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SUMMARY

1. Responses of zooplankton to nutrient enrichment and fish predation were studied in 1998 and 1999 by carrying out parallel mesocosm experiments in six lakes across Europe.
2. Zooplankton community structure, biomass and responses to nutrient and fish manipulation showed geographical and year-to-year differences. Fish had a greater influence than nutrients in regulating zooplankton biomass and especially the relative abundances of different functional groups of zooplankton. When fish reduced the biomass of large crustaceans, there was a complementary increase in the biomasses of smaller crustacean species and rotifers.
3. High abundance of submerged macrophytes provided refuge for zooplankton against fish predation but this refuge effect differed notably in magnitude among sites.
4. Large crustacean grazers (*Daphnia*, *Diaphanosoma*, *Sida* and *Simocephalus*) were crucial in controlling algal biomass, while smaller crustacean grazers and rotifers were of minor importance. Large grazers were able to control phytoplankton biomass even under hypereutrophic conditions (up to 1600 μg TP L^{-1}) when grazer biomass was high (>80–90 μg dry mass L^{-1}) or accounted for >30% of the grazer community.
5. The littoral zooplankton community was less resistant to change following nutrient enrichment in southern Spain, at high temperatures (close to 30 °C), than at lower temperatures (17–23 °C) characterising the other sites. This lower resistance was because of a greater importance of nutrients than zooplankton in controlling algal biomass.
6. Apart from the reduced role of large crustacean grazers at the lowest latitude, no consistent geographical patterns were observed in the responses of zooplankton communities to nutrient and fish manipulation.

Keywords: eutrophication, fish predation, littoral zooplankton, mesocosm experiments, nutrient enrichment

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Introduction

The outcomes of impacts like nutrient enrichment are reflected in community structure and interactions within the food web. The trophic structure of communities can be seen as the partitioning of biomass

into different levels. It is basically determined by available resources and primary productivity but is also regulated by feedbacks from higher trophic levels, which in turn affect resource availability (Arditi & Ginzburg, 1989; Power, 1992; Leibold *et al.*, 1997; Polis, 1999; Persson *et al.*, 2001). Theories of food-web dynamics focus on the number of trophic levels. They predict relatively discontinuous, alternating (high/low/high) changes in biomasses along the food chain and an increasing number of trophic levels with increasing productivity (Hairston, Smith & Slobodkin, 1960; Fretwell, 1977; Oksanen *et al.*, 1981; Scheffer, 1991).

Such cascading effects between trophic levels have successfully explained incongruous plant/herbivore biomass variation in terms of resource availability. Evidence for strong vertical structuring of aquatic food webs has been gained through manipulations of upper trophic levels (Carpenter, Kitchell & Hodgson, 1985; Carpenter *et al.*, 1987; Horppila *et al.*, 1998; Jeppesen *et al.*, 1998b; Kairesalo *et al.*, 1999) and nutrient availability (Hansson, 1992; Persson *et al.*, 1992; Sarnelle, 1992). However, nutrient manipulations have shown a surprisingly high variability, including positive correlations of two adjacent trophic levels and responses unrelated to the number of trophic levels in the food web (Hansson, 1992; Mazumder, 1994; Brett & Goldman, 1997; Leibold *et al.*, 1997).

Variation in the responses of aquatic plankton communities to nutrient enrichment has indicated the importance of considering not only the number of trophic levels but also the nature of the organisms within them (Leibold *et al.*, 1997; Persson *et al.*, 2001) and variation in the efficiency of consumers to exploit their prey (Power, 1992). Compensatory community responses may account for much of the observed variation, and dampen trophic cascades when species vulnerable to predation are replaced by less vulnerable species. For instance, primary producer biomass may increase with increasing nutrient availability when species composition shifts to inedible species, even in the presence of efficient grazers (Mazumder, 1994; Abrams & Walters, 1996; McCauley *et al.*, 1999). Such declines in the edibility of food with increasing primary productivity may lead to ratio-dependent interactions and proportional changes in biomass at each trophic level (Arditi & Ginzburg, 1989; Power, 1992).

In shallow lakes and the littoral zones of larger lakes, spatial heterogeneity produced by submerged vegetation, coupled with movements of organisms between open water, vegetated, and benthic environments may provide important compensatory mechanisms. Through trophic interactions, this spatial heterogeneity affects water quality and may also buffer effects of nutrient enrichment (Scheffer *et al.*, 1993; Jeppesen *et al.*, 1998c; Scheffer, 1999).

The resistance of aquatic ecosystems to perturbations, such as nutrient enrichment, are controlled by variable environmental factors that are often difficult to predict. These include climate, and its manifestations in weather, an aspect that has received little explicit consideration in field studies, which have mainly been conducted in temperate ecosystems. Most biological processes are temperature dependent (McCauley & Murdoch, 1987; Lehman, 1988; Petchey *et al.*, 1999). Production of zooplankton has been shown to increase with temperature, while biomass accumulation is more dependent on resource availability (Shuter & Ing, 1997). Primary productivity at higher temperatures may become more controlled by nutrient turnover rate. Temperature may influence demography (Arditi & Ginzburg, 1989). Thus, with rising temperature, the inherently greater growth rate of algae compared with that of zooplankton might lead to reduced control of algal biomass by zooplankton. In addition, the threshold food requirement of zooplankton increases with water temperature and, for metabolic reasons, with increasing animal size (Lehman, 1988). This may mean a lower share of large-bodied, typically more efficient grazers in the zooplankton community at higher temperatures. Thus, the resource base may have a major role in the functioning of food webs as temperature increases, such that the cascading top-down effect of fish via zooplankton grazers on phytoplankton could become less important.

In this study, the response of zooplankton communities to experimental manipulation of nutrients and fish was studied in shallow-lake systems by comparing nutrient and plankton dynamics at six field sites across Europe.

