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Responses to fish predation and nutrients by plankton at different levels of taxonomic resolution

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SUMMARY

1. To improve mechanistic understanding of plankton responses to eutrophication, a mesocosm experiment was performed in the shallow littoral zone of a south Swedish lake, in which nutrient and fish gradients were crossed in a fully factorial design.
2. Food chain theory accurately predicted total biomass development of both phyto- and zooplankton. However, separating zooplankton and algae into finer taxonomic groups revealed a variety of responses to both nutrient and fish gradients.
3. That both nutrients and fish are important for phytoplankton dynamics was seen more clearly when viewing each algal group separately, than drawing conclusions only from broad system variables such as chlorophyll *a* concentration or total phytoplankton biovolume.
4. In some taxa, physiological constraints (e.g. sensitivity to high pH and low concentrations of free CO₂) and differences in competitive ability may be more important for the biomass development than fish predation, grazing by herbivorous zooplankton, and nutrient availability.
5. We conclude that food chain theory accurately predicted responses in system variables, such as total zooplankton or algal biomass, which are shaped by the dynamics of certain strong interactors ('keystone species'), such as large cladocerans, cyanobacteria and edible algae (<50 µm), whereas responses at finer taxonomic levels cannot be predicted from current theory.

Keywords: eutrophication, fish predation, food web, lake, phytoplankton, zooplankton

Introduction

Many lakes in the world suffer from high loadings of nutrients of anthropogenic origin, mainly from sewage and agriculture (e.g. Moss, 2000). A conspicuous feature of eutrophicated lakes is high concentrations of algae, making the water green and turbid and sometimes toxic owing to toxin-producing cyanobacteria (Lampert, 1981; Rohrlack, Henning & Kohl, 1999). Such turbid and toxic water makes the lake less valuable as a natural resource, for use as drinking water, and for recreation and fishing (Kitchell, 1992; Hansson & Bergman, 1998; Moss, 2000). Another

important feature, albeit less obvious to the occasional visitor to a eutrophic lake, is that the number of zooplanktivorous cyprinid fish increases with eutrophication (Jeppesen *et al.*, 1996). Hence, two main actors on the scene of a eutrophicated lake are phytoplankton (algae) and cyprinid fish.

A well-defined theoretical basis exists with respect to food chains in terrestrial systems (Hairston, Smith & Slobodkin, 1960; Fretwell, 1977; Oksanen *et al.*, 1981). It has been extended to aquatic systems, linking nutrient loading and fish abundance to system variables, such as the total biomass of zooplankton or phytoplankton (Carpenter *et al.*, 1987; Persson *et al.*, 1988; Hairston & Hairston, 1993, 1997), and these theoretical predictions have repeatedly withstood empirical test (Carpenter *et al.*, 1987; Persson *et al.*, 1988; Hansson, 1992; Mazumder, 1994; Hansson *et al.*,

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1998a). However, knowledge is limited regarding the validity of theoretical predictions when system variables are separated into finer taxonomic levels, such as genera or groups of genera. Some groups of phytoplankton, such as toxin-producing cyanobacteria, are less desirable than others, so it is important to quantify responses at this finer taxonomic level. Similar arguments may be put forward with respect to zooplankton, where certain genera, such as *Daphnia*, are known to be highly efficient grazers and may be more important for the whole lake dynamics than several other groups. Furthermore, many cyprinid fish, such as roach (*Rutilus rutilus* L.) are known to become very abundant in eutrophic lakes and to exert a strong predation pressure on zooplankton. Thus, some groups of organisms may constitute 'keystone taxa' with respect to their impact on food-web structure, whereas other groups may have a negligible impact.

Our approach here is to broaden the understanding of food-web dynamics by quantifying not only responses in total organism biomasses ('system variables'), but to analyse responses of key groups also at finer scales. The selected groups include cyanobacteria, green algae, and *Daphnia*, as well as less predominant organisms. Experiments were carried out as part of a series of parallel studies, collectively called the International Mesocosm Experiment (IME), at six sites across Europe (Stephen *et al.*, 2004a). The experimental design was chosen to mimic a gradient of lakes from low productivity, with no influence of fish, to highly eutrophic lakes with strong impact of fish. A main aim of our study was to demonstrate which mechanisms are of greatest importance for eutrophication in which situation, and to make predictions about how lake ecosystems will respond to measures against eutrophication. The specific question addressed in the present study is whether predictions, derived from food chain theory, of linear responses along nutrient gradients are valid only for total biomasses at different trophic levels, or if these responses can be tracked at finer taxonomic levels.

Material and methods

Study site

The experiment was carried out in Lake Krankesjön (55°42'N, 13°28'E), which is situated 15 km east of

Lund, southern Sweden. The lake is shallow (mean and maximum depths of 1.5 and 3 m, respectively) and considered moderately eutrophic with a mean summer (May to September) total phosphorus (TP) concentration of 38 µg L⁻¹, total nitrogen concentration of 1.3 mg L⁻¹ and chlorophyll *a* concentration of 17 µg L⁻¹. For more details, see Blindow (1992) and Hargeby *et al.* (1994).

Experimental design

Thirty-six cylindrical enclosures with a diameter of 1 m were constructed in water of 1-m depth about 200 m from the shore of Lake Krankesjön in 1999. A system of catwalks was built between enclosures to avoid damage to enclosures and to facilitate sampling. Enclosures were made in plastic of identical material and shape to those used at the other sites of the IME (Stephen *et al.*, 2004a). Three biomass levels of fish and six levels of nutrient addition were crossed in a fully factorial design, giving a total of 18 treatments, which were replicated twice within two blocks. Treatments were randomly distributed within each block. Sheet metal wind and wave breakers were attached to poles around the enclosure setup, following the destruction of a previous experiment in 1998 by storms. Nets, removable during sampling, were put over the enclosures to avoid nutrient input and predation on fish by birds. Pretreatment samples were taken on 21 June 1999, about a week after the enclosures had been constructed, but before any treatment was applied. On 24 June, fish and nutrients were added, and samples were then collected once a week: on 28 June, 5, 12 and 19 July, and on 2 August 1999. Full details of treatments and methods, standardised across all sites, are given in Stephen *et al.* (2004a,b).

