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Responses of phytoplankton to fish predation and nutrient loading in shallow lakes: a pan-European mesocosm experiment

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SUMMARY

1. The impacts of nutrients (phosphorus and nitrogen) and planktivorous fish on phytoplankton composition and biomass were studied in six shallow, macrophyte-dominated lakes across Europe using mesocosm experiments.
2. Phytoplankton biomass was more influenced by nutrients than by densities of planktivorous fish. Nutrient addition resulted in increased algal biomass at all locations. In some experiments, a decrease was noted at the highest nutrient loadings, corresponding to added concentrations of 1 mg L^{-1} P and 10 mg L^{-1} N.
3. Chlorophyll *a* was a more precise parameter to quantify phytoplankton biomass than algal biovolume, with lower within-treatment variability.
4. Higher densities of planktivorous fish shifted phytoplankton composition toward smaller algae (GALD < 50 μm). High nutrient loadings selected in favour of chlorophytes and cyanobacteria, while biovolumes of diatoms and dinophytes decreased. High temperatures also may increase the contribution of cyanobacteria to total phytoplankton biovolume in shallow lakes.

Keywords: fish, food-web interactions, mesocosm experiments, nutrients, phytoplankton composition

Introduction

Many factors may play a role in controlling the composition of phytoplankton communities and phytoplankton biomass in shallow lakes. Among them are nutrient loading, grazing by zooplankton, which in turn can be influenced by fish predation, abundance of macrophytes, and climate. The relative importance of bottom-up and top-down controls can vary widely among both lakes and years (Jeppesen *et al.*, 1997), and because of the high complexity of food webs in shallow lakes it is difficult to establish simple cause-effect relationships.

Nutrients generally increase total algal biomass and the percentage of chlorophytes and cyanobacteria in cool-temperate shallow lakes (Jensen *et al.*, 1994; Scheffer *et al.*, 1997; Jeppesen *et al.*, 2000). Reports on warmer shallow lakes are scarcer. In shallow lakes of Florida, phytoplankton composition changes along a trophic gradient, with green algae tending to domin-

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ate in oligotrophic lakes and cyanobacteria dominating in eutrophic and hypertrophic lakes; diatoms are relatively abundant in mesotrophic lakes (Canfield *et al.*, 1984; Duarte, Agustí & Canfield, 1992). For other warmer regions, it has been reported that cyanobacteria are abundant in both clear and turbid shallow lakes (Romo *et al.*, 2004), which is consistent with an alleged cyanobacterial preference for higher temperatures (Reynolds, 1984; Komárek, 1985).

Aquatic macrophytes play a key role in structuring food webs in shallow lakes and in maintaining water transparency by direct and indirect effects on phytoplankton growth (Scheffer *et al.*, 1993; Jeppesen *et al.*, 1998; Van Donk & Van de Bund, 2002). Empirical studies undertaken in temperate and some subtropical areas have shown that water transparency is generally high in lakes with high macrophyte cover (Jeppesen *et al.*, 1990; Canfield & Hoyer, 1992). Phytoplankton communities in macrophyte beds are often dominated by small and motile forms such as cryptophytes, while algae with high sinking rates (e.g. diatoms and green algae) are less well represented (Balls, Moss & Irvine, 1989; Van Donk *et al.*, 1990).

Macrophytes compete for nutrients and other resources with phytoplankton and periphyton (Ozimek, Gulati & Van Donk, 1990; Van Donk *et al.*, 1993). They also reduce resuspension (Barko & James, 1998) and increase sinking losses and shading for the phytoplankton. Macrophytes are also very important for higher trophic levels, providing refuges for zooplankton against their predators (Timms & Moss, 1984; Schriver *et al.*, 1995; Jeppesen *et al.*, 1998) and structuring fish communities in shallow eutrophic lakes (Lammens, 1989; Persson *et al.*, 1993; Persson & Eklöv, 1995). Grazing by zooplankton tends to result in a shift of phytoplankton species towards algae with higher growth rates or a higher grazing resistance or both (Leibold, 1989).

Furthermore, macrophytes can produce allelopathic substances affecting phytoplankton and periphyton (Wium-Andersen, Christophersen & Houen, 1982; Jasser, 1995), and perhaps also higher trophic levels (Lauridsen & Lodge, 1996; Burks, Jeppesen & Lodge, 2000).

The relative importance of the above-mentioned factors is likely to vary with climate, lake morphology and variation in plant community composition and density (Moss, Madgwick & Phillips, 1997; Scheffer,

1998) and also with nutrient status of lakes (Jeppesen *et al.*, 1999).