We hypothesised (i) that algal biomass is controlled by large crustacean grazers and (ii) that large grazers increase in number or biomass with nutrient enrichment, unless they are controlled by planktivorous fish or submerged macrophytes provide refuges against

fish predation (Jeppesen *et al.*, 1998a; Scheffer, 1999). We aimed to find a threshold fish density below which zooplankton escapes control by fish. Furthermore, we hypothesised (iii) that the loss of large crustaceans is compensated by an increase in the biomass of smaller zooplankton, which exert weaker control of phytoplankton biomass. As we surmised that the resistance of plankton community structure and dynamics to nutrient enrichment is temperature dependent, we hypothesised (iv) that at warmer southern sites, control of phytoplankton by large crustacean zooplankton and the cascading effects of fish via zooplankton on phytoplankton are less important than nutrient control, and vice versa at colder northern sites.

Methods

Study sites and experimental design

Similar mesocosm experiments were carried out simultaneously in 1998 and 1999 (Stephen *et al.*, 2004a). The study lakes were shallow (mean depth about 1 m over the whole lake or over extensive lake areas) and potentially rich in submerged macrophyte vegetation. Experiments were run at 1-m depth at six nearshore sites in Lake Vesijärvi (Finland), Lake Krankesjön (Sweden), Little Mere (England), Lake Naardermeer (The Netherlands), Lake Sentiz (Leon, northern Spain) and Lake Xeresa (Valencia, southern Spain). Hereafter, the study sites are referred to as Finland, Sweden, England, Netherlands, Leon and Valencia.

The mesocosms were 1-m diameter open cylinders made of clear polyethylene suspended from wooden or plastic frames at the surface and sealed into the littoral sediment. The experiments had a fully factorial block design with three levels of fish biomass (0, 4 and 20 g fresh mass m^{-2}) in both years and four levels of nutrient enrichment (0, 1, 5 and 10 mg N L^{-1} combined with 0, 0.1, 0.5 and 1.0 mg P L^{-1}) in 1998 and six levels (0, 0.3, 0.6, 0.9, 1.5 and 3.0 mg N L^{-1} combined with 0, 0.03, 0.06, 0.09, 0.15 and 0.3 mg P L^{-1}) in 1999. Hereafter, the fish treatments are referred to as fish-free, low-fish and high-fish, and nutrient treatments as N0, N1, N2, N3, N4 and N5. The fish were locally the most appropriate planktivorous species of small individual size (5–10 cm). The low-fish treatment had at least two individuals. Each treatment had three

replicates in 1998 and two replicates in 1999, i.e. altogether 36 mesocosms in each year per site. The highest nutrient additions in 1998 resulted in unrealistically hypereutrophic conditions and mortality of fish in one location (see below). We therefore narrowed the nutrient range in 1999.

Samples for zooplankton were taken weekly for 6 weeks with a plastic tube (inner diameter 4 cm) from the whole water column of the mesocosms. Pretreatment samples for zooplankton were taken just before the first nutrient addition and the introduction of fish. Separate sub-samples were taken randomly from different places in each mesocosm to obtain 10 L of water. After mixing this pooled sample, a 3-L sub-sample was filtered through a 25-μm mesh net, and a 7-L sub-sample through a 50-μm mesh net to obtain rotifer and crustacean zooplankton samples, respectively. Zooplankton samples were preserved in 70% ethanol or 4% formaldehyde (final concentration). After counting and length measurement of zooplankton, crustacean biomasses were calculated from either length-dry weight (Bottrell *et al.*, 1976) or length-carbon regressions (Vasama & Kankaala, 1990; Luokkanen, 1995; A. Lehtovaara, pers. comm.). Rotifer carbon contents were obtained from Latja & Salonen (1978) and Telesh, Rahkola & Viljanen (1998). Dry weight of zooplankton was calculated assuming that the carbon content is 40% of dry weight, the average of values reported by Wiebe, Boyd & Cox (1975) and Latja & Salonen (1978). More details of the study lakes, mesocosm experiments, fish species used, sampling protocol and analyses of the samples are available in Stephen *et al.* (2004a,b), and in individual papers describing the results of the experiments (Fernández-Aláez *et al.*, 2004; Hansson *et al.*, 2004; Hietala, Vakkilainen & Kairesalo, 2004; Romo *et al.*, 2004; Stephen *et al.*, 2004b; Van de Bund & Van Donk, 2004).

Data analysis

Zooplankton taxa were grouped into four functional groups: (i) Rotifers; (ii) small crustacean grazers (≤0.5 mm), mainly *Bosmina* spp., *Ceriodaphnia* spp., chydorid cladocerans and copepod nauplii; (iii) large crustacean grazers (>0.5 mm), mainly *Daphnia* spp., *Diaphanosoma brachyurum* Liéven, *Sida crystallina* O.F. Müller, *Simocephalus* spp. and diaptomid calanoid copepods; and (iv) raptorial feeders (*Polyphemus*

pediculus L. and cyclopoid copepods). Time-weighted averages (pretreatment data not included) for zooplankton biomass, total phosphorus (TP) and chlorophyll *a* concentrations (chl *a*) were used for each replicate mesocosm. Macrophyte abundance was classified as negligible, sparse and abundant. The corresponding numeric values used are 0, 1 and 2.

The following data were excluded from analyses: (i) results of fish treatments with the two highest nutrient additions in 1998 in Finland, owing to the complete mortality of fish in those mesocosms; (ii) results from Sweden in 1998 because heavy storms destroyed the mesocosms; (iii) the Netherlands rotifer data because rotifers were counted only in week 6 in 1998; (iv) the 1999 fish treatments in Leon because of high fish mortality and because dense growth of *Myriophyllum alterniflorum* L. made it necessary to take water samples from an artificially separated macrophyte-free area in the centre of each mesocosm; and (v) the England mesocosms in 1999, which accidentally received a 10-fold higher phosphorus enrichment than planned.

