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REPORT

Complementary impact of copepods and cladocerans on phytoplankton

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Abstract

The differences in the impact of two major groups of herbivorous zooplankton (Cladocera and Copepoda) on summer phytoplankton in a mesotrophic lake were studied. Field experiments were performed in which phytoplankton were exposed to different densities of two major types of herbivorous zooplankton, cladocerans and copepods. Contrary to expectation, neither of the two zooplankton groups significantly reduced phytoplankton biomass. However, there were strong and contrasting impacts on phytoplankton size structure and on individual taxa. Cladocerans suppressed small phytoplankton, while copepods suppressed large phytoplankton. The unaffected size classes compensated for the loss of those affected by enhanced growth. After contamination of the copepod mesocosms with the cladoceran *Daphnia*, the combined impact of both zooplankton groups caused a decline in total phytoplankton biomass.

Keywords

Cladocera, Copepoda, herbivory, phytoplankton, size spectrum, top-down control, zooplankton.

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INTRODUCTION

Cladocerans and copepods are crustacean taxa which are major components of mesozooplankton (zooplankton with a body size of 0.2–2 mm), and contribute significantly to grazing pressure on phytoplankton. Strong top-down effects on phytoplankton, including order-of-magnitude reductions of phytoplankton biomass, have been reported for cladoceran-dominated zooplankton in lakes (Lampert 1978, 1988; Sommer *et al.* 1986) and for copepod-dominated zooplankton in the sea (Bautista *et al.* 1992). While copepods tend to dominate crustacean zooplankton in most marine habitats, cladocerans, particularly *Daphnia* spp., do so in many lakes. Copepods generally prefer larger food particles than cladocerans, although a wide overlap in the food spectrum is generally found (Gliwicz 1980; Geller & Müller 1981; Kleppel 1993; Sommer *et al.* 2000, 2001). Here, we report field mesocosm experiments from a lake in which phytoplankton has been subjected to different densities of both zooplankton groups. The phytoplankton response was analysed at the level of total biomass, size classes and individual taxa.

METHODS

We installed 24 mesocosms in the moderately nutrient-rich lake Schöhsee (northern Germany) in which natural phytoplankton and microzooplankton were exposed to logarithmically scaled gradients of cladoceran (*Daphnia hyalina X galeata*) and copepod (*c.* 1/2 *Eudiaptomus* spp. and 1/2 copepodide stages of cyclopoid copepods) seeding density. The mesocosms consisted of transparent polyethylene enclosures, 3.4 m^3 in volume and 3.2 m in depth. On 7 August 2000, mesocosms were filled by lake water sieved through 50 μm plankton gauze in order to remove mesozooplankton, and fertilized by phosphorus in order to ensure a balanced total N : total P ratio (Redfield ratio 16 : 1; here: 34.86 μM N, 2.18 μM P). Balancing of the nutrient ratio was performed in order to study the impact of both zooplankton groups on the stoichiometry of N and P recycling, which will be published in a subsequent study. After 2 days of phytoplankton growth, logarithmically scaled gradients of zooplankton density were established by adding *Daphnia hyalina X galeata* from the stock cultures of the Max-Planck-Institute of Limnology, Plön, Germany, to the cladoceran treatments and copepods from wild catches to the copepod treatments. Cladocerans were removed from copepod catches by heavy bubbling with air for 7 h. The inoculum of *Daphnia* comprised the entire size spectrum from neonates to maximal sized adults (0.8–2.4 mm), while the copepod size spectrum ranged from early copepodide stages to maximal sized adults (0.4–1.5 mm). The cladoceran gradient consisted of seeding densities of 1.25, 2.5, 5, 10, 20 and 40 individuals per litre; the copepod gradient consisted of seeding densities of 5, 10, 20, 40, 80 and 160 individuals per litre. Each treatment was replicated, except for the lowest zooplankton densities of each gradient. Two enclosures received no zooplankton addition and served as controls. The seeding densities were chosen in order to produce a similar range of zooplankton biomasses, calculated from the mean individual dry mass values taken from the literature: *Daphnia hyalina*, 17 µg from stock cultures (Santer 1990); copepods, 4 µg calculated from *Eudiaptomus* mean length (Kiefer 1978) and a widely used length–weight regression (Bottrell *et al.* 1976). The maximal seeding densities of each gradient are about double the seasonal abundance maxima (Fußmann 1996).