A few studies are available showing how nutrient, fish and macrophyte interactions together affect phytoplankton in shallow lakes (Meijer *et al.*, 1990; Schriver *et al.*, 1995; Beklioglu & Moss, 1996; Jeppesen *et al.*, 2000). Schriver *et al.* (1995) observed in a mesocosm experiment that at increasing fish densities, zooplankton dominance shifted from large-sized cladocerans to cyclopoids, while phytoplankton shifted from small fast-growing species to cyanobacteria and dinoflagellates. Gragnani, Scheffer & Rinaldi (1999) formulated a theoretical model suggesting that, in the absence of a correlation between planktivorous fish predation and selective feeding by zooplankton, cyanobacteria tend to be favoured by intermediate but not by high grazing pressure. It has been suggested that the effects of zooplankton grazing and fish community structure on phytoplankton are stronger in eutrophic than in mesotrophic shallow lakes (Leibold, 1989; Sarnelle, 1993; Jeppesen *et al.*, 2000).

The aim of the present study was to elucidate how the impact of nutrient additions (phosphorus and nitrogen) and planktivorous fish on phytoplankton composition and biomass in shallow, macrophyte-dominated lakes changes across sites at the continental scale. More specific information on each experiment, including phytoplankton data, is reported in Fernández-Alaez *et al.* (2004), Hansson *et al.* (2004), Hietala, Vakkilainen & Kairesalo (2004), Stephen *et al.* (2004b), Van de Bund & Van Donk (2004), and Romo *et al.* (2004). Methods and other background details are given in Stephen *et al.* (2004a,b). The specific objective of this paper is to integrate and analyse main trends in phytoplankton communities emerging from comparative analysis of experimental results among locations, taking into account climatic variations among sites ranging from northern to southern Europe.

Methods

Enclosure experiments

Eleven mesocosm experiments were performed in 1998 and 1999, in six shallow, macrophyte-dominated lakes in five European countries: Vesijarvi in Finland, Krankesjön in Sweden, Little Mere in the U.K., Naardermeer in the Netherlands, Lake Sentiz in

northern Spain (León), and Lake Xeresa in southern Spain (Valencia). Key information about background conditions in the lakes is summarised in Table 1 of Stephen *et al.* (2004a).

Enclosures were polyethylene cylinders with a diameter of 1 m enclosing up to 750 L of lake water, including sediment and vegetation. Experiments were very similar between locations in a given year. Each experiment consisted of 36 enclosures, with distinct fish and nutrient treatments for each year (Stephen *et al.*, 2004a). In 1998, there were three zooplanktivorous fish levels (from 0 to 20 g fresh mass m^{-2}), and four nutrient levels (from no nutrient addition to weekly nitrate and phosphate additions sufficient to create an additional immediate concentration of up to 10 mg L^{-1} N and 1 mg L^{-1} P), with three replicates for each treatment. In 1999, fish treatments were the same as in 1998, but there were six instead of four nutrient levels (from no nutrient addition to weekly additions enough to create an additional immediate concentration of 3 mg L^{-1} N and 0.3 mg L^{-1} P), with two replicates for each treatment. Appropriate zooplanktivorous fish species were used in different locations (Table 1 of Stephen *et al.*, 2004a). Enclosures were put in place several days before adding the fish and applying the first nutrient addition; pre-existing fish were removed by electrofishing. The duration of

the experiments was 5 weeks in 1998 and 6 weeks in 1999. Weekly samples were taken for water chemistry, phytoplankton and zooplankton.

Phytoplankton was sampled from all enclosures using a tube sampler of at least 5-cm in diameter. Chlorophyll *a* was extracted from filters into 90% ethanol in a 75 °C water bath for 5 min and measured spectrophotometrically. Details followed international standard ISO 10260 modified into Finnish standard SFS 5772. A sample of the mixed tube sample water was preserved with Lugol's iodine solution for subsequent phytoplankton counts using an inverted microscope. An agreed protocol was used to standardise counting effort among laboratories and determination of biovolumes by optical measurement. In this paper, phytoplankton composition is presented as the relative contribution of the main algal groups (green algae, cyanobacteria, cryptophytes, diatoms, and others) to the total phytoplankton biovolume. The Greatest Axial or Linear Dimension (GALD; Reynolds, 1984) was used to categorise the phytoplankton size distribution into two groups, separating small (GALD < 50 μm) and large (GALD > 50 μm). The focus of the analysis in this paper is on the general patterns emerging from comparison of the eleven experiments.

Statistical methods

Time-weighted averages were calculated for each enclosure in each experiment. Week number was used as a weighting factor (Stephen *et al.*, 2004a; Van de Bund & Van Donk, 2004). Overall data were log-transformed (chlorophyll *a* and total biomass) or arcsine-transformed (contributions to total biovolume) in order to meet requirements for analysis of variance (ANOVA). Data were analysed separately for the 2 years by a two-way ANOVA, with fish and nutrient as treatment variables. Separate analyses of the 2 years were necessary because the experimental set-up differed between years. Additionally, data from individual experiments were analysed using two-way ANOVA with fish and nutrients as treatment variables.