All statistical analyses were made using SPSS for Windows (version 10.0). Treatment effects were tested separately for the two study years with MANOVA followed by univariate ANOVA with type III sums of squares and Tukey's test. Time-weighted average biomasses of the four zooplankton functional groups were the response variables, study sites were treated as block effects and nutrient and fish treatments were the factorial variables. The homogeneity of variances was tested with Levene's test and the normality of the data was tested with a Kolmogorov–Smirnov test and on the basis of the residual plots. Log_{10}-transformation was used to normalise the distribution of the data before the statistical analyses. However, the criteria for homoscedasticity were not met even after log-transformation, thus increasing the risk for type I error. Pillai's trace was used in multiple comparisons as this test of significance is the most robust to violations of assumptions (Scheiner, 1993). Non-transformed values are presented in the figures and tables.

In order to study the treatments between study years and sites, the responses of similar nutrient treatments in both study years were compared, i.e. with 0.1 mg P L^{-1}; 1.0 mg N L^{-1} in 1998 and 0.09 mg P L^{-1}; 0.9 mg N L^{-1} in 1999. Between-year comparisons were not possible for the whole enrichment regime owing to the different enrichment levels. The

responses of time-weighted mean zooplankton biomasses (BIOM) to nutrient enrichment and fish density were studied by calculating biomass response factors as follows: log_{10} [(BIOM$_{treatment}$ + 1)/ (BIOM$_{control}$ + 1)]. The response factors were calculated for both fish and nutrient treatments. Using linear regression, the zooplankton biomass response factors were tested for the effects of mean water temperature and macrophyte abundance as independent variables. This approach allowed inclusion of the Netherlands results, which could not be treated in the MANOVA owing to the lack of rotifer data. The use of response factors harmonised the data and, thus enabled comparison of nutrient effects on zooplankton biomasses under different water temperature and macrophyte density (Table 1).

General trends in the relation of zooplankton functional group biomass to chl *a* were studied at individual study sites. Results of both study years were pooled and analysed after log_{10} transformation using linear regression with chl *a* as the dependent variable and zooplankton biomass as the independent variable. Further analyses focused on the relationship between chl *a* and TP at different biomasses of large crustacean grazers. Before all regression analyses, normality of the data was tested on the basis of the residual plots. The overall potential of large crustacean grazers to control phytoplankton was further analysed at different productivity levels, measured as TP concentration, by using the whole data set without separating it into different study sites. Chl *a* was compared with the large crustacean grazer biomass and their percentage of the total grazer biomass. Further analyses aimed to discover whether possible threshold biomasses and shares of large grazers might provide a buffer mechanism and an efficient control of phytoplankton biomass over the whole range of TP concentrations.

Results

Weather conditions in northern and central Europe differed greatly between the study years: summer 1998 was cool and windy, whereas summer 1999 was warm (Table 1). The southernmost study site, Valencia, had the highest water temperature (29 °C) in both years. The ranges of the time-weighted mean chl *a* and TP concentrations in all experiments and treatments were 3–870 and 15–3700 µg L^{-1}, respectively, thus covering a wide range of mesotrophic to

Table 1 Characteristics of mesocosms at six shallow-lake sites across Europe during experiments in 1998 and 1999

Site	Year	Temperature (°C)	TP (µg L⁻¹)	Chl *a* (µg L⁻¹)	Total biomass (µg dry mass L⁻¹)	Dominant zooplankton taxa	Macrophyte abundance	Dominant macrophyte taxa
Finland	1998	17.3 (15–21)	632 (40–3700)	70 (7–200)	1500 (300–4500)	Rotifers, *Mesocyclops, Thermocyclops, Polyphemus pediculus* L.	Negligible	*Lemna trisulca* L.
Finland	1999	21.3 (17–23)	140 (50–400)	25 (8–65)	670 (330–1280)	*Ceriodaphnia, Diaphanosoma brachyurum* Liéven, *Daphnia longispina* O.F Müller, chydorid cladocerans, cyclopoid copepods	Abundant	*Lemna trisulca*
Sweden	1999	21.3 (19–23)	70 (25–280)	50 (3–380)	1240 (430–2840)	*Daphnia, Bosmina, Ceriodaphnia, Eudiaptomus*	Negligible	*Myriophyllum, Chara*
England	1998	17.8 (17–19)	500 (120–1250)	50 (10–240)	450 (200–100)	*Daphnia hyalina* Leydig, *Eudiaptomus gracilis* G.O. Sars, *Bosmina, Ceriodaphnia,* cyclopoid copepods, rotifers	Sparse	*Potamogeton, Elodea canadensis* Michx, *Ceratophyllum demersum* L.
Netherlands	1998	18.5 (17–22)	100 (15–350)	20 (3–90)	40 (1–300)*	*Ceriodaphnia, Bosmina, Eudiaptomus*	Sparse	*Chara*
Netherlands	1999	22.4	100 (20–360)	30 (6–85)	30 (2–220)*	*Ceriodaphnia, Daphnia galeata* Sars, *Diaphanosoma brachyurum*	Abundant	*Chara*
Leon (northern Spain)	1998	23.2 (20–26)	430 (70–1080)	45 (5–120)	3400 (1270–8400)	*Ceriodaphnia,* chydorid cladocerans, *Daphnia longispina, Eucyclops, Macrocyclops, Megacyclops*	Abundant	*Myriophyllum alterniflorum* L.
Leon (northern Spain)	1999	19.7 (18–21)	65 (40–130)	20 (10–35)	760 (160–2450)	*Ceriodaphnia,* chydorid cladocerans, *Eucyclops, Macrocyclops, Megacyclops*	Abundant	*Myriophyllum alterniflorum*
Valencia (southern Spain)	1998	28.7 (28–29)	400 (20–1150)	230 (3–870)	600 (50–1800)	Rotifers, *Ceriodaphnia, Acanthocyclops*	Abundant	*Chara*
Valencia (southern Spain)	1999	28.7 (27–30)	80 (15–230)	50 (6–180)	555 (65–1260)	Rotifers, *Ceriodaphnia, Acanthocyclops, Thermocyclops*	Abundant	*Chara*

*Biomass available only for total crustacean zooplankton.

