

---

## The impact of metazooplankton on the structure of the microbial food web in a shallow, hypertrophic lake

Klaus Jürgens<sup>1,3</sup> and Erik Jeppesen<sup>2</sup>

<sup>1</sup>Max Planck Institute of Limnology, PO Box 165, D-24302 Plön, Germany and <sup>2</sup>National Environmental Research Institute, PO Box 314, DK-8600 Silkeborg, Denmark

<sup>3</sup>To whom correspondence should be sent

---

**Abstract.** Shallow, hypertrophic Lake Søbygård is characterized by strong fluctuations in the plankton community structure over short time scales, and cascading predation effects from higher to lower trophic levels. We examined the coupling between the classical and microbial food web for a 1 month period, during which the typical zooplankton summer succession from rotifers (mainly *Brachionus* spp.) to cyclopoid copepods and daphnids occurred. In addition to the analysis of the plankton succession, we performed mesocosm experiments, comparing the microbial community structure in treatments with and without metazooplankton. We focused on the development of different functional groups within the microbial food web: total heterotrophic bacteria, filamentous bacteria (as grazing-resistant forms), prokaryotic and eukaryotic, autotrophic picoplankton (pAPP and eAPP), heterotrophic nanoflagellates (HNF) and ciliates. During the first experiment, the metazooplankton was dominated by rotifers which exerted only moderate top-down control on small ciliates, HNF and APP. Cascading predation effects were visible after the collapse of the rotifer population; enhanced protozoan grazing resulted in a decrease in single-celled bacteria and an increase in filamentous bacteria. During the second experiment, characterized by dominance of *Cyclops vicinus*, strong alterations in the microbial food web structure occurred. The most obvious effects were an efficient predation control of planktonic ciliates by copepods and a shift of the picoplankton towards filamentous bacteria and a very high biomass of pAPP, which we interpret as a result of enhanced protozoan grazing pressure on bacterioplankton. After the peak in cyclopoids, *Daphnia* spp. became the dominant zooplankton taxa which resulted in the well known strong predation effects on all microbial components. The two experiments confirm that metazooplankton species composition is an important structuring factor for the microbial food web. Two functional groups deserve special attention: filamentous bacteria and pAPP. Filamentous bacteria, which attained nearly 50% of total heterotrophic bacterial biomass during the second period, seem to be a sensitive indicator of the overall planktonic food web structure and showed significant responses in the enclosures. With a special staining procedure, we analysed the abundance of filamentous bacteria in Lugol-fixed samples collected since 1984 during periods when either daphnids, cyclopoids or rotifers dominated the metazooplankton community. On average, high abundance of filaments was always associated with *Cyclops*-dominated situations, low numbers with *Daphnia* dominance and high, but rather variable numbers with rotifer dominance. A general close correlation of pAPP with filamentous bacteria might indicate that pAPP also possess a reduced edibility for protozoans.

---

### Introduction

The microbial food web, including autotrophic and heterotrophic organisms in the pico- and nanosize range (bacteria and protists), often accounts for a major portion of biomass production and nutrient recycling in the pelagic zone of both freshwater and marine environments. It is one of the major goals in aquatic microbiology to understand the factors regulating the productivity and biomass of these microbial communities. As for bacteria, the controlling factors can be limitation by resources (dissolved organic or inorganic nutrients), predation (by phagotrophic protists or cladocerans) and viral lysis [e.g. (Sanders *et al.*, 1992; Jürgens, 1994; Weinbauer and Höfle, 1998)]. The limiting factors and types of

grazers are, however, determined and modulated by the overall planktonic food web structure (Pace *et al.*, 1990; Vaqué and Pace, 1992). Research studies, especially from freshwater systems, have shown that the microbial food web is not a separate 'loop' but is connected to the classical grazer food chain in many direct and indirect ways (Riemann and Christoffersen, 1993).

Herbivorous zooplankton is known to influence the structure and dynamics of phytoplankton communities (Sterner, 1989). It has become evident that the impact of metazooplankton on the microbial food web is of similar importance, functioning in two different ways: first, indirectly, by mediating the resource supply for bacteria via phytoplankton dynamics and accelerated DOC release due to grazing (Jumars *et al.*, 1989); second, directly, by predation on microbial food web components, mainly the different groups of protists which fall into the prey size spectrum of most zooplankton species [reviews by (Arndt, 1993; Sanders and Wickham, 1993; Jürgens, 1994)]. The latter point, especially, has been examined in more detail in recent years, and has revealed many species-specific effects of the metazooplankton-protist link. It has been shown that nearly all metazooplankton taxa, with the notable exception of cyclopoid copepods, impose strong predation pressure on heterotrophic nanoflagellates (HNF) (Sanders *et al.*, 1994; Jürgens *et al.*, 1996). Cyclopoid and calanoid copepods are known to be efficient selective grazers of planktonic ciliates (Burns and Gilbert, 1993; Wiackowski *et al.*, 1994; Wickham, 1995) and *Daphnia* effects the whole microbial food web, from ciliates to larger sized bacteria and picoalgae (Porter *et al.*, 1988; Jürgens, 1994).