Results

Overall fish and nutrient effects

In general, the variables quantifying phytoplankton biomass and composition responded much stronger

Table 1 Overall treatment effects on phytoplankton biomass and composition in enclosure experiments in 1998 and 1999

Variable	Year	Fish (F)	Nutrients (N)	F × N
Chlorophyll *a*	1998[†]	n.s.	↑***	n.s.
	1999	n.s.	↑***	n.s.
Total biomass	1998	↑*	↑***	n.s.
	1999	n.s.	n.s.	n.s.
% Chlorophytes	1998	n.s.	↑***	n.s.
	1999	n.s.	n.s.	n.s.
% Cyanobacteria	1998	n.s.	↓*	*
	1999	n.s.	n.s.	n.s.
% Cryptophytes	1998	n.s.	n.s.	n.s.
	1999	n.s.	n.s.	n.s.
% Diatoms	1998	n.s.	↓*	n.s.
	1999	n.s.	↓**	n.s.
% GALD < 50 μm	1998	n.s.	n.s.	n.s.
	1999	↕**	n.s.	n.s.

[†]No data available for Leon.

Two-way ANOVA results: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant.

Arrows indicate direction of change with treatment level for fish and nutrient main effects: ↑, increase; ↓, decrease; ↕, no consistent trend.

to nutrient addition than to the fish treatments (Table 1). Fish effects were not significant for most variables, except total phytoplankton biovolume (1998 only), and the contribution of small-size algae (GALD < 50 μm; 1999 only). Chlorophyll *a* concentration (both years) and the relative contribution of chlorophytes to total phytoplankton biovolume (1998 only) increased consistently with increasing nutrient addition. The contribution of diatoms to the total phytoplankton biomass decreased with increasing nutrients in both years. Nutrient treatment effects on the contribution of cyanobacteria and chlorophytes were only significant in 1998.

The lack of consistency in overall treatment effects is largely because of considerable differences between individual experiments, both among locations and between years. These differences are described in more detail below.

Chlorophyll a *concentration and total phytoplankton biovolume*

To examine the high spatial and temporal variability, we compared chlorophyll *a* levels in control enclosures (no fish and no nutrients added). Control chlorophyll *a* levels were relatively low in Spain, the Netherlands and Sweden, and much higher in England and Finland, and they were considerably higher in 1999 than in 1998 in all locations (Table 2). In England, chlorophyll *a* values were extremely high in 1999 owing to unusually high initial concentrations in the lake (Stephen *et al.*, 2004b). The magnitude of the treatment effect in the different experiments was quantified by calculating the ratio of the highest and

Table 2 Chlorophyll *a* concentrations (time-weighted averages) in control enclosures (neither fish nor nutrients added), and the ratio of chlorophyll *a* concentrations in the highest and lowest treatment in eleven enclosure experiments

Location	Control chlorophyll *a* (μg L^{-1})		Effect size (highest/lowest)	
	1998	1999	1998	1999
Valencia	6.7	11	131	14
Leon	no data	13	no data	4
the Netherlands	4.9	7.3	14	11
England	15	384	9	4
Sweden	no data	4.9	no data	77
Finland	11	25	13	4

lowest chlorophyll concentration (averaged by treatment) for each location. This ratio varied between experiments and between years (Table 2). The effect of the treatments tested was substantial throughout but particularly pronounced in Valencia in 1998 and in Sweden in 1999 (mostly because of nutrient additions), and relatively low in Leon, England and Finland in 1999.

The general pattern for chlorophyll *a* concentrations and total phytoplankton biovolumes was similar in most experiments (Figs 1 & 2). Within-treatment variability was much lower for chlorophyll *a* than for total algal biovolume. A likely cause for this observation is that the latter variable is much more difficult to standardise between different laboratories, and could be biased by differences in cell size calculations for the different algal taxa (Rott, 1981).