Mean values with ranges in parentheses are provided for temperature. Time-weighted mean values with ranges in parentheses are given for total phosphorus (TP) and chlorophyll *a* (Chl *a*) concentrations and for total zooplankton biomasses.
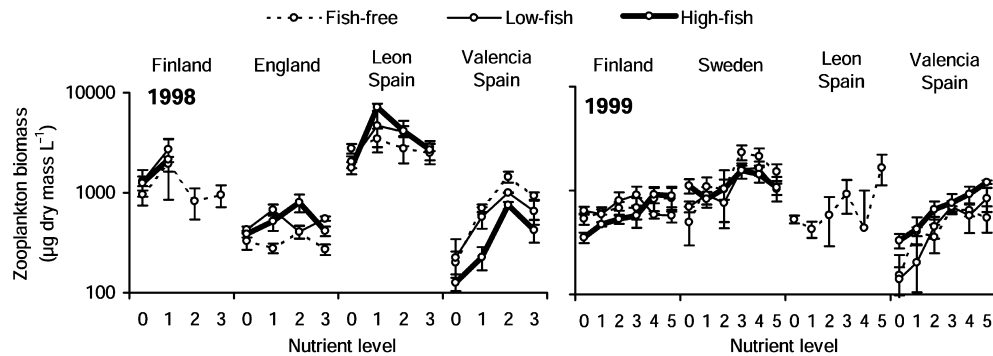
Fig. 1 Time-weighted mean (±SE) of total zooplankton biomass in different nutrient and fish treatments of a series of mesocosm experiments carried out at six shallow-lake sites across Europe in 1998 and 1999. Nutrient and fish treatments are given in sequential order according to the levels detailed in the text.

hypereutrophic conditions (Table 1). The species composition and abundance of macrophyte and zooplankton communities varied among sites and years and there was no obvious geographical pattern (Table 1). The cladoceran community was mostly characterised by small genera such as *Ceriodaphnia*, *Bosmina* and chydorid cladocerans, which often occurred together with cyclopoid copepods. *Daphnia* was found only in a few experiments, while *Diaphanosoma* was a quite common large-bodied cladoceran at many sites (Table 1). Total zooplankton biomasses reached higher values in 1998, when nutrient manipulations included greater enrichment than in 1999 (Table 1, Fig. 1). They also showed an increasing trend with nutrient enrichment in both years and in 1998, when the greatest enrichment exceeded that in 1999, the highest biomasses were often measured at intermediate nutrient levels (Fig. 1).

Nutrient enrichment had a marginally significant multivariate effect on zooplankton only in 1998, when it increased the biomass of small crustacean grazers and, in all sites except Valencia, decreased that of raptorials (Table 2; Fig. 2). The responses of large crustacean grazers to nutrient enrichment were mostly negligible in fish-free treatments, and negative in fish treatments (Fig. 2). Thus, neither the main effect of nutrients nor the joint effect of nutrients and fish were significant on large grazers.

Fish had a highly significant multivariate effect on zooplankton in both years (Table 2). In general, the biomass of rotifers increased, and those of all other functional groups decreased with increasing fish density (Table 2; Fig. 2). Differences in the biomass of almost all zooplankton groups were significant between no-fish and high-fish treatments as well as between no-fish and low-fish treatments (Tukey's test).

Table 2 MANOVA and ANOVA results of effects of treatments (nutrients and fish) and block (site) on time-weighted averages of zooplankton biomass in mesocosm experiments carried out at four shallow-lake sites across Europe in 1998 and 1999

Year	Source of effect	Pillai's trace			Anova	Rotifers		Small crusta-cean grazers		Large crusta-cean grazers		Raptorials	
		d.f.	F	P	d.f.	F	P	F	P	F	P	F	P
1998	Nutrient	12	1.8	0.052	3	1.4	0.245	2.9	0.038	1.2	0.308	3.1	0.030
	Fish	8	14.0	<0.001	2	33.1	<0.001	10.6	<0.001	44.0	<0.001	19.1	<0.001
	Nutrient × fish	24	0.8	0.743	6	1.4	0.221	0.9	0.519	1.2	0.326	0.6	0.748
	Site	12	41.8	<0.001	3	98.3	<0.001	52.0	<0.001	57.7	<0.001	28.4	<0.001
1999	Nutrient	20	0.9	0.594	5	0.7	0.652	1.2	0.322	1.4	0.242	1.3	0.271
	Fish	8	9.1	<0.001	2	34.2	<0.001	14.2	<0.001	9.6	<0.001	12.5	<0.001
	Nutrient × fish	40	0.7	0.903	10	0.4	0.922	0.2	0.992	1.9	0.057	0.3	0.985
	Site	12	27.0	<0.001	3	52.2	<0.001	90.2	<0.001	398.5	<0.001	120.4	<0.001