During spring 1999, macrophytes, mainly *Myriophyllum spicatum* L. and *Chara tomentosa* L., were sparse. In order to increase macrophyte densities in the enclosures, *M. spicatum* was planted prior to the experiment by moving plants from a nearby pond to the enclosures and attaching a piece of modelling clay to the stem to make the plant sink. The plants rapidly developed roots, which penetrated the sediment. Despite this successful establishment, macrophytes grew sparsely during our experiment.

Phosphorus and nitrogen were added to enclosures weekly as KH₂PO₄ and Ca(NO₃)₂ 4H₂O according to

the standard protocol of the IME (Stephen *et al.*, 2004a,b). The desired added levels were 0, 30, 60, 90, 150 and 300 $\mu\text{g PO}_4\text{-P L}^{-1}$ and 0, 0.3, 0.6, 0.9, 1.5 and 3.0 $\text{mg NO}_3\text{-N L}^{-1}$. Response to these treatment variables was closely mirrored in actual concentrations in the enclosures (Fig. 1), indicating that nutrient leakage from the enclosures was negligible.

Prior to the experiment, all enclosures were electrofished and netted to remove any fish. Fish were caught by electrofishing in the lake and stored in tanks until the start of the experiment, when 1+ roach with a length of 40–60 mm were added. The low-fish treatment received three to four fish with a total wet weight of 3 g, and the high-fish treatment between 13 and 21 fish with a total wet weight of 13 to 16 g. These abundances were slightly lower than the desired levels of 4 and 20 g wet weight, respectively (Stephen *et al.*, 2004a,b). On each sampling occasion, enclosures were checked for dead fish, which were immediately

removed. Death rates were low and generally a total of zero to five fish were replaced per week. As 1+ roach were very difficult to catch during late summer, a corresponding biomass of 0+ fish (length: about 40 mm) was used to replace any dead fish.

All statistical analyses are based on time-weighted averaging, which rests on the assumption that changes caused by the treatments will increase in importance with time, followed by two-way analysis of variance (ANOVA) on these means. Calculation of weighted averages (WAs) is based on the following equation:

$$\text{WA} = [(1 \times V_1) + (2 \times V_2) + (3 \times V_3) + (4 \times V_4) + (5 \times V_5) + (6 \times V_6)] / (1 + 2 + 3 + 4 + 5 + 6),$$

where V with a subscript number is any variable on sampling dates 1–6. For further rationale regarding this approach, see Stephen *et al.* (2004a,b). Results presented in all graphs are also based on WAs. Generally, graphs are drawn with nutrient levels as the independent variable (x -axis), and data on the y -axis presented for the three fish treatments. Either a linear or exponential model was then fitted to the data. If neither of these models was applicable, the data points were connected with a line in order to illustrate, for example, a bell-shaped response to nutrient addition.

Because several ANOVA tests were performed, there was a risk of invoking a Type I error, i.e. rejecting a true null hypothesis. To adjust for such errors, we applied a sequential Bonferroni test (Rice, 1989). We used a k (total number of tests) of 26, which makes this approach very conservative and the risk of instead making a Type II error (accepting a false null hypothesis) becomes high. Therefore, we provide both original and Bonferroni-adjusted P -values (Tables 1 and 2).

Data on organisms were divided into 'system variables', including total biomass and chlorophyll a concentration, and 'group variables', which are the different taxa expressed on a finer taxonomic level, such as family or size class.

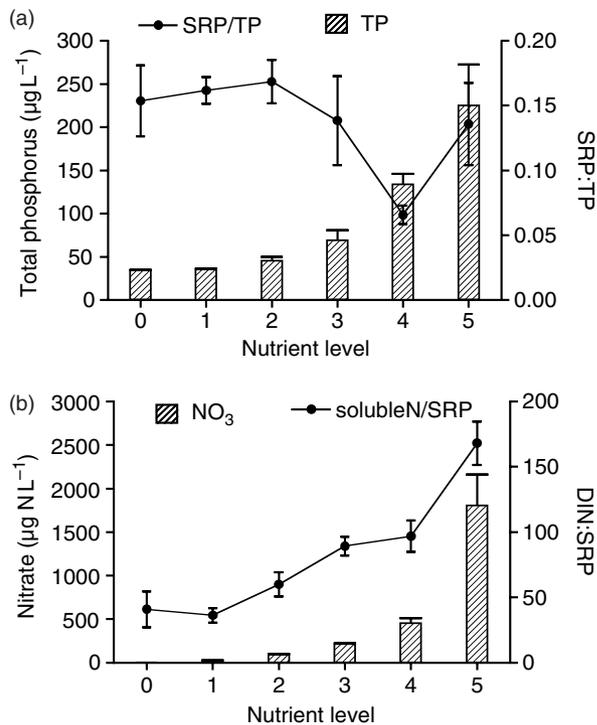


Fig. 1 (a) Concentration (mean \pm 1 SE) of total phosphorus (TP; $\mu\text{g L}^{-1}$) and ratio between soluble reactive phosphorus (SRP) and TP at increasing nutrient additions. (b) Concentration (mean \pm 1 SE) of nitrate and ratio between dissolved inorganic nitrogen (DIN = $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$) and SRP at increasing nutrient additions. Fish treatments were pooled as there was no difference among them; this results in $n = 6$ for each nutrient treatment.