This may explain why a higher number of significant treatment effects was found for chlorophyll *a* (Table 3). The ANOVA of individual experiments
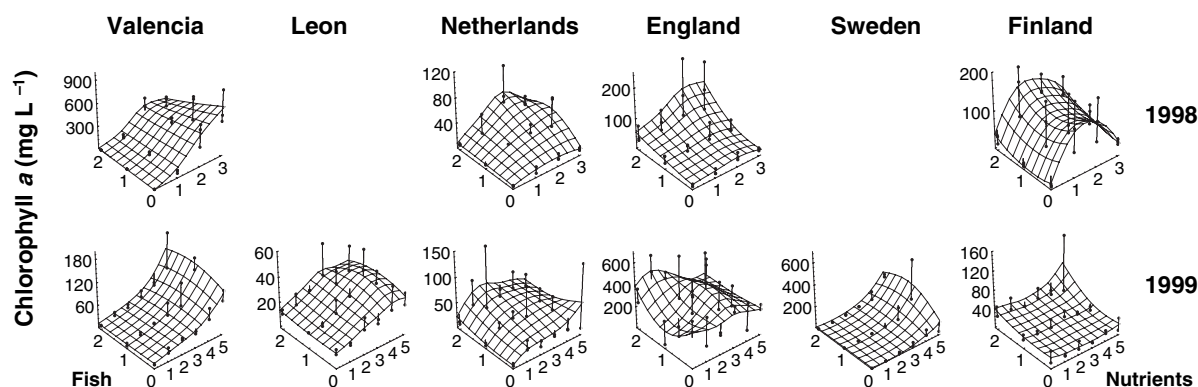

Fig. 1 Effect of fish and nutrient treatments on chlorophyll *a* concentrations (time-weighted averages) in two series of enclosure experiments performed in 1998 and 1999 in six macrophyte-dominated shallow lakes distributed across Europe.

Fig. 2 Effect of fish and nutrient treatments on total phytoplankton biovolume (time-weighted averages) in two series of enclosure experiments performed in 1998 and 1999 in six macrophyte-dominated shallow lakes distributed across Europe.
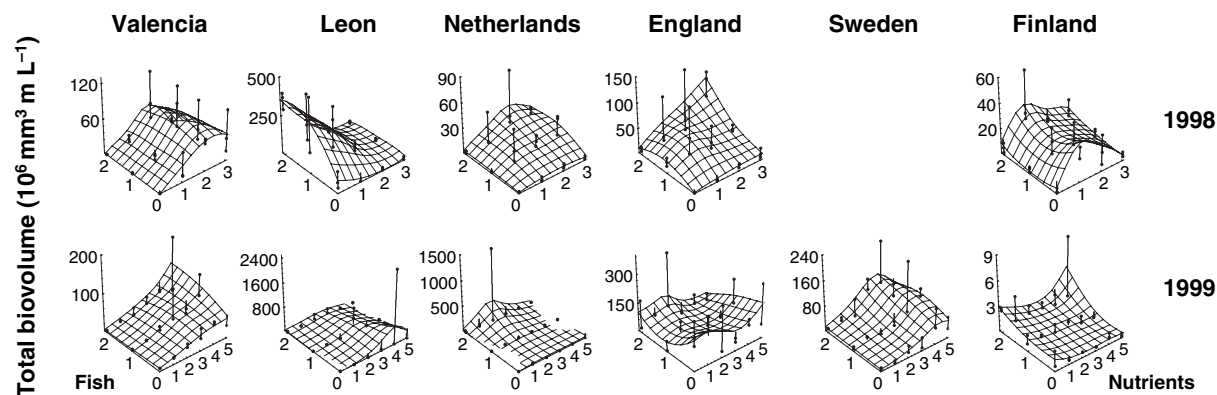
showed highly significant increases of chlorophyll *a* levels with increasing nutrient addition in seven of 10 experiments, and significant increases for total phytoplankton biovolume in four of 12 experiments (Table 3). There was an increase in algae with nutrients except with the highest nutrient level (10 mg L^{-1} N and 1 mg L^{-1} P; Figs 1 & 2). In one experiment (Leon in 1998), there was a highly significant decrease in total phytoplankton biovolume with nutrient addition, owing to the decrease of large colonial algae (Fernández-Alaez *et al.*, 2004), and in two others (Leon 1999 and Finland 1998) there was a significant effect, but with no consistent trend (Table 3).

In 1998, there were significant fish effects on chlorophyll *a* in all four experiments from which chlorophyll data are available. In the Valencia experi-

ment, chlorophyll *a* concentrations decreased with fish density, while in the Netherlands, England and Finland there was an increase (Table 3). In the Netherlands mesocosms in 1998, the fish effect was much stronger at the higher nutrient additions, resulting in a significant interaction effect between the fish and nutrient treatments (Table 3). In 1999, only in the Finnish experiment was there a significant increase of chlorophyll *a* with increasing fish density (Table 3).