Samples for quick, low precision phytoplankton counts were taken at 2–3-day intervals in order to determine the time of in-depth analysis. The first in-depth analysis was performed with the phytoplankton samples on 17 August, just before significant cross-contamination occurred between both gradients. The second in-depth analysis was performed on 28 August (termination of the experiments) to utilize the cross-contamination to analyse the combined effects of both zooplankton groups. Phytoplankton were counted according to the inverted microscope technique. If possible, 400 individuals per taxon were counted, which gives 95% confidence limits of ± 10% (Lund *et al.* 1958). Biomass was estimated as biovolume, which was calculated according to appropriate geometric models (Hillebrand *et al.* 1999) after microscopic measurement of at least 20 individuals per taxon. Phytoplankton biovolumes were defined in two different ways: for total and size class biomass calculations, only cell volumes excluding gelatinous coverings were used; for phytoplankton size–grazing relationships and for the assignment of species to size classes, the effective particle volume was used, which is the colony size for colonial species and includes gelatinous coverings.

RESULTS

Results on 17 August

Total phytoplankton biomass showed no significant response to zooplankton seeding density in either zooplankton gradient (copepod gradient: $P = 0.44$; cladoceran gradient: $P = 0.49$). However, the two different zooplankton taxa had strongly different impacts on the size structure of the phytoplankton community (Fig. 1). With increasing *Daphnia*, there was a reduction of biomass of small phytoplankton, particularly of the smallest size class (<100 µm^3). Losses of small phytoplankton were compensated by a positive response of large phytoplankton (>10 000 µm^3). In the copepod gradient, the biomass of the large phytoplankton decreased with increasing copepod density, while the biomass of small phytoplankton algae compensated for the negative response.

The analysis of the response at the individual species level was performed by a multiplicative regression analysis according to the model $y = ax^b$, where y is the biomass of a phytoplankton species and x is the seeding density of zooplankton plus half of the minimal seeding density. The transformation of x was necessary in order to include the controls with zero seeding density in the regressions. The exponent b was taken as a measure of positive or negative impact of zooplankton type on phytoplankton species. As shown for the small diatom *Stephanodiscus parvus* (Fig. 2), there was an opposite response to the two zooplankton gradients (Table 1). The majority of phytoplankton species were negatively affected by *Daphnia* and positively affected by copepods or vice versa. The only

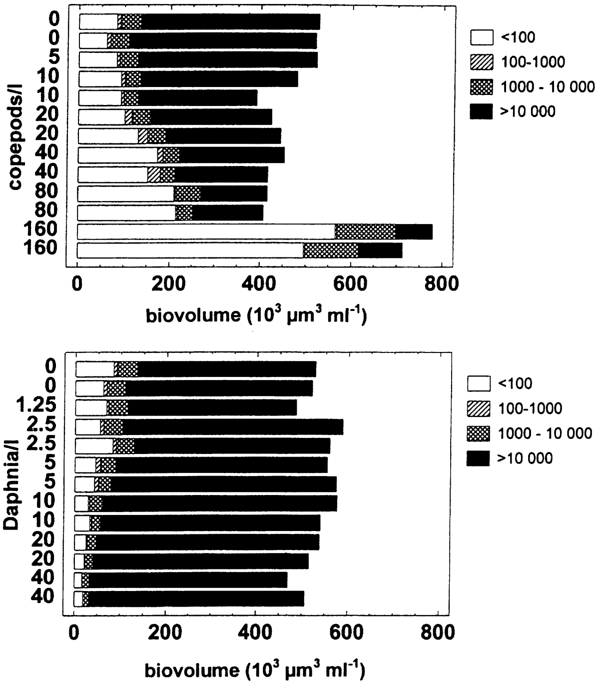

Figure 1 Zooplankton impact on phytoplankton biomass and size structure. Phytoplankton biovolume (in 10^3 µm^3/ml) in mesocosms with different seeding densities of copepods and *Daphnia* after 8 days of grazing. Cumulative plot of particle volume size classes: white, < 100 µm^3; hatched, 100–1000 µm^3; cross-hatched, 1000–10 000 µm^3; black, > 100 000 µm^3.