Results are based on $\log_{10}$-transformed variables. The Netherlands' results were excluded from this analysis.
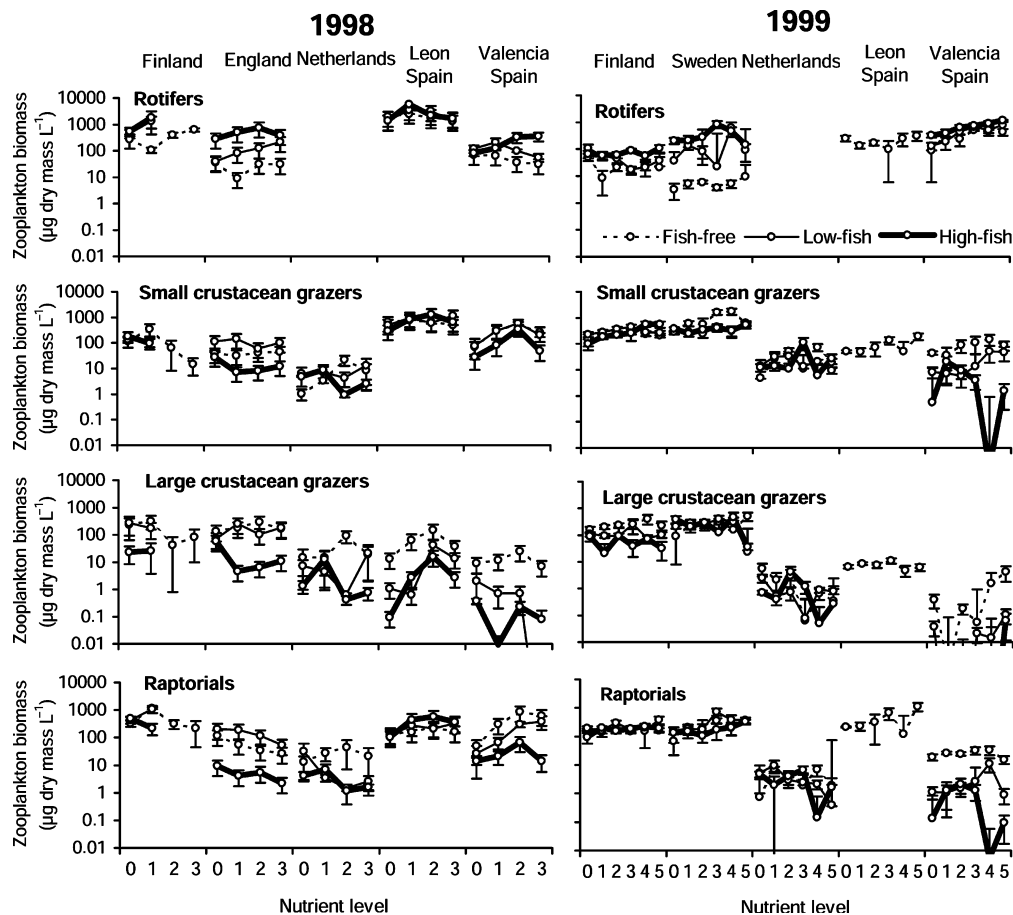
Fig. 2 Time-weighted mean (±SE) biomasses of four zooplankton functional groups in different nutrient and fish treatments of a series of mesocosm experiments carried out at six shallow-lake sites across Europe in 1998 and 1999. Nutrient and fish treatments are given in sequential order according to the levels detailed in the text.

Zooplankton biomass differed among study sites, resulting in a highly significant multivariate main effect (Table 2). Lowest total biomasses were found in Valencia, the southernmost site, which differed greatly from Leon, the other Spanish site, and also from almost all other sites (Figs 1 & 2). Conversely, at the two northernmost sites in Finland and Sweden, the zooplankton communities resembled each other in 1999 (Fig. 2). The response factors of total zooplankton biomass showed a consistently positive effect of nutrients, whereas the effect of fish was variable (Fig. 3). Similarly to MANOVA and ANOVA, the response factors of distinct functional groups showed strong effects of fish and less clear effects of nutrients. In the absence of fish, crustacean grazers and raptorials responded positively to nutrient enrichment, rotifers mostly negatively. In fish treatments,

however, the pattern was much more variable with both positive and negative responses without clear geographical trends. In Valencia, zooplankton biomasses increased with nutrients in all fish treatments, with the exception of large crustacean grazers. The Netherlands results were generally in line with those of the other sites (Fig. 3). An exception was the positive responses of large crustacean grazers in high-fish treatments. The response factors of small crustacean grazers to nutrients were significantly related to mean water temperature (linear regression analysis; $R^2 = 0.26$; $F_{1,27} = 9.08$; $P = 0.006$). High water temperatures in 1999 (Table 1) apparently enhanced the reproduction of small crustaceans in Finland, Sweden and The Netherlands. At the high temperature in Valencia, small crustaceans increased with nutrients in both study years (Fig. 3).
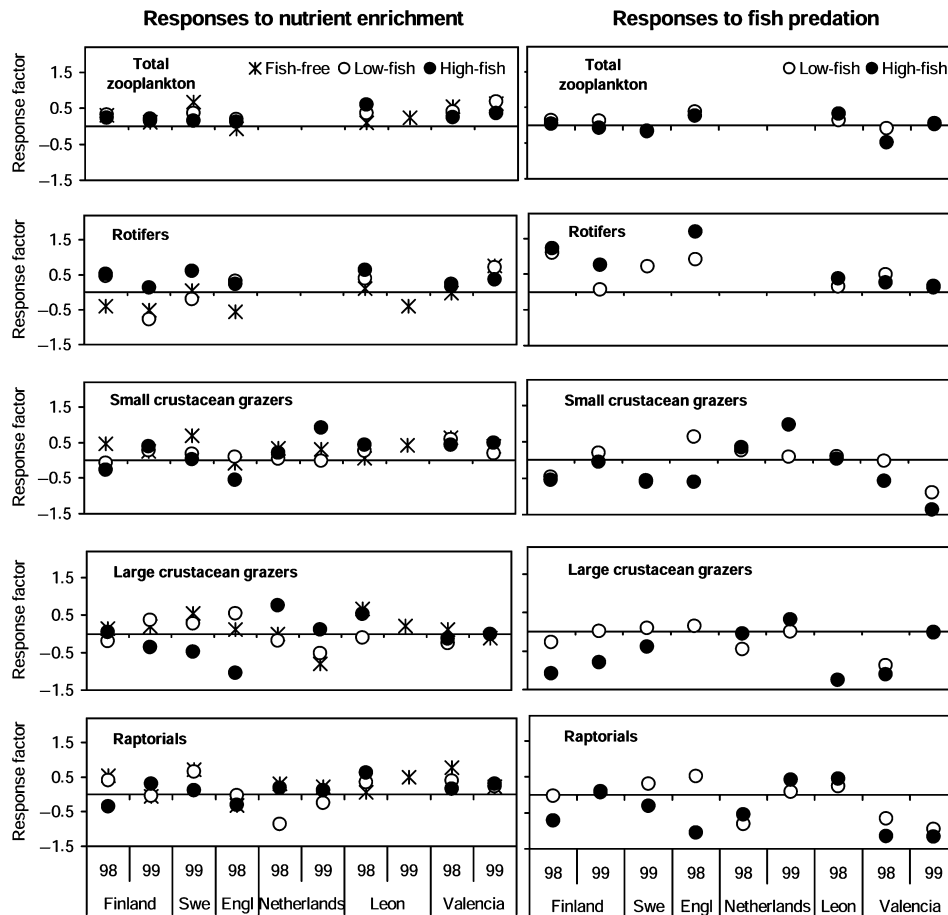
Fig. 3 Response factors of the biomasses of total zooplankton and four zooplankton functional groups for nutrient (left) and fish (right) treatments of a series of mesocosm experiments carried out at six shallow-lake sites across Europe in 1998 and 1999 with similar nutrient additions, i.e. 0.1 mg P + 1 mg N L^{-1} in 1998 and 0.09 m P + 0.9 mg N L^{-1} in 1999. Abbreviations: Swe, Sweden; Engl, England.