Results

Results were summarised by use of a standard set of variables treated in all IME experiments (Table 2). Data from either predation (fish) or nutrient enrichment showed a variety of treatment responses. As a

Table 1 Portion (%) of significant differences attributable to nutrient or fish treatments in a mesocosm experiment carried out in the littoral zone of a Swedish lake

| Type of response variable | Manipulated factor | |
|---------------------------|--------------------|---------|
| | Nutrients | Fish |
| Water chemistry | 100 (100) | 0 (0) |
| Primary producers | 70 (50) | 30 (8) |
| Zooplankton | 38 (11) | 63 (33) |

Values in parentheses represent the same data adjusted with sequential Bonferroni tests (Rice, 1989). Significances are based on two-way ANOVA and a probability at the 0.05 level.

consequence, none of the chemical variables, which included pH and concentrations of TP, soluble reactive phosphorus (SRP), nitrate and ammonium, were affected by fish density (Table 1). In contrast, all

chemical variables were affected by nutrient addition. With respect to primary producers (including macrophytes, periphyton and various phytoplankton groups), 70% of the significant effects were attributable to nutrient additions, and 30% to fish. Moving higher up in the food web, to the zooplankton, skews the portion of significances further towards fish effects (63%). Similar trends remain when data are Bonferroni-adjusted, although percentages of significant effects are lower (Table 1).

Water temperatures were high and relatively constant throughout the summer, ranging between 18.5 and 23.4 °C. The concentration of TP increased from about 20 to above 200 µg L⁻¹ along the gradient of nutrient additions (Fig. 1), and variances were low among replicates. SRP concentration increased only

Table 2 Results from a two-way ANOVA with fish and nutrients as independent variables and various chemical, physical and biological dependent variables

| Dependent variable | Fish (<i>P</i>) | Tukey (fish) | Nutrient (<i>P</i>) | Tukey (nutrients) |
|-------------------------------|-------------------|---------------|-----------------------|---|
| Water chemistry | | | | |
| Total phosphorus | NS | – | 0.001 | 0–5; 1–5; 2–5; 3–5; 4–5; 0–4, 1–4; 2–4; 3–4; 0–3; 1–3 |
| SRP | NS | – | 0.001 | 0–5; 1–5; 2–5; 3–5; 4–5; 0–4; 0–3 |
| NO ₃ -N | NS | – | 0.001 | All except 3–4 |
| NH ₄ -N | NS | – | NS | – |
| pH | NS | – | 0.001 | 0–5; 1–5; 2–5; 3–5; 0–4; 1–4; 2–4; 3–4; 0–3; 0–2 |
| Algae | | | | |
| Phytoplankton chl <i>a</i> | NS | – | 0.001 | 0–5; 1–5; 2–5; 3–5; 0–4; 1–4; 2–4; 0–3; 1–3; 0–2 |
| Total phytoplankton biovolume | (0.020) | 0–2 | 0.001 | 0–5; 0–4; 1–4; 2–4; 0–3 |
| Chlorophycota | NS | – | NS | – |
| Cryptophyceae | NS | – | 0.001 | 0–5; 1–5; 2–5; 3–5; 0–4; 0–3 |
| Bacillariophyceae | 0.001 | 0–2; 1–2 | (0.005) | 0–4; 0–2 |
| Cyanophycota | (0.027) | 0–2 | 0.001 | 4–5; 0–4; 1–4; 2–4; 0–3 |
| Chrysophyceae | (0.016) | 0–2 | NS | – |
| Dinophyceae | (0.026) | 0–1 | NS | – |
| Euglenophyceae | NS | – | NS | – |
| GALD <50 µm | 0.001 | 0–1; 0–2 | (0.041) | – |
| GALD >50 µm | NS | – | 0.001 | 4–5; 0–4; 1–4; 2–4; 3–4; 0–3; 0–2 |
| Periphyton chl <i>a</i> | NS | – | 0.001 | 0–5; 0–4; 0–3; 0–2; 0–1 |
| Zooplankton biovolume | | | | |
| Total zooplankton | NS | – | (0.010) | 0–3 |
| Rotifers | 0.001 | 0–1; 0–2; 1–2 | NS | – |
| Cladocera <500 µm | NS | – | NS | – |
| Cladocera >500 µm | 0.001 | 0–1; 0–2 | NS | – |
| Cyclopoid copepods | NS | – | NS | – |
| Calanoid copepods | NS | – | 0.002 | 0–5; 1–5; 2–5; 3–5 |
| Copepod nauplii | 0.002 | 0–1; 0–2 | NS | – |
| Raptorial zooplankton | NS | – | NS | – |
| Open-water filterers | (0.012) | 0–1 | (0.019) | 0–3 |

Greatest Axial Linear Dimension (GALD) is the greatest axial linear dimension. The 0.05 significance level was used and NS denotes non-significant differences. No interactions between nutrients and fish were significant, except for Cryptophyceae (*P* = 0.05). Prior to analysis, all dependent variables were tested for normality and log transformed when needed. Results of pairwise comparisons (Tukey's test) are also given. Nutrient treatments are assigned 0, 1, 2, 3, 4, 5 and fish 0, 1 and 2. Note that *P*-values within parentheses are not formally significant when sequential Bonferroni test is applied (critical value for *k* = 26: 0.00213; Rice, 1989).

negligibly between nutrient treatments 0 and 4, but then increased about threefold between the two highest nutrient treatments. The SRP/TP ratio, which may be used as an indicator of the ratio between available and TP, thereby decreased considerably between nutrient treatments 0 and 4, but then again increased at the highest nutrient level (Fig. 1). The nitrate concentration followed the same trend as TP with a regular increase along the nutrient gradient (Fig. 1). This was not the case with ammonium, however, which showed no trend between nutrient treatments 0 and 4, and only a minor increase between the two highest nutrient levels. Concentrations of all nutrients, except ammonium, were strongly related to nutrient additions, whereas none showed any significant response to fish (Table 2). The pH in mesocosms showed an increase from 7.5 to 8.3 with nutrient addition, but no trend in response to fish abundance (Table 2).