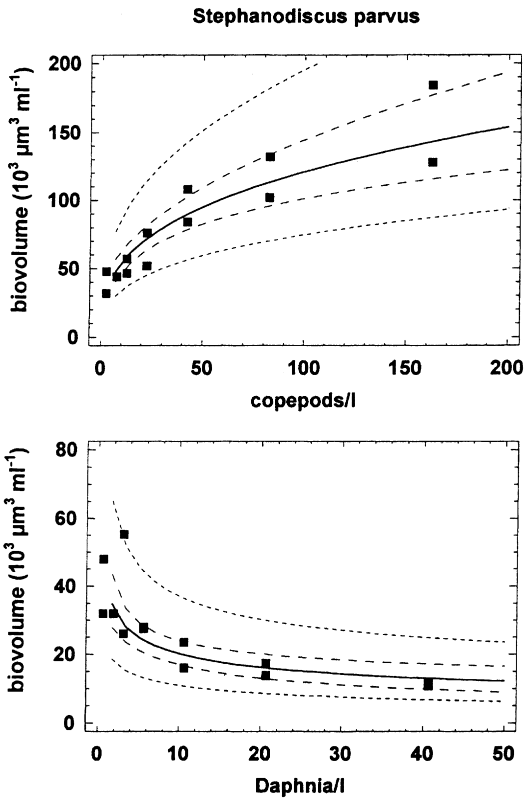

Figure 2 Zooplankton impact on *Stephanodiscus parvus* biomass. Response of *Stephanodiscus parvus* biovolume to copepod and *Daphnia* seeding densities after 8 days of grazing. For regression equation, see Table 1.

exceptions occurred for species of intermediate size (3600–4000 μm^3) and for three large phytoplankton species: the gelatinous green algae *Sphaerocystis schroeteri* and *Quadrigula pfitzeri* (positive impact by both zooplankton) and the colonial flagellate *Dinobryon sociale* (negative impact by both zooplankton).

Results on 28 August

After 17 August, a cross-contamination of both gradients, in particular a contamination of the copepod gradient by *Daphnia*, became obvious. A decline in phytoplankton biomass was found in these treatments, which had high abundances of both zooplankton types. The response was tested by a multiple regression analysis with stepwise variable selection (F-to-remove = 4.0; backward selection). The dependent variables were phytoplankton biomass (in 10^3 μm^3/ml) and size class biomass (< 4000 μm^3; > 4000 μm^3). The independent variables were copepod density (x_1; in individuals per litre), *Daphnia* density (x_2) and the product $x_1 \times x_2$ (x_3). The selected models (Table 2) show a significant negative impact of copepods on large algae, a significant negative impact of *Daphnia* on small algae and a significant negative impact of the copepod × *Daphnia* product on total phytoplankton biomass, while all other independent variables were excluded as insignificant.

DISCUSSION

Except for the analysis of the effect of cross-contamination, our statistical analysis was restricted to the seeding densities

Table 1 Impact of zooplankton type on phytoplankton species measured by coefficient b in a regression model, $y = ax^b$, where y is the phytoplankton biomass (10^3 μm^3/ml) and x is the zooplankton seeding density (/l).