Phytoplankton composition

Chlorophytes, cyanobacteria, cryptophytes and diatoms together comprised over 95% of the total phytoplankton biomass in most of the enclosure

Table 3 Treatment effects on chlorophyll *a* concentration (Chl-*a*) and total phytoplankton biomass (Biomass) in individual experiments

Location	Parameter	Fish 1998	Fish 1999	Nutrients 1998	Nutrients 1999	F × N 1998	F × N 1999
Valencia	Chl-*a*	↓*	n.s.	↑***	↑***	n.s.	n.s.
	Biomass	n.s.	n.s.	↑***	n.s.	n.s.	n.s.
Leon	Chl-*a*	no data	n.s.	no data	↑***	no data	n.s.
	Biomass	n.s.	n.s.	↓***	↕*	n.s.	n.s.
the Netherlands	Chl-*a*	↑***	n.s.	↑***	↑**	*	n.s.
	Biomass	↑**	n.s.	↑***	n.s.	n.s.	n.s.
England	Chl-*a*	↑***	n.s.	n.s.	n.s.	n.s.	n.s.
	Biomass	↑***	↓*	↑**	n.s.	n.s.	n.s.
Sweden	Chl-*a*	no data	n.s.	no data	↑***	no data	n.s.
	Biomass	no data	n.s.	no data	↑***	no data	n.s.
Finland	Chl-*a*	↑*	↑**	↑***	n.s.	n.s.	n.s.
	Biomass	n.s.	↑*	↕***	n.s.	n.s.	n.s.

Two-way ANOVA results: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant.
Arrows indicate direction of change with treatment level for fish and nutrient main effects: ↑, increase; ↓, decrease; ↕, no consistent trend.
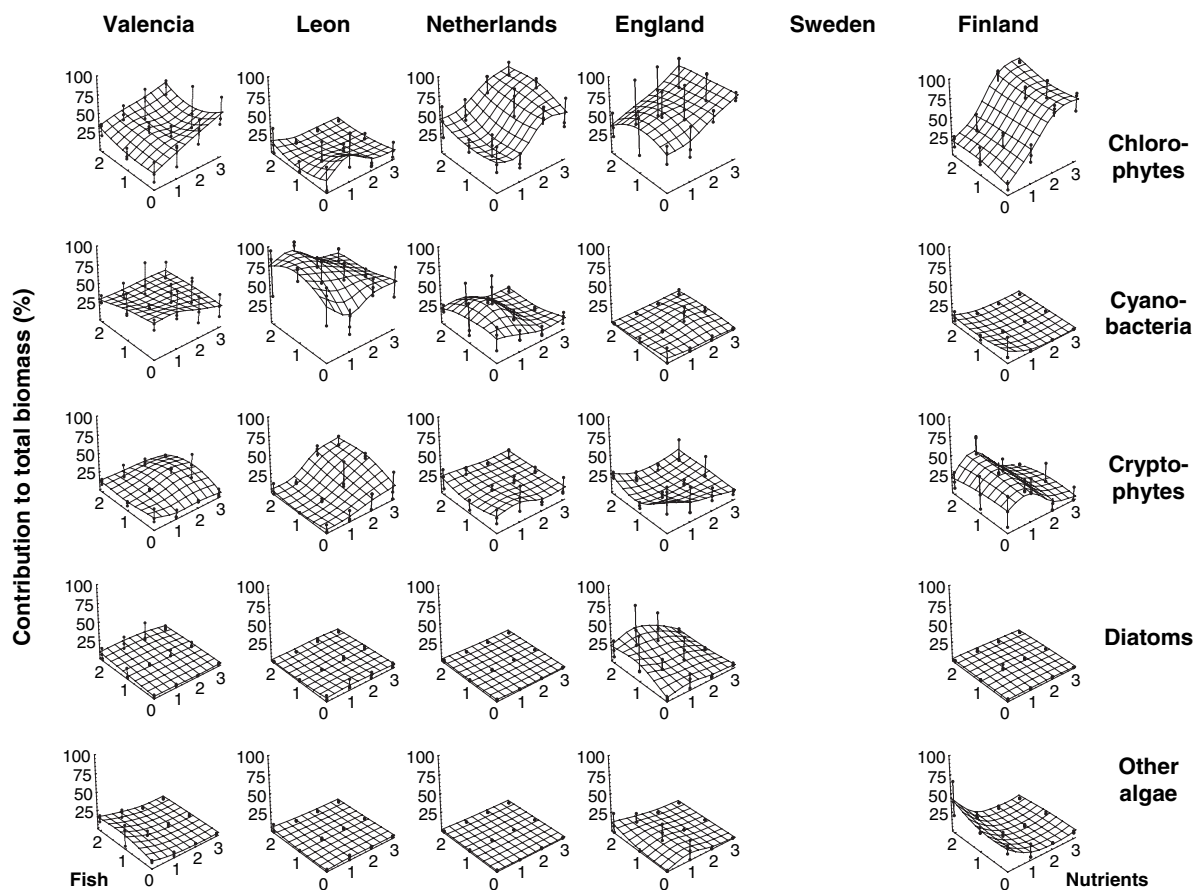
Fig. 3 Effect of fish and nutrient treatments on the contribution of chlorophytes, cyanobacteria, cryptophytes, diatoms and other algae to total phytoplankton biovolume (time-weighted averages) in a series of enclosure experiments performed in 1998 in six macrophyte-dominated shallow lakes distributed across Europe.

experiments in 1998 (Fig. 3) and 1999 (Fig. 4). Dinophytes were occasionally important in both Valencia experiments, as well as in the 1999 Leon experiment. Chrysophytes and euglenophytes were relatively important in both Finnish experiments but not in others.