System variables

The phytoplankton chlorophyll *a* concentration was strongly related to nutrient enrichment, but showed no significant response to fish additions (Table 2). Moreover, chlorophyll *a* concentrations tended to increase with nutrient additions and were similar among fish treatments up to the highest nutrient level. The low-fish treatment gave the highest, and the absence of fish gave the lowest average chlorophyll *a* concentration (Fig. 2). In contrast to chlorophyll *a* concentration, total phytoplankton biovolume only showed an increase up to nutrient level 4, but then declined considerably at the highest nutrient treatment (Fig. 2). The increase was most pronounced, and also started at lower nutrient levels, at the highest fish abundance (Fig. 2). ANOVA showed that both fish and nutrients affected the total phytoplankton biovolumes, although the fish effect was not significant when data were Bonferroni-adjusted (Table 2). As the majority of the cyanobacteria in our experiment were large species and constituted a dominant group with respect to biovolume, the pattern shown by cyanobacteria strongly affected the pattern apparent for total phytoplankton (Fig. 2).

Periphyton chlorophyll *a* concentration also showed an increase along the experimental nutrient gradient, a trend that was most pronounced at high fish densities, although the increase was less dramatic

than for phytoplankton (Fig. 3; Table 2). The third primary producer, submerged macrophytes, showed high variability in biomass, but at higher nutrient levels macrophyte biomass tended to decline in all treatments (Fig. 3).

Total zooplankton biomasses showed only a tendency towards a positive response to increased nutrient levels in the presence of fish (Fig. 2). Nutrient additions in the absence of fish affected the total biomass of zooplankton significantly, although this effect disappears when Bonferroni-adjustment is applied (Table 2). Nevertheless, in the absence of fish the zooplankton biomasses increased almost linearly up to nutrient level 3 before they declined at higher nutrient concentrations (Fig. 2).

Group variables

The main phytoplankton groups showed three different patterns in response to nutrient additions (Fig. 4). Responses include an exponential increase (e.g. chlorophytes and cryptophytes; Fig. 4), an increase to a certain nutrient level followed by a decline at higher concentrations (cyanobacteria, diatoms), and a direct linear decline (chrysophytes; Fig. 4). The exponential responses in chlorophytes and cryptophytes were not affected by fish (Table 2). Diatoms and cyanobacteria (decline at high nutrient additions) were affected by both fish and nutrients, whereas chrysophytes (linear decline) were only affected by fish abundance (Table 2). Chrysophytes were the only algae that declined at increasing nutrient concentration, a trend that was most pronounced at the highest fish density (Fig. 2). The biomass development of dinoflagellates was affected by fish, but not nutrients, whereas euglenophytes showed no response to either fish or nutrient additions (Table 2). If Bonferroni-adjustment is applied, the only significant fish effect is on diatoms (Bacillariophyceae). Separating algae into two size classes with a Greatest Axial Linear Dimension (GALD) larger and smaller than 50 µm, respectively, shows that the smaller, edible algae were affected by both fish and nutrients, whereas only nutrients but not fish had an effect on the larger inedible algae (Table 2; Fig. 4). In the treatment without fish, biovolumes of none of the phytoplankton groups except cyanobacteria were affected by nutrient addition. Moreover, responses were generally strongest at the highest fish densities (Fig. 4).