Interactions between zooplankton and phytoplankton

Linear regressions of log-transformed data showed in general a positive or negligible relationship between chl *a* and biomass of both rotifers and small crustacean grazers (Table 3). Chl *a* decreased with increasing biomass of small crustacean only in Finland and England. In contrast, the biomasses of large crustacean grazers were inversely related to chl *a*, except in Valencia where chl *a* was unrelated to the biomass of large crustacean grazers (Table 3). Thus, the potential of large grazers to control phytoplankton biomass was shown at all study sites except Valencia.

Extremely high chl *a* concentrations (≥ 190–200 μg L^{-1}) were observed when large grazers accounted for less than about 30% of total zooplankton biomass (Fig. 4a). At higher grazer biomasses,

there was only one outlying point. A similar picture was obtained when comparing chl *a* concentration with the biomass of large grazers, with only two outlying points at grazer biomasses >80–100 μg dry mass L^{-1} (Fig. 4b). At a biomass of large grazers >80 μg dry mass L^{-1}, chl *a* concentration increased only slightly with increasing TP concentration (slope = 0.22, R^2 = 0.09, P = 0.005, n = 83; linear regression analysis of log-transformed data) and the mean chl *a* concentration was 27 μg L^{-1} with a SE of ±3 (Fig. 4d). At lower grazer biomasses, there was a significant relationship between chl *a* and TP (slope = 0.56, R^2 = 0.35, P < 0.001, n = 167) and the mean chl *a* concentration was 96 μg L^{-1} with a SE of ±11.

With a >30% share of large grazers, the slope between chl *a* and TP was 0.27 (R^2 = 0.18, n = 52)

Table 3 Results of linear regressions describing relationships between zooplankton biomass and chl a ($\log_{10}$-transformed) determined in a series of mesocosm experiments at six shallow-lake sites across Europe

Dependent variable	Site, years	d.f.	slope	R^2	F	P
Rotifers	Finland, 1998 & 1999	59	0.29	0.29	24.1	<0.001
	Sweden, 1999	35	0.17	0.07	2.6	0.12
	England, 1998	35	0.35	0.53	38.8	<0.001
	Leon, 1998 & 1999	47	0.21	0.09	4.5	0.039
	Valencia, 1998 & 1999	71	−0.13	0.01	0.6	0.43
Small crustacean grazers	Finland, 1998 & 1999	59	−0.19	0.07	4.1	0.046
	Sweden, 1999	35	0.26	0.03	0.9	0.36
	England, 1998	35	−0.25	0.18	7.6	0.009
	Netherlands, 1998 & 1999	71	0.12	0.03	1.9	0.18
	Leon, 1998 & 1999	47	0.20	0.06	3.2	0.08
	Valencia, 1998 & 1999	71	0.32	0.20	17.9	<0.001
Large crustacean grazers	Finland, 1998 & 1999	59	−0.19	0.12	7.6	0.008
	Sweden, 1999	35	−0.67	0.30	14.3	0.001
	England, 1998	35	−0.33	0.50	34.4	<0.001
	Netherlands, 1998 & 1999	71	−0.41	0.33	34.3	<0.001
	Leon, 1998 & 1999	47	−0.35	0.37	27.3	<0.001
	Valencia, 1998 & 1999	71	0.28	0.04	2.7	0.11

(Fig. 4c). Predictability of phytoplankton control was not increased further by raising the threshold biomass of large grazers from 80 to 100 µg DW L^{-1}. Moreover, the lower number of observations containing these high biomasses likely augmented the possibility of coincidence. In contrast, when the biomass thresholds were lowered, the slope increased, e.g. to 0.26 ($R^2 = 0.11$) with a large grazer biomass of ≥60 µg DW L^{-1}.

Similarly, when the 30% threshold of large grazers was shifted to ≥20 and ≥40%, the slopes changed to 0.34 ($R^2 = 0.21$, $n = 71$) and 0.26 ($R^2 = 0.18$, $n = 42$), respectively.