Species	Particle size (μm^3)	Copepods		*Daphnia*	
		b	r^2	b	r^2
Unident. nanoflagellates	33	0.43 ± 0.07	0.78†	−0.47 ± 0.07	0.78†
Stephanodiscus parvus	60	0.35 ± 0.04	0.86‡	−0.31 ± 0.06	0.75†
Rhodomonas minuta	65	0.54 ± 0.09	0.76†	−0.58 ± 0.10	0.76†
Cryptomonas spp.	1200	0.46 ± 0.05	0.87‡	−0.34 ± 0.06	0.77†
Phacotus lenticularis	3600	−0.06 ± 0.07	0.05ns	−0.49 ± 0.11	0.63*
Rhizochrysis spp.	3900	−0.58 ± 0.17	0.52*	−0.69 ± 0.11	0.78†
Stephanodiscus alpinus	4000	0.03 ± 0.07	0.02ns	−0.40 ± 0.08	0.68†
Cryptomonas rostr.	4000	−0.46 ± 0.04	0.85‡	−0.31 ± 0.06	0.69†
Quadrigula pfitzeri	6800	0.71 ± 0.18	0.58*	0.48 ± 0.12	0.59*
Peridinium bipes	18000	−0.44 ± 0.06	0.83‡	0.19 ± 0.03	0.77†
Ceratium hirundinella	45000	−0.47 ± 0.07	0.80‡	0.12 ± 0.2	0.76†
Sphaerocystis schroeteri	47700	0.65 ± 0.08	0.87‡	0.72 ± 0.8	0.88‡
Microcystis spp.	141000	−1.08 ± 0.12	0.87‡	0.29 ± 0.06	0.71†
Dinobryon sociale	165000	−0.28 ± 0.04	0.82‡	−0.22 ± 0.06	0.52*
Anabaena flos-aquae	220000	−0.85 ± 0.08	0.90‡	0.40 ± 0.07	0.77†

$*P < 0.05$; $\dagger P < 0.01$; $\ddagger P < 0.001$.

Table 2 Final models selected from a multiple regression of phytoplankton biomass (10^3 μm^3/ml) on copepod density (N_{cop}/l), *Daphnia* density (N_{dap}/l) and the product of both zooplankton densities.

Dependent variable	Significant independent variable	*a*	*b*	r^2	*P*
Total phytoplankton	Copepods × *Daphnia*	188.4 ± 10.4	−0.048 ± 0.014	0.37	0.0022
Small phytoplankton	*Daphnia*	89.2 ± 9.98	−1.13 ± 0.39	0.28	0.0092
Large phytoplankton	Copepods	98.3 ± 10.7	−0.92 ± 0.26	0.38	0.0019

of zooplankton. Qualitatively similar results would have been obtained if we had used the final or mean zooplankton density as the independent variable. In spite of significant zooplankton growth in the low density treatments and slight declines in the high density treatments, there was still a highly significant log–log correlation between the initial and final densities (*Daphnia*: $r^2 = 0.77$, $P < 0.001$; copepods: $r^2 = 0.68$; $P < 0.01$) on 28 August. This means that the logarithmic scaling of the gradients remained intact throughout the entire experimental period, while the cross-contamination forced us to restrict the single zooplankton analysis to the first 7 days.

We emphasize that the exponent *b* in Table 1 is not a measure of grazing alone. It is a composite measure of impact which includes indirect effects as well, such as the recycling of nutrients, removal of competitors or removal of protozoan grazers of phytoplankton. Both zooplankton types feed on protozoa (Stoecker & Capuzzo 1990; Jürgens 1994), but more protozoan feeding is expected for copepods because of their preference for bigger and motile food (Burns & Schallenberg 1996; Adrian & Schneider-Olt 1999).

There were three species which did not fit into the general relationship between phytoplankton particle size and zooplankton impact (Fig. 3): the gelatinous green algae *Sphaerocystis schroeteri* and *Quadrigula pfitzeri*, and the colonial flagellate *Dinobryon sociale.* The gelatinous phytoplankton profited from both zooplankton types. Such phytoplankton are known to be poorly digestible even if they can be ingested by zooplankton and to profit from nutrient enrichment during gut passage (Porter 1976; Sterner 1989). *Dinobryon* was negatively affected by both zooplankton types. This might result from the fragile character of *Dinobryon* colonies. Colonies are large (165 000 μm^3) and might form attractive concentrations of food biomass for copepods, but individual cells break loose easily and are well within the edible range for *Daphnia* (175 μm^3).