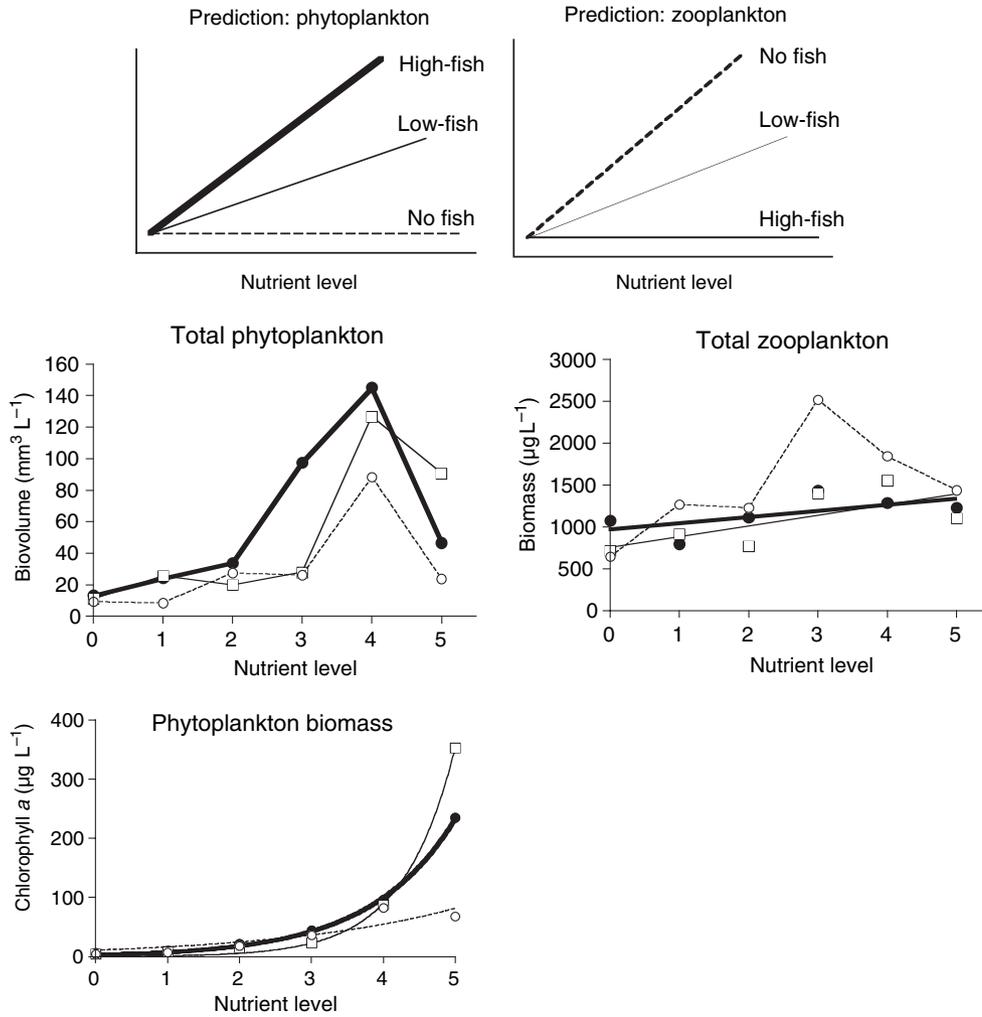


Fig. 2 Predicted phytoplankton and zooplankton responses to fish abundances along a nutrient gradient compared with total phytoplankton biovolume, chlorophyll *a* concentration, and total zooplankton biovolume recorded in mesocosms. Fish levels are: no fish (dotted line), low fish (thin line) and high fish density (thick line).

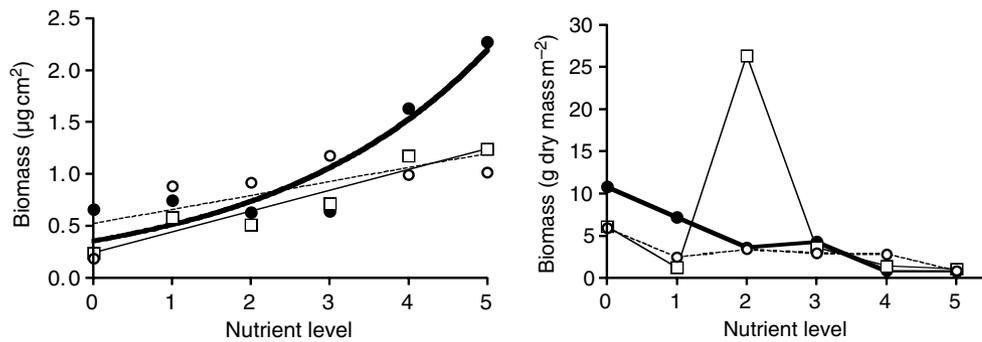


Fig. 3 Responses of periphytic algae ($\mu\text{g chlorophyll } a \text{ cm}^{-2}$) and macrophytes (g dry mass m^{-2}) along a nutrient gradient. Fish levels are: no fish (dotted line), low fish (thin line) and high fish density (thick line).