Changes in chl a over the TP concentration range were explained better by the abundance of large grazers than by the presence or absence of fish (Fig. 4e). There was a significant relationship between
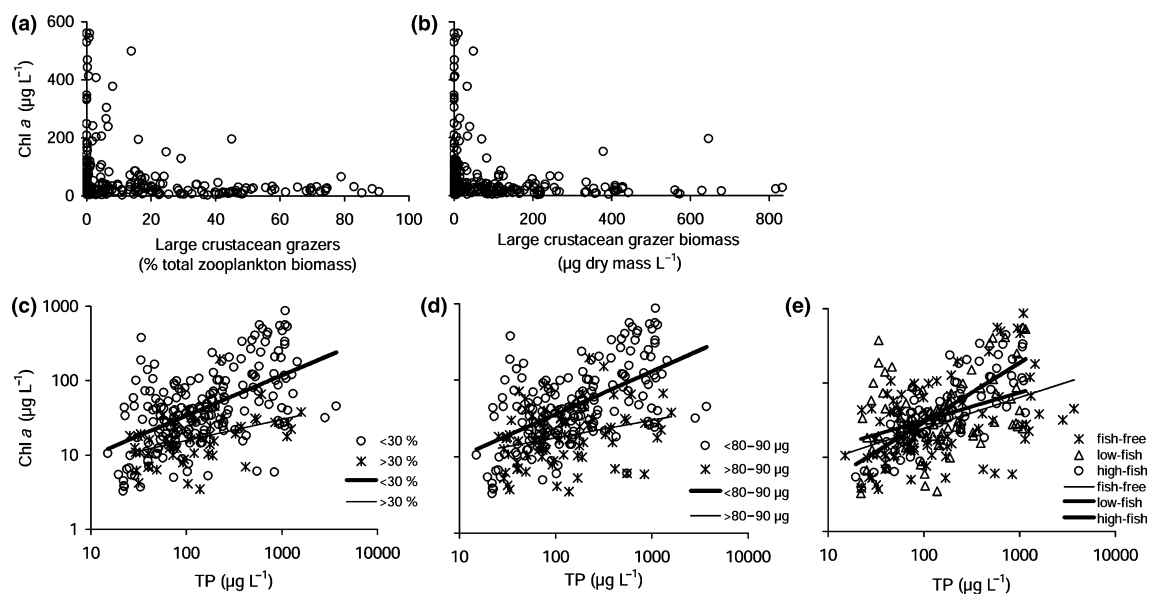

Fig. 4 Relationship between chlorophyll a concentration (chl a) and the (a) biomass share and (b) absolute biomass of large crustacean grazers; chl a and the total phosphorus concentration (TP) in treatments with a biomass of large grazers below and above (c) 30% of the total zooplankton biomass and (d) 80–90 µg dry mass L^{-1}; and (e) chl a and TP at three fish densities. Data are from mesocosm experiments carried out at six shallow-lake sites across Europe in 1998 and 1999. Note the logarithmic presentation of both axes in Fig. 4c–e.

chl *a* and TP even in fish-free treatments (slope = 0.41, $R^2 = 0.21$, $P < 0.001$, $n = 95$). This relationship did not markedly change in low-fish treatment (slope = 0.37, $R^2 = 0.13$, $P = 0.001$, $n = 77$), but a stronger response was observed in the high-fish treatment (slope = 0.78, $R^2 = 0.78$, $P < 0.001$, $n = 77$).

Discussion

The results of this pan-European mesocosm experiment emphasise the importance of consumer control over resource control in determining zooplankton biomass in shallow lake systems. Thus, our results are in agreement with the general view that (large) herbivores respond strongly to predator manipulations in aquatic food webs (Brett & Goldman, 1997; Mehner *et al.*, 2002; Shurin *et al.*, 2002). Rotifers increased with nutrient enrichment in fish treatments, while small crustacean grazers showed positive responses to nutrient enrichment largely independently of the presence or absence of fish. Small zooplankton species apparently benefited from the mostly negative responses of their larger competitors and raptorial predators to the presence of fish. Thus, within zooplankton communities different functional groups responded inconsistently to the treatments. Such compensation suggests a ratio-dependent functional response and is consistent with the view that nutrient enrichment leads to proportional increases at all trophic levels (Arditi & Ginzburg, 1989; Leibold *et al.*, 1997).

The traditional equilibrium theory of food chain dynamics and its modifications (Hairston *et al.*, 1960; Fretwell, 1977; Oksanen *et al.*, 1981; Scheffer, 1991) emphasises the control of lower trophic levels by consumers, and predicts that effects of nutrient enrichment are transferred into biomass at the top trophic level and even-numbered levels below it. Accordingly, zooplankton biomass increases with enhanced primary productivity in two-level systems (phytoplankton and herbivorous zooplankton) but does not change in three-level systems (phytoplankton, herbivorous zooplankton and planktivorous fish). In contrast to this theory, the higher zooplankton biomass we observed in fish treatments compared with fish-free treatments, especially in 1998, shows that total zooplankton biomass may increase with nutrients independently of the number of trophic links in the food chain. Several field and laboratory studies of aquatic food chains have shown similar increases with nutrient enrichment in both two- and three-level systems (Mazumder, 1994; Leibold *et al.*, 1997; Persson *et al.*, 2001). In our study, available food resources appeared to determine total zooplankton biomass, whereas fish regulated relative abundances of different functional groups. This emphasises the importance of compositional changes within trophic levels and reveals the limitation of simple food-chain and ratio-dependent models to capture such changes, as pointed out by Leibold *et al.* (1997) and Hulot *et al.* (2000). In addition, our result that algal biomass was positively related with TP concentration even in the presence of efficient grazers has often been observed in previous studies (Hansson, 1992; Mazumder, 1994; Brett & Goldman, 1997; Leibold *et al.*, 1997; Shuter & Ing, 1997; Persson *et al.*, 2001). It reflects the positive response of adjacent trophic levels and, therefore, is not consistent with traditional food-chain theory.

The variability of responses to fish and nutrient manipulations was also influenced by substantial variation in community structure among sites. For instance, in Leon, the biomass of large crustacean grazers in 1998 increased with nutrients even when fish density was high, most probably through the refuge effect of the abundant growth of *Myriophyllum* (Timms & Moss, 1984; Jeppesen *et al.*, 1998a; Scheffer, 1999; Burks *et al.*, 2002). In shallow lakes and littoral zones, refuges provided to zooplankton by macrophytes work as an important buffering mechanism to changes in fish predation pressure (Jeppesen *et al.*, 1998c). *Chara* beds have also been considered as good refuges for zooplankton against fish predation (Diehl, 1988; Jeppesen *et al.*, 1998a), but our results do not support this view as *Chara* at the study sites in Valencia and Netherlands did not prevent efficient foraging of fish for large crustacean grazers.

There were substantial differences in zooplankton community structure and biomass among study sites, and even between study years at the same site. Nonetheless, some consistent patterns were evident. Valencia in southern Spain differed from the other sites in that zooplankton had a minor influence on phytoplankton dynamics. Nutrient enrichment apparently increased algal growth rate more than that of zooplankton and thus disrupted top-down control of algae (cf. Arditi & Ginzburg, 1989; Power, 1992). In addition, as Power (1992) pointed out, if grazers were subject to predation, the grazer control of phytoplankton would further weaken relative to resource control.