After exclusion of the three species mentioned above, particle size explained 77% of the variance of copepod impact and 74% of *Daphnia* impact. The two polynomial regressions fitted to the data in Fig. 3 form almost mirror images, indicating that copepod and *Daphnia* impacts are opposite, except for the region where the curves cross. It is known that copepods can ingest larger food items than *Daphnia*, but generally a broad overlap in the size range of 5–30 μm cell length has been found (Gliwicz 1980; Geller & Müller 1981; Kleppel 1993; Adrian & Schneider-Olt 1999; Sommer *et al.* 2001). Therefore, the small amount of overlap in the spectra of positively and negatively affected algae is a surprise, especially because several of the species positively impacted by copepods (e.g. *Cryptomonas* spp., 22 μm length, 1200 μm^3 volume; *Rhodomonas minuta*, 7.5 μm, 65 μm^3) are known to be suitable food algae from culture experiments (Santer 1994). However, edibility in culture experiments with a monospecific diet implies grazing in a mixed diet only in non-selective filter-feeders like *Daphnia*, but not in zooplankton feeding more selectively by individual particle capture (DeMott 1986, 1988). The smaller edible algae might have been underrepresented in the diet of the copepods, because it was more profitable to capture the larger food particles, which were available in sufficient amounts (> 75% of total biomass > 10 000 μm^3 at the start of the experiments and in the controls). Alternatively, copepods might have grazed on those species, but grazing might have been overcompensated by indirect positive effects, e.g. nutrient recycling from large algae and removal of protozoa. We do not expect that the results would have been fundamentally different if we had used other *Daphnia*

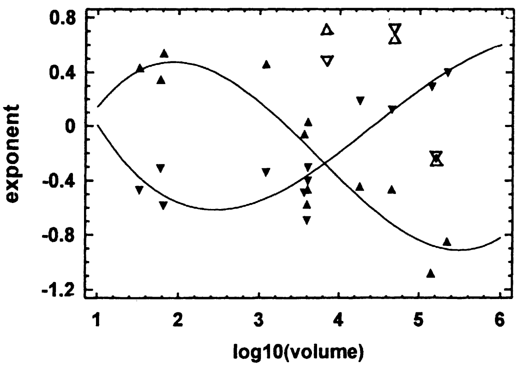

Figure 3 Zooplankton impact vs. phytoplankton particle size. Impact of zooplankton (exponent *b* from Table 1) as a function of phytoplankton particle volume (*V*, μm^3): copepods, ▲, △; *Daphnia*, ▼, ▽; open symbols, not included in regressions (1, *Quadrigula pfitzeri*; 2, *Sphaerocystis schroeteri*; 3, *Dinobryon sociale*). Regression equations are third-order polynomials: copepods: $b = -1.19 + 1.96 \log^{10} V - 0.69(\log^{10} V)^2 + 0.062(\log^{10} V)^3$; $r^2 = 0.77$; $P = 0.0019$; *Daphnia*: $b = 1.39 - 1.88 \log^{10} V + 0.52 (\log^{10} V)^2 - 0.04 (\log^{10} V)^3$; $r^2 = 0.74$; $P = 0.0029$.

strains or wild populations. The feeding size spectrum of the used strain is typical of the size spectrum of most medium-sized *Daphnia* spp. which are usually found in lakes with moderate fish pressure (Gliwicz 1980; Geller & Müller 1981).