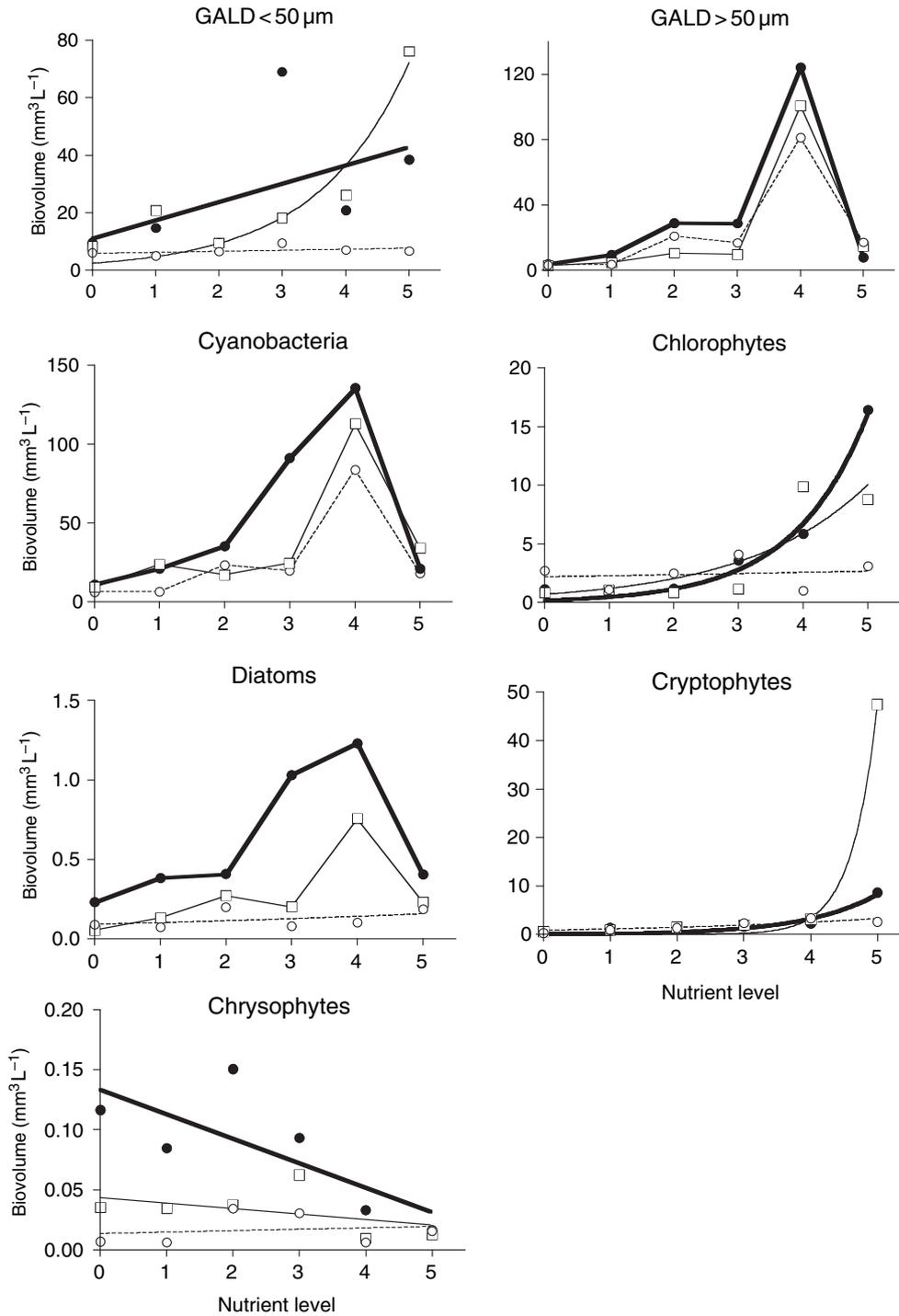


Fig. 4 Biovolume of various phytoplankton groups at different fish concentrations plotted along a nutrient gradient. Functional groups include edible (GALD <50 μm) and inedible algae (GALD >50 μm). Taxonomic groups include chlorophytes, cyanophytes, cryptophytes, diatoms and chrysophytes. Fish levels are: no fish (open circles, dotted line), low fish (squares, thin line) and high fish density (filled circles, thick line).

The pattern shown by total zooplankton was almost exclusively determined by cladocerans larger than 500 μm , a group that was very scarce in the presence of fish (Fig. 5), which led to a highly significant response to fish abundance (Table 2). However, a decline in cladocerans smaller than 500 μm occurred only at the highest fish treatment, whereas at moderate predation pressure (i.e. at low fish density), these small cladocerans showed maximum biomass (Fig. 5). As for small cladocerans, cyclopoid copepods showed the highest biomass at intermediate nutrient concentrations in the treatments with no fish and low fish

abundances. At high fish abundances, however, cyclopoid copepods showed an exponentially increasing trend with nutrient addition (Fig. 5). The biomass of calanoid copepods, in contrast, decreased with increasing nutrient concentration in all fish treatments (Fig. 5; Table 2), resulting in a notable increase in the ratio between cyclopoid and calanoid copepods as productivity increased. The smallest zooplankters, such as rotifers and copepod nauplii, generally showed significantly higher biomass in the presence, than in the absence, of fish (Fig. 5), in contrast to relationships between fish and large zooplankton

