and macroinvertebrate metrics at 26 sites in Lake Ohrid measured in 2009 and 2010. “Quantity” equals the sum of the cubed abundances (see Materials and Methods); values for NO_3-N, TP, conductivity and bod5 were back-transformed after averaging logarithmic values.

	average	**min**	**max**
NO_3-N [mg/l]	36.2	10.5	124
TP [µg/l]	7.16	3.68	50
pH	8.48	7.75	8.70
conductivity [µS/cm]	208	195	239
dissolved oxygen [mg/l]	9.96	8.23	11.64
bod5 [mg/l]	1.58	0.87	3.59
macrophyte index	3.21	1.95	4.78
number of taxa macrophytes	7	2	13
abundance *Cladophora glomerata*	2.92	0	5
quantity Charales	28.19	0	107
quantity non-charophyte macrophytes	65.50	1	153
diatom trophic index	2.11	1.50	2.70
number of taxa diatoms	30.96	18	41
macroinvertebrate ICM	0.22	0.06	0.52
number of taxa macroinvertebrates	5.85	2	13
% grazers	0.62	0	1.43
% shredders	21.33	0	58.15
% gatherers	23.66	0	91.77
% filter feeders	0.51	0	1.57

Tab. 2. Spearman correlation coefficients between water chemistry and the biological metrics measured in Lake Ohrid; bold numbers are significant at $p < 0.05$.

	log(bod5+1)	log(NO_3)	log(TP)	pH	log(cond.)	dissolved oxygen	macrophyte index	abundance *C. glomerata*	quantity non-charophyte macrophytes	diatom trophic index	% grazers	% shredders	% gatherers	% filter feeders
log(NO_3)[mg/l]	-0.03													
log(TP)[µg/l]	0.24	**0.40**												
pH	-0.02	-0.01	0.27											
log(conductivity)	-0.11	**0.40**	0.02	0.08										
dissolved oxygen [mg/l]	-0.11	-0.11	-0.19	-0.05	0.03									
macrophyte index	0.11	0.20	0.31	0.10	0.32	0.13								
abundance *Cladophora glomerata*	0.26	-0.02	**0.54**	0.08	-0.11	0.28	0.37							
quantity non-charophyte macrophytes	0.33	-0.10	0.11	-0.11	-0.05	0.20	**0.50**	**0.44**						
diatom trophic index	0.00	-0.04	0.10	-0.12	-0.21	0.20	-0.03	0.27	0.18					
% grazers	-0.19	-0.07	-0.01	-0.16	0.05	-0.08	0.27	0.13	0.18	0.02				
% shredders	**0.51**	0.14	0.14	-0.17	0.01	-0.20	0.31	-0.13	**0.38**	-0.26	0.21			
% gatherers	0.27	0.30	0.26	-0.13	0.29	-0.30	0.15	-0.11	0.00	0.19	0.10	**0.39**		
% filter feeders	-0.17	-0.05	**-0.39**	-0.03	0.06	0.01	0.03	-0.01	-0.03	-0.10	-0.11	-0.22	-0.25	
macroinvertebrate ICM	-0.26	-0.20	-0.35	0.01	-0.17	0.08	0.14	**-0.46**	0.22	-0.22	0.20	0.21	-0.19	-0.06