The second surprise was the inability of even very dense, but unmixed, zooplankton populations to depress phytoplankton biomass. In particular, *Daphnia* grazing has frequently been reported as a sufficient reason for phytoplankton biomass depressions under otherwise good growth conditions. Such biomass minima are found either during the spring clear water phase (Lampert 1978, 1988; Sommer *et al.* 1986) or for more extended periods when artificial removal of planktivorous fish ("biomanipulation") leads to an increase and a subsequent dominance of large-bodied *Daphnia* spp. (Shapiro & Wright 1984). However, failures of biomanipulation with subsequent dominance of large algae, particularly cyanobacteria, have been reported as well (Benndorf 1990). Usually, in such studies, the role of subdominant zooplankton taxa has been neglected, and grazing impact has been totally ascribed to the dominant *Daphnia* spp. However, as in our case, summer phytoplankton in meso- and eutrophic lakes frequently includes enough large, inedible phytoplankton for compensatory growth if they are not controlled by macrophageous herbivores. Separating the effects of the different zooplankton types was the major reason for choosing experiments at the mesocosm scale instead of comparative field studies or whole lake manipulations. A total exclusion of zooplankton functional types is impossible when zooplankton is manipulated indirectly, e.g. by fish removal. Even in our mesocosm experiments, control over the independent variable could only be maintained during the first 7 days. Thus, we were only able to study the short time response of phytoplankton without permitting much feedback from changed patterns of zooplankton growth. A longer duration of the experiment would have increased the chances of initially undetectable and well-defended phytoplankton species to increase and to dominate the biomass.

The extent to which lower trophic levels are controlled by higher ones has a long tradition in both terrestrial and aquatic ecology, starting from Hairston *et al.*'s (1960) famous "green world" question. The domination of biomass by plants has either been explained by plant defence against herbivory (the dominant terrestrial paradigm) or by predator control of herbivores (the dominant limnological paradigm; Carpenter *et al.* 1985). In a recent review, Pace *et al.* (1999) have found examples of both from all kinds of ecosystems. It is a general feature of the plant defence hypothesis that herbivores should be able to control plant species composition, but not plant biomass (Power 1992; Strong 1992). Our results indicate that the possibility of herbivore control of plant biomass does not only depend on plant functional diversity (well-defended vs. less-defended plants), but also on the functional diversity of herbivores (in our case microphageous vs. macrophageous herbivores).

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REFERENCES

Adrian, R. & Schneider-Olt, B. (1999). Top-down effects of crustacean zooplankton on pelagic microorganisms in a mesotrophic lake. *J. Plankton Res.*, 21, 2175–2190.

Bautista, B., Harris, R.P., Tranter, P.R.G. & Harbour, D. (1992). In situ copepod feeding and grazing rates during a spring bloom dominated by *Phaeocystis* sp. in the English Channel. *J. Plankton Res.*, 14, 691–703.

Benndorf, J. (1990). Conditions for effective biomanipulation; conclusions derived from whole-lake experiments in Europe. *Hydrobiologia*, 200, 187–203.

Bottrell, H.H., Duncan, A., Gliwicz, Z.M., Grygierek, E., Herzig, A., Hillbricht-Ilkowska, A., Kurasawa, H., Larsson, P. & Weglenska, T. (1976). A review of some problems in zooplankton production studies. *Norw. J. Zool.*, 24, 419–456.

Burns, C.W. & Schallenberg, M. (1996). Relative impact of cladocerans, copepods and nutrients on the microbial food web of a mesotrophic lake. *J. Plankton Res.*, 18, 683–714.

Carpenter, S.R., Kitchell, J.F. & Hodgson, D.R. (1985). Cascading trophic interactions and lake productivity. *Bioscience*, 35, 634–639.

DeMott, W.R. (1986). The role of taste in food selection by freshwater zooplankton. *Oecologia*, 69, 334–340.

DeMott, W.R. (1988). Discrimination between algae and artificial particles by freshwater and marine copepods. *Limnol. Oceanogr.*, 33, 397–408.

Fußmann, G. (1996). Die Rolle der Rotatorien im Pelagial eines mesotrophen Sees durch Bottom-up und Top-down Prozesse: Freilandbeobachtungen und Enclosure-Experimente. Diploma Thesis. Kiel: University of Kiel.