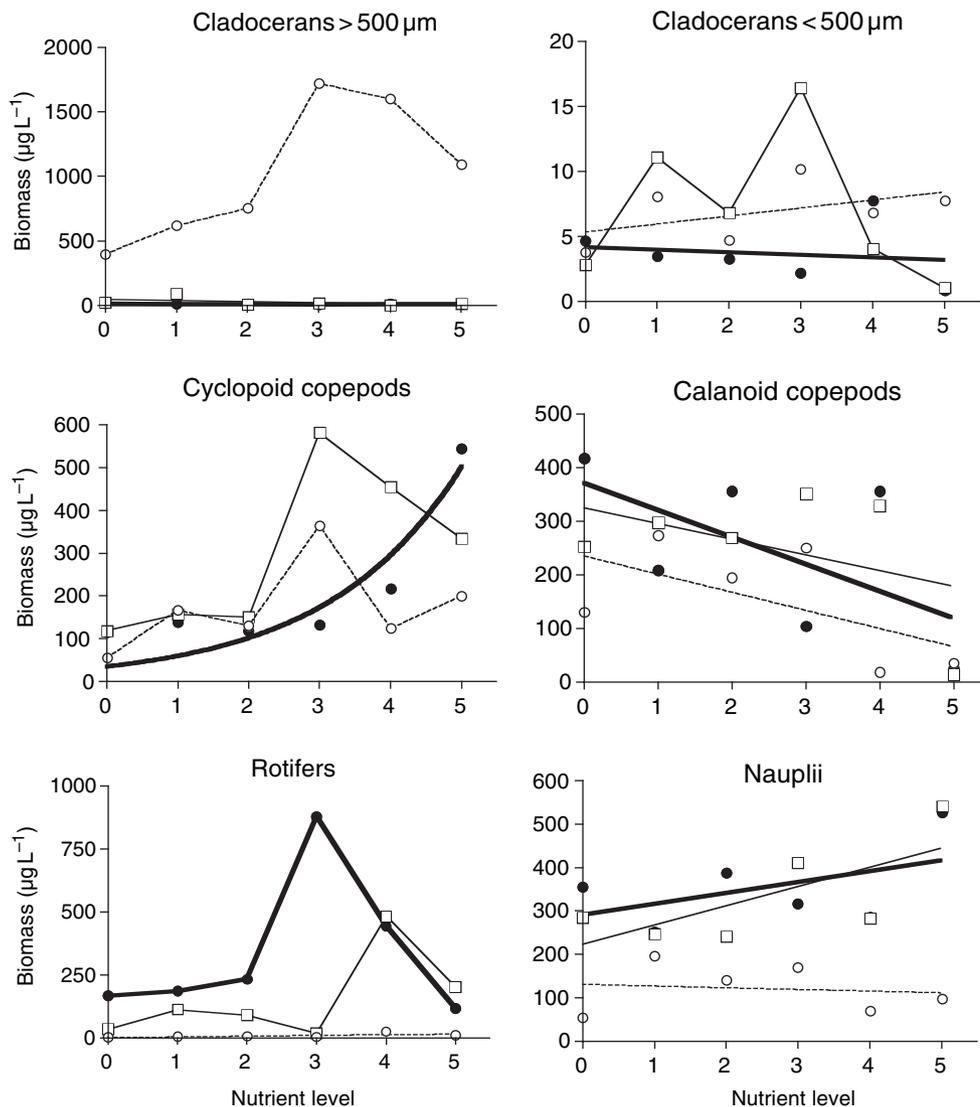


Fig. 5 Biovolume of various zooplankton groups at different fish concentrations plotted along a nutrient gradient. Groups include cladocerans >500 μm and <500 μm , cyclopoid and calanoid copepods, rotifers and copepod nauplii. Fish levels are: no fish (open circles, dotted line), low fish (squares, thin line) and high fish density (filled circles, thick line).

(Table 2). Rotifers especially had very low biovolumes in the absence of fish, irrespective of nutrient treatment. However, at the highest fish density, rotifers declined after a maximum biovolume at intermediate nutrient levels (Fig. 5). A similar pattern occurred at low fish abundances, although the maximum biovolumes were reached at higher nutrient concentrations than at the highest fish abundances (Fig. 5).

Discussion

Food chain theory predicts that with increasing nutrient levels, algal biomass does not necessarily increase as a greater part of the algal biomass production is removed by a growing herbivorous zooplankton community. However, if planktivorous fish are present, algal biomass will increase proportionately, as the planktivores will keep the herbivores at a low level (Carpenter *et al.*, 1987; Persson *et al.*, 1988; Hansson, 1992; Hairston & Hairston, 1993, 1997). This theory has predicted natural dynamics reasonably well with respect to broad system variables such as total abundance and biomass (Hairston & Hairston, 1997; Hansson *et al.*, 1998a). However, separation of system variables into finer taxonomic levels, such as to families or even genera, may be of utmost importance in developing water management plans, because some organism groups (e.g. *Daphnia*) are desired, whereas others (e.g. cyanobacteria) are generally not. In order to improve the accuracy of management plans, and disentangle mechanisms behind nutrient and predation effects, we analysed the data from our mesocosm experiment both on a system level (total biovolumes/concentrations) and on more detailed taxonomic and size-class levels.

In addition to food chain effects such as predation and grazing, community composition and development in lakes are strongly affected by nutrient dynamics. With respect to nuisance algal blooms, the ratio between total nitrogen (TN) and TP has been pointed out as important, especially with respect to cyanobacteria, which rarely form blooms at ratios above 29 (by weight; Smith, 1983). In our experiment, cyanobacteria declined in all fish treatments at the highest nutrient treatment, a decline that had a strong impact also on system variables such as total phytoplankton biomass (Fig. 2). One reason why cyanobacteria often dominate at low TN : TP ratio is that many of them can fix atmospheric nitrogen. However,

cyanobacteria that apparently do not fix nitrogen, such as *Microcystis*, which was a dominant genus in our experiment, also often dominate at low TN : TP ratios. This phenomenon has been explained by the inferior competitive ability of these cyanobacteria for phosphorus, restricting their dominance to situations where nitrogen limits most other algal groups (Tilman, Kilham & Kilham, 1982; Smith, 1983). Possibly, their recruitment from sediments (Ståhl-Delbanco, Hansson & Gyllström, 2003), where elevated nutrient levels may provide initial high cell concentrations, is also relevant. In our experiment, all significant effects on chemical variables were attributable to nutrient additions alone (Table 1), although fish can affect nutrient levels by both bioturbation (Lammens, 1988) and excretion (Vanni & Layne, 1997; Attayde & Hansson, 2001; Vanni, 2002).

Total nitrogen was not included in our sampling protocol, making it impossible to calculate the exact TN/TP ratio in our treatments. However, the gradual increase in the ratio between readily available nitrogen (measured as $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$) and phosphorus (SRP) recorded in our experiment (Fig. 1), would eventually lead to a decline in cyanobacteria. The increase in this ratio may be because of a faster consumption of phosphorus than nitrogen by algae and by an increase in the ammonium concentration. Most likely, the critical ratio constraining cyanobacteria would have been reached somewhere between nutrient enrichment levels 4 and 5, where cyanobacteria, accordingly, showed a considerable decline (Fig. 4). In our experiment, chlorophytes and cryptophytes were the superior competitors at high nitrogen to phosphorus ratios (Fig. 4).

The decreasing trend in SRP/TP ratio between nutrient treatments 0 and 4 indicates that phosphate was rapidly taken up by organisms, suggesting that phosphorus was the growth-limiting nutrient along this section of the nutrient gradient. At very high phosphorus additions (nutrient treatment 5), the concentration and availability of SRP increased, as illustrated by the increase in SRP/TP ratio. The SRP/TP ratio was almost a mirror image of the cyanobacterial biovolume development, suggesting that these algae may have been responsible for the dip in SRP/TP at nutrient treatment 4, where cyanobacteria showed a biomass maximum (Figs 1 & 4). At nutrient treatment 5, however, cyanobacteria declined despite the SRP/TP ratio being higher than in treatment 4, an

observation that may be explained by a less favourable N/P ratio (see above), allowing other algal groups to dominate. Similar shifts from dominance by cyanobacteria to green algae at very high nutrient levels were also recorded in several of the parallel experiments in other countries (van de Bund *et al.*, 2004), and seems to be a general feature of shallow lakes (Jensen *et al.*, 1994).