Nutrient and fish effects differed widely among experimental locations. In 1998, there was a significant overall increase of chlorophytes and a decrease of cyanobacteria and diatoms with increasing nutrient additions (Table 1), coinciding with a shift in size distribution towards larger cells (GALD > 50 μm). In 1999, nutrient addition also significantly affected the contribution of chlorophytes, cyanobacteria and diatoms to the total phytoplankton biomass, but not in a consistent direction (Table 1).

The contribution of chlorophytes to the total phytoplankton biomass tended to increase with nutrient addition in most of the experiments (Table 4). Fish affected chlorophyte contribution significantly in a few experiments only, with no consistent pattern in the direction of change. Relative biomass of cyanobacteria decreased with fish addition in two experiments (Valencia 1999 and England 1999), with no significant effect in the other experiments (Table 4). This was due mainly to changes in species composition from colonial or filamentous species to smaller cyanobacteria with increasing abundance of planktivorous fish. Nutrient addition had significant effects on cyanobacterial contribution to total phytoplankton biomass in all experiments, but again there was no consistent pattern in the direction of change.

Effects of fish on the percentage of cryptophytes in the total phytoplankton biomass were only significant in three experiments (Table 4). Nutrient addition significantly affected cryptophytes in six experiments,
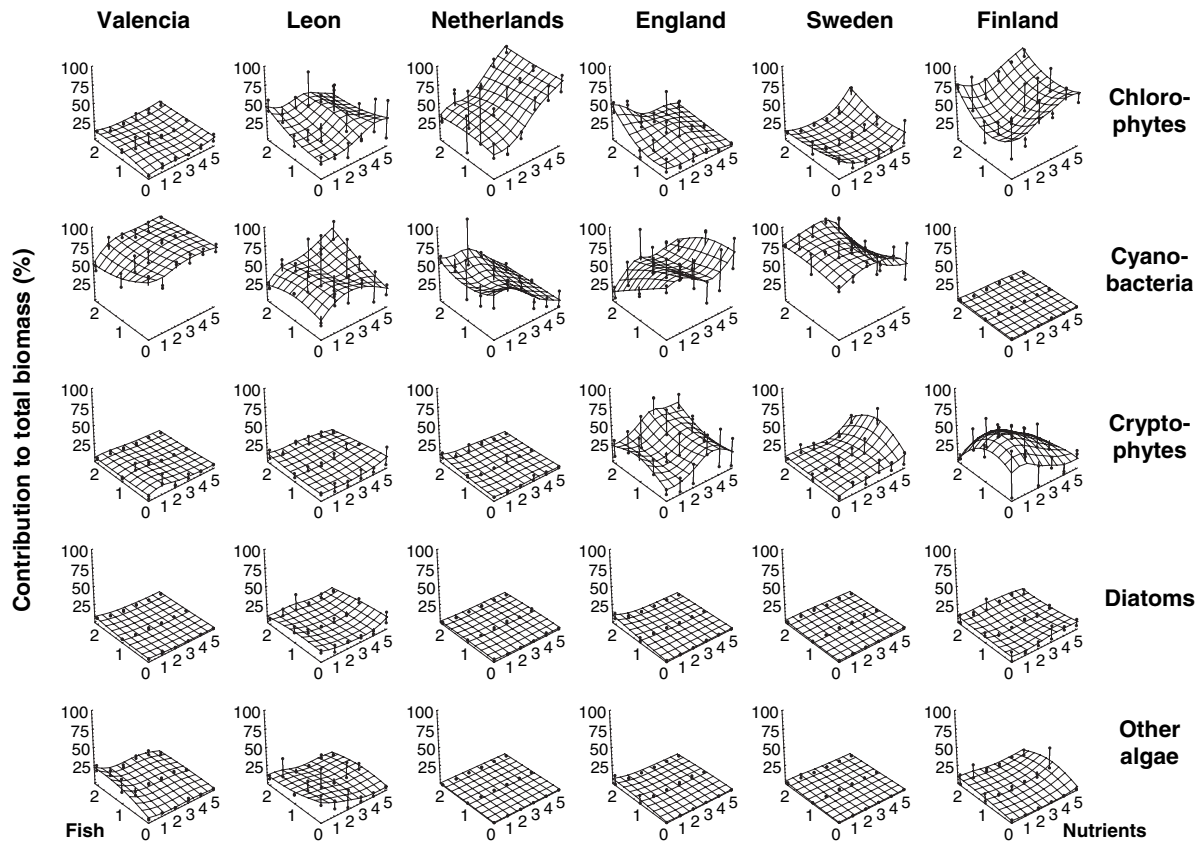
Fig. 4 Effect of fish and nutrient treatments on the contribution of chlorophytes, cyanobacteria, cryptophytes, diatoms and other algae to total phytoplankton biovolume (time-weighted averages) in a series of enclosure experiments performed in 1999 in six macrophyte-dominated shallow lakes distributed across Europe.