Our result supports this hypothesis because fish efficiently reduced the biomasses of large grazers in Valencia and the remaining small grazers were unable to control algal biomass.

Daphnia has been suggested as a keystone species and its abundance as a useful indicator to predict the grazing impact of zooplankton on phytoplankton (Hansson, 1992; Sarnelle, 1992; Mazumder, 1994; Persson *et al.*, 2001). Supporting evidence for food-chain theory has been gained especially from open-water systems with *Daphnia* as the key grazer (Carpenter *et al.*, 1985, 1987; Jeppesen *et al.*, 1998b). In our experiments, however, *Daphnia* was relatively sparse and virtually absent in fish treatments. Its biomass was therefore pooled with that of other large grazers. The potential of large grazers to control phytoplankton biomass was shown at all study sites except Valencia. The share of large crustacean grazers of the whole grazer biomass had a clear inverse relationship with chl *a* concentration. A consistent result was obtained when chl *a* concentration was related to the biomass of large grazers. When abundant, these large grazers efficiently controlled phytoplankton biomass even under hypertrophic conditions (up to 1600 μg TP L^{-1}). Otherwise, chl *a* concentration increased steeply with increasing TP concentration. Sarnelle (1992), moreover, found even stronger differences in the chl *a*/TP relationship in systems with both high and negligible biomass of *Daphnia*. Our empirically obtained threshold biomass (80–90 μg dry mass L^{-1}) is close to that (ca 100 μg dry mass L^{-1}) at which Hansson (1992) found an efficient control of algal biomass by grazers.

Increase in the biomass of small crustaceans and rotifers was generally associated with an increase in chl *a*, indicating the low ability of small zooplankton to control total phytoplankton biomass. In Finland and England, however, even small grazers such as *Ceriodaphnia* were apparently able to restrain algal biomass. In Finland, this control was probably enhanced by high abundance of the free-floating macrophyte, *Lemna trisulca* L. (Hietala *et al.*, 2004). Rotifers are highly specialised suspension-feeders and are not able to control total algal biomass, at least in eutrophic systems, because they feed mainly on nanoplankton such as bacteria, flagellates, ciliates, and small algae (Pourriot, 1977; Gilbert & Bogdan, 1984). In addition, rotifers probably recycle nutrients more effectively than larger crustaceans (Vanni, 2002) and therefore further enhance the availability of nutrients to algae.

Nonetheless, the food-web role of rotifers is emphasised under turbid conditions. Through providing food for young-of-the-year (YOY) fish, they may maintain a high recruitment of zooplanktivorous fish. The proliferation of small crustaceans and rotifers in our experiment was probably related to their lower susceptibility to fish predation. Additionally, their shorter generation times compared with larger crustaceans allowed the smaller zooplankton to respond quickly to nutrient enrichment. On the contrary, it is unlikely that life-history constraints affected the nutrient enrichment responses of the larger zooplankton during the 5-week experiments as with an average growth rate of 0.3 day^{-1} populations turned over at least 15 times. Suppression of large herbivores at higher water temperature (Beisner, McCauley & Wrona, 1997) and/or in enriched systems (McCauley *et al.*, 1999) has been explained by increases in inedible algae. However, no such consistent dominance of inedible algae (i.e. forms having a Greatest Axial or Linear Dimension, GALD, of ≥50 μm) was observed in our enriched or high-temperature mesocosms (Van de Bund *et al.*, 2004).

A steep slope between chl *a* and TP concentration suggests a cascading effect of fish through herbivorous zooplankton on phytoplankton in systems with three trophic levels (Hansson, 1992; Mazumder, 1994). Our results from high-fish treatments agree with these predictions, although the slope in low-fish treatments was less steep and similar to that in fish-free treatments. However, the abundance of large crustacean grazers explained the relationship between chl *a* and TP better than did the mere number of trophic levels. This can be explained by the fact that our experimental systems involved several hypereutrophic fish-free systems with low biomass of large grazers, as well as several low-fish systems with high biomass of large grazers. In addition, small crustacean grazers that were not heavily preyed upon by fish probably had at least a complementary role in controlling phytoplankton biomass. Thus, our results show that high fish biomass (20 g dry mass m^{-2}) leads to generally low grazer biomass and consistently to high algal biomass with increasing nutrient availability. However, when fish biomass is low (4 g dry mass m^{-2}) grazer biomass can be high and control algal biomass in shallow-lake systems.

In conclusion, fish had a greater role than nutrients in regulating zooplankton biomass and especially

relative abundances of zooplankton functional groups. Large crustacean grazers were of crucial importance in controlling algal biomass even under hypereutrophic conditions provided that their biomass proportion or absolute biomass was high. This result supports our first hypothesis that algal biomass is controlled by large crustacean grazers. However, our second hypothesis that large crustaceans increase in number or biomass with nutrient enrichment either in the absence of fish or when protected from fish predation by submerged macrophytes, was not supported. Although abundant submerged macrophytes often provided refuges for zooplankton against fish predation as expected, differences in the magnitude of the refuge effect occurred at different sites. These may be because of differences in macrophyte species, fish species or other factors that varied among sites. In agreement with our third hypothesis, the biomass of large crustaceans was reduced when fish were present and smaller zooplankton species with minor or complementary importance in controlling phytoplankton biomass became more abundant. Our fourth hypothesis that at southern, warmer sites control of primary producers by large crustacean zooplankton and cascading effects of fish via zooplankton grazers are less important than nutrients, and vice versa at the northern cooler sites, was supported by the Valencia results; the potential of zooplankton, especially that of large crustacean grazers, to control phytoplankton biomass was evident at all other sites. Apart from the reduced role of large crustacean grazers in Valencia, no consistent geographical patterns were observed in the responses of zooplankton communities to nutrient and fish manipulation.

Acknowledgments

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