Geller, W. & Müller, H. (1981). The filtration apparatus of cladocera. Filter mesh sizes and their implications on food selectivity. *Oecologia*, 49, 316–321.

Gliwicz, Z.M. (1980). Filtering rates, food size selection, and feeding rates in cladocerans — another aspect of interspecific competition in filter-feeding zooplankton. In: *Evolution and Ecology of Zooplankton Communities*, eds Kerfoot, W.C. Hanover, N.H: University Press of New England, pp. 282–291.

Hairston, N.G., Smith, F.E. & Slobodkin, L.B. (1960). Community structure, population control, and competition. *Am. Naturalist*, 94, 421–425.

Hillebrand, H., Dürselen, C.D., Kischtel, D., Pollingher, U. & Zohary, T. (1999). Biovolume calculations for pelagic and benthic microalgae. *J. Phycol.*, 35, 403–424.

Jürgens, K. (1994). Impact of *Daphnia* on planktonic microbial food webs. — A review. *Mar. Microbial Food Webs*, 8, 295–324.

Kiefer, F. (1978). *Freilebende Copepoda. Die Binnengewässer*, Vol. 26/2. Stuttgart: Schweizerbarth.

Kleppel, G.S. (1993). On. the diet of calanoid copepods. *Mar. Ecol. Prog. Ser.*, 99, 183–195.

Lampert, W. (1978). Climatic conditions and planktonic interactions as factors controlling the regular succession of spring algal bloom and extremely clear water in Lake Constance. *Verh. Inten. Verein. Limnol.*, 20, 969–974.

Lampert, W. (1988). The relationships between zooplankton biomass and grazing. A review. *Limnologica*, 19, 11–20.

Lund, J.W.G., Kipling, C. & LeCren, E.D. (1958). The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia*, 11, 143–170.

Pace, M.L., Cole, J.J., Carpenter, S.R. & Kitchell, J.F. (1999). Trophic cascades revealed in diverse ecosystems. *Trends Ecol. Evol.*, 14, 483–488.

Porter, K.G. (1976). Enhancement of algal growth and productivity by grazing zooplankton. *Science*, 192, 1332–1334.

Power, M.E. (1992). Top down and bottom up forces in food webs: Do plants have primacy? *Ecology*, 73, 733–746.

Santer, B. (1990). Lebenszyklusstrategien cyclopoider Copepoden. PhD Thesis. Kiel: University of Kiel.

Santer, B. (1994). Influences of food type and concentration on the development of *Eudiaptomus gracilis* and implications for the interactions between calanoid and cyclopoid copepods. *Arch. Hydrobiol.*, 131, 141–159.

Shapiro, J. & Wright, D.I. (1984). Lake restoration by biomanipulation: Round Lake, Minnesota, the first two years. *Freshwater Biol.*, 14, 371–383.

Sommer, F., Stibor, H., Sommer, U. & Velimirov, B. (2000). Grazing by mesozooplankton from Kiel Bight, Baltic Sea, on different sized algae and natural size fractions. *Mar. Ecol. Prog. Ser.*, 199, 43–53.

Sommer, U., Gliwicz, Z.M., Lampert, W. & Duncan, A. (1986). The PEG-model of seasonal succession of planktonic events in lakes. *Arch. Hydrobiol.*, 106, 433–471.

Sommer, U., Stibor, H., Katechakis, A., Sommer, F. & Hansen, T. (2001). Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production : primary production. *Hydrobiologia*, in press.

Sterner, R.W. (1989). The role of grazers in phytoplankton succession. In: *Plankton Ecology. Succession in Plankton Communities*, eds. Sommer, U. Heidelberg: Springer, pp. 107–170.

Stoecker, D.H. & Capuzzo, J.M. (1990). Predation on protozoa and its importance to zooplankton. *J. Plankton Res.*, 12, 891–908.

Strong, D.R. (1992). Are trophic cascades all wet? Differentiation and donor control in diverse ecosystems. *Ecology*, 73, 747–754.

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