although again no general pattern emerged. The contribution of diatoms was generally quite small (Figs 3 & 4). The diatom contribution decreased with added nutrients in four experiments (Table 4). In general, increasing planktivorous fish densities had a positive effect on diatom relative biomass. Phytoplankton size composition (Table 5) was significantly altered by fish in five experiments, with a trend towards larger cells (GALD > 50 μm) in Valencia (1999), Leon (1998) and England (1999). Nutrient addition had a significant effect on size composition in four experiments, but only in Valencia (1999) was there a consistent trend towards larger cells with increasing nutrient addition.

Discussion

Although our results showed a marked variability both among locations and between years, some general patterns are apparent. Generally, total phytoplankton biomass was more influenced by nutrients than by the presence of planktivorous fish. However, strong fish effects occurred in specific experiments. Top-down control of phytoplankton biomass was particularly important in England (1998), the Netherlands (1998), Leon (1998) and Finland (1999), but only when nutrient levels were low. Regressions between chlorophyll *a* concentration and biomasses of zooplankton grazers (Vakkilainen *et al.*, 2004) led to comparable conclusions. In England in 1999, with initially high standing stocks, the zooplankton did not control phytoplankton biovolume. In general, increases in nutrients resulted in increased algal biomass, but in some experiments depletion of overall phytoplankton biomass occurred with very high nutrient additions.

Differences in macrophyte densities between locations appear to have influenced phytoplankton composition during our experiments. In the Spanish experiments (especially the 1999 Valencia experiment), macrophyte biomass was relatively high,

Table 4 Treatment effects on the contribution of chlorophytes, cyanobacteria, cryptophytes and diatoms in individual enclosure experiments carried out in 1998 and 1999 at six shallow-lake sites across Europe

Location	Algal taxon	Fish (F) 1998	Fish (F) 1999	Nutrients (N) 1998	Nutrients (N) 1999	F × N 1998	F × N 1999
Valencia	Chlorophytes	n.s.	↓*	↑*	↑**	n.s.	*
	Cyanobacteria	n.s.	↓*	n.s.	↑**	n.s.	*
	Cryptophytes	↕*	n.s.	n.s.	↕***	n.s.	n.s.
	Diatoms	↑*	↑*	n.s.	↓***	n.s.	n.s.
Leon	Chlorophytes	↓*	n.s.	n.s.	n.s.	n.s.	n.s.
	Cyanobacteria	n.s.	n.s.	↓*	n.s.	**	n.s.
	Cryptophytes	n.s.	n.s.	↑***	n.s.	n.s.	n.s.
	Diatoms	↓**	n.s.	n.s.	n.s.	n.s.	n.s.
the Netherlands	Chlorophytes	n.s.	n.s.	↑***	↑***	n.s.	n.s.
	Cyanobacteria	n.s.	n.s.	↕***	↓***	**	n.s.
	Cryptophytes	n.s.	n.s.	n.s.	↕*	n.s.	n.s.
	Diatoms	n.s.	n.s.	↓***	n.s.	n.s.	n.s.
England	Chlorophytes	n.s.	↑*	↑*	n.s.	n.s.	*
	Cyanobacteria	n.s.	↓**	n.s.	↕*	n.s.	n.s.
	Cryptophytes	↕*	n.s.	n.s.	n.s.	n.s.	n.s.
	Diatoms	n.s.	↑*	n.s.	↓***	n.s.	*
Sweden	Chlorophytes	no data	n.s.	no data	↕**	no data	n.s.
	Cyanobacteria	no data	n.s.	no data	↕**	no data	n.s.
	Cryptophytes	no data	n.s.	no data	↕**	no data	n.s.
	Diatoms	no data	↑*	no data	n.s.	no data	n.s.
Finland	Chlorophytes	n.s.	↕*	↑***	↑*	n.s.	n.s.
	Cyanobacteria	n.s.	n.s.	↓***	n.s.	n.s.	n.s.
	Cryptophytes	n.s.	↕*	↑***	↓*	n.s.	n.s.
	Diatoms	n.s.	n.s.	↓*	n.s.	n.s.	n.s.