Despite cyanobacteria generally having far higher biovolumes than other algal groups, their bell-shaped response to nutrient enrichment in terms of biovolume (Fig. 4) was not mirrored in chlorophyll *a* concentration (Fig. 2). This is most probably because of our basing biovolume estimates on colonies and not single cells. As *Microcystis* was one of the dominant cyanobacterial genera, the measurements of biovolume also included the mucus surrounding the cells and thus enhancing the biovolumes. The relationship of this group with chlorophyll *a* is therefore likely to be weaker than for other algal groups. Consequently, in our experiment, cyanobacteria were not responsible for the pattern in chlorophyll *a* concentrations at high nutrient additions, which may be expected from the high biovolumes. Instead, chloro- and cryptophytes were the algae forming large populations at the highest nutrient concentrations (Figs 2 & 4).

The decline in chrysophytes with increasing nutrient concentrations may be a competitive effect as most other algae increased with nutrient addition (Fig. 4). Alternatively, it may reflect a physiological constraint associated with the high pH and reduced availability of free CO₂ at high nutrient levels. Chrysophytes showed the highest biomass at no or low nutrient addition and at the highest fish abundance, constituting a situation where both competition and predation pressure is low (low biomass of other algae and low zooplankton abundance). Chrysophytes are often associated with oligotrophic conditions (Reynolds, 1984) and generally increase in abundance, as well as in species numbers, when eutrophic lakes are restored to less nutrient-rich conditions (Cronberg, 1982).

No algal group except for cyanobacteria showed any positive response to nutrient addition in treatments without fish (Fig. 4). Instead, all the algal biomass produced was rapidly consumed by a growing zooplankton assemblage. This is in accordance with predictions from food chain theory (Hairston *et al.*, 1960; Fretwell, 1977; Oksanen *et al.*, 1981; Car-

penter *et al.*, 1987; Persson *et al.*, 1988; Hansson, 1992). However, large cladocerans reached their maximum biomass already at intermediate nutrient levels. Despite higher nutrient additions applied in nutrient treatments 4 and 5, growth rates of algae were apparently not high enough to feed further increases in herbivore biomass (Fig. 5).

In contrast to planktonic and periphytic algae, macrophytes decreased in biomass at high nutrient addition, most probably because of shading from the other primary producers. Such a decline in macrophyte biomass is a common feature following eutrophication (Phillips, Eminson & Moss, 1978; Lauridsen, Jeppesen & Søndergaard, 1993; Strand, 1999). As the macrophyte density in our experiment was rather low, its effect on other organisms and on trophic interactions may have been negligible.

Large cladocerans disappeared almost completely in the presence of fish, whereas smaller crustaceans generally were most abundant at intermediate fish densities. The smallest zooplankters, such as nauplii and rotifers, showed a positive response to fish, which may be because of a release from competition with large zooplankters. An additional, non-exclusive, explanation may be a reduction in predation from large cladocerans as illustrated by Gilbert (1988) and Jack & Gilbert (1994). In any case, it is clear that not all zooplankton groups respond similarly to the presence of fish. For the largest (cladocerans >500 µm), and smallest (rotifers) zooplankters there was even a gradual, albeit opposite, response to fish biomass. These data suggest that the general predictions of strong negative effects of planktivorous fish on herbivorous zooplankton may not be valid for all taxonomic groups and size classes of zooplankton.

Although less clear than for zooplankton, a gradual, but opposite, response pattern to fish was also detected among most algal groups, including cyanobacteria, diatoms, chrysophytes and partly also for chlorophytes (Fig. 4). Most algal groups, however, were more affected by nutrient addition. Thus, dependent on which algal group dominates in a specific lake and season, the effect on system variables such as total phytoplankton biomass may alter between fish and nutrients, suggesting that there may be temporal and spatial differences in the most important governing factor. Total phytoplankton biovolumes gave a more accurate estimate of the phytoplankton dynamics than chlorophyll *a*, showing that

not only nutrient addition, but also cascading grazing effects (fish predation on zooplankton, which in turn graze algae) may affect phytoplankton dynamics. This notion is strengthened by the stronger fish treatment effects (i.e. zooplankton grazing rates) on edible, than less edible algae (>50 µm; Fig. 4). That both nutrients and fish are important for phytoplankton dynamics is seen more clearly when viewing each group separately, than drawing conclusions only from broad system variables such as chlorophyll *a* concentration or total phytoplankton biovolume (Fig. 2).

A major conclusion from our experiment is that current theory fails to predict the biomass development of individual taxonomic or functional groups of phyto- and zooplankton along gradients of fish density and nutrient concentration. Almost all possible response curves were recorded among the groups investigated, including no response, exponential growth, a bell-shaped pattern, as well as a linear decline. The reason for this may be that other variables than those included in current theory (predation and nutrients) are important in determining biomass development of organisms (Hairston & Hairston, 1993, 1997; Hansson *et al.*, 1998a; Persson *et al.*, 2001). Such variables may include chemical constraints (e.g. N : P ratio; Smith, 1983; Ståhl-Delbanco *et al.*, 2003), climate features and changes (Hargeby, Blindow & Hansson, 2004), inedible prey, omnivory (Diehl, 1993), predator refuges (Sih, 1987), size-selective predation (Brooks & Dodson, 1965) and competition (Tilman, 1981; Hansson, 1988). However, predictions from theory fitted reasonably well with our data both on broad system variables and dominant taxa and size classes, such as total biovolumes of zoo- and phytoplankton and large cladocerans. Thus, results from our mesocosm experiment are consistent with patterns observed in shallow lakes, suggesting that they may serve as an adequate basis to develop management and restoration plans, such as biomanipulation by removal of zooplanktivorous fish (Shapiro, Lamarra & Lynch, 1975; Hansson *et al.*, 1998b; Mehner *et al.*, 2002).

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