Two-way ANOVA results: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant. Arrows indicate direction of change with treatment level for fish and nutrient main effects: ↑, increase; ↓, decrease; ↕, no consistent trend.

resulting in reduced fish predation pressure on zooplankton (Fernández-Alaez *et al.*, 2004; Romo *et al.*, 2004). The high macrophyte biomass enhanced phytoplankton diversity, with coexistence of both motile and non-motile algal forms such as cryptophytes, diatoms and chlorophytes in León (Fernández-Alaez *et al.*, 2004), and chroococcal cyanobacteria and cryptophytes in Valencia (Romo *et al.*, 2004). Both quiescence of the water and reduced fish access within the macrophyte beds may have contributed to these results.

Temperature could have influenced the dominance of algal groups. In central and northern Europe, water temperatures were considerably lower in 1998 than in 1999 (18 °C and 21 °C, respectively). León in northern Spain had an inverse pattern (23.4 °C in 1998, 19 °C in 1999). In all but one of these locations the contribution of cyanobacteria was considerably higher in the year with the highest temperature (Figs 3 & 4). The only exception was Finland, where the relative percentage of cyanobacteria was always very low. In Valencia, where water temperature was equally high in both

years (29 °C), cyanobacteria were well represented during both experiments. Similar increases in the contribution of cyanobacteria with increasing water temperature have been reported in shallow northern lakes (Bailey-Watts & Kirika, 1999), which is consistent with the relatively high temperature growth optimum of cyanobacteria (Reynolds, 1984; Komárek, 1985; Romo, 1994).

Increasing nutrient loadings in shallow lakes often lead to dominance of chlorophytes and cyanobacteria (Scheffer *et al.*, 1997). Jensen *et al.* (1994) found that cyanobacteria and chlorophytes were the predominant groups when nutrient concentrations increased in Danish shallow lakes, and argued that chlorophytes will outcompete cyanobacteria in hypertrophic conditions (>1 mg L^{-1} TP), owing to faster growth of chlorophytes under continuous input of nutrients from external and internal sources. This general pattern was also found in the present enclosure experiments; under eutrophic conditions (especially in the 1998 experiments, when higher nutrient levels were tested), the contribution of chlorophytes to total

Table 5 Treatment effects on contribution of small cells (GALD < 50 μm) to total phytoplankton biomass in individual enclosure experiments carried out in 1998 and 1999 at six shallow-lake sites across Europe

Location	Fish (F)		Nutrients (N)		F × N	
	1998	1999	1998	1999	1998	1999
Valencia	n.s.	↓***	n.s.	↓***	n.s.	n.s.
Leon	↓**	n.s.	n.s.	n.s.	*	n.s.
the Netherlands	n.s.	n.s.	n.s.	n.s.	*	n.s.
England	n.s.	↓*	↑*	n.s.	n.s.	n.s.
Sweden	no data	↕**	no data	↕***	no data	n.s.
Finland	n.s.	↕*	↕**	n.s.	n.s.	n.s.

Two-way ANOVA results: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s. – not significant.
Arrows indicate direction of change with treatment level for fish and nutrient main effects: ↑, increase; ↓, decrease; ↕, no consistent trend.

phytoplankton biomass increased with increasing nutrient enrichment.

Planktivorous fish were associated with a decrease in the contribution of cyanobacteria and chlorophytes to the total phytoplankton biovolume. However, when absolute biovolumes of each algal group are considered, cyanobacteria, chlorophytes and cryptophytes generally increased with both nutrient concentrations and planktivorous fish densities, whereas total biovolumes of diatoms and dinophytes decreased with fertilisation but increased with higher densities of planktivorous fish. Furthermore, cyanobacteria and cryptophytes clearly increased with nutrients in most locations, and presence of planktivorous fish also increased the biomass of cyanobacteria in three of six locations (Sweden, Leon and Valencia), in accordance with results from some other mesocosm experiments (Schriver *et al.*, 1995; Beklioglu & Moss, 1996). These positive effects of both fish and nutrients on cyanobacteria support the idea (Gragnani *et al.*, 1999) that cyanobacteria tend to dominate in eutrophic situations with high fish stocks but disappear when fish and nutrients are reduced. In practice, selectivity of zooplankton grazing favours cyanobacterial dominance in situations with high fish densities where zooplankters typically are small and feed selectively on specific algae (Romo *et al.*, 2004).

In conclusion, whilst nutrient addition had predictable and relatively consistent effect on phytoplankton biomass and composition, the effect of planktivorous fish was much more difficult to predict and depended very much on local conditions, including climatic variations among sites.

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