It is a matter of debate how the strong links between metazooplankton and protists, and protists and picoplankton, may form a trophic cascade which affects planktonic bacteria. Experimental field mesocosm studies in which metazooplankton composition and biomass were manipulated revealed only little and contradictory evidence for such a trophic cascade. In some studies, the experimental manipulations of higher trophic levels did not result in clear changes in bacterial abundance (Pace and Funke, 1991; Wickham, 1998), whereas in others, the presence of *Daphnia* resulted in enhanced control of bacterial biomass (Riemann, 1985; Christoffersen *et al.*, 1993). The cascading trophic interactions which extend from the largest to the smallest organisms might be truncated at some lower level due to compensatory interactions (Pace *et al.*, 1998). On the other hand, the aggregated variables, bacterial abundance and biomass, might not be sufficient for assessing cascading predatory effects on bacterioplankton because changes in predation impact can result in shifts of bacterial community composition rather than in changes of total biomass. In mesocosm experiments in meso- to eutrophic lakes, it has been observed that removal of metazooplankton, or a shift from *Daphnia* to copepods, results in an increased abundance of bacterivorous protists. This, in turn, triggers the development of grazing-resistant bacterial morphotypes and a shift in bacterial community composition with only minor changes in bacterial biomass (Jürgens *et al.*, 1994, 1999a).

Seasonal shifts in species composition of freshwater zooplankton are expected to be reflected in structural and functional changes in the microbial food web. Although we now have data on predatory effects of different zooplankton taxa

on microbial components, it is still not possible to predict the structure, function and dynamics of the microbial food web in response to changes in metazooplankton, owing to the many direct and indirect interactions within the microbial food web, and the diverse interactions with the phytoplankton–zooplankton grazing food chain.

Hypertrophic lakes are especially suitable for the study of trophic interactions between metazooplankton and the microbial food web due to their relatively simple food web structure, which is generally dominated by few species in high numbers, and with marked oscillations of the whole food web (Sommaruga and Robarts, 1997; Jeppesen *et al.*, 1997b). Shallow, eutrophic lakes are widespread in Denmark and have been studied extensively for several decades. One of the best studied systems is Lake Søbygård in Central Jutland, for which more than 18 years of field data have been analysed (Jeppesen *et al.*, 1998).

The goal of the present study was to examine the impact of metazooplankton on the structure of the microbial food web in Lake Søbygård by manipulating zooplankton biomass and composition in mesocosms. This approach has frequently been used to study trophic interactions between classical and microbial food webs in lakes (Christoffersen *et al.*, 1993; Brett *et al.*, 1994; Jürgens *et al.*, 1994; Burns and Schallenberg, 1996). We performed mesocosm experiments during two consecutive stages in the plankton succession and compared the effects of different zooplankton treatments on the development of the microbial components. We focused on presumed important functional groups of the microbial food web, i.e., groups of species of very different taxonomy but with an assumed similar ecological position (mainly related to their size) within the planktonic food web. These groups were: phagotrophic protozoans (ciliates, HNF), heterotrophic bacteria (differentiated into normal morphotypes and filamentous forms) and autotrophic picoplankton (APP, differentiated into prokaryotic and eukaryotic cells). In a previous paper (Jürgens *et al.*, 1999b), we presented results on the population dynamics and species composition of planktonic ciliates. Here, we focus on the cascading effects on autotrophic and heterotrophic pico- and nanoplankton. As filamentous bacteria proved to be a characteristic functional component of the microbial food web, we developed a staining protocol which allowed the enumeration of filaments in Lugol-fixed samples from previous periods. This gave further evidence that the appearance of filamentous bacteria is coupled via predatory cascades to the planktonic food web structure.

## Method

### *Study site and description of mesocosm experiments*

Lake Søbygård is a shallow, hypertrophic lake with a surface area of 38 ha, a mean and maximum depth of 1.0 and 1.9 m, respectively, and a hydraulic retention time of 15 to 20 days. The average concentration of chlorophyll *a* during summer is between 130 and 730  $\mu\text{g l}^{-1}$ , with decreasing values in recent years (Jeppesen *et al.*, 1990, 1998). Further details about the lake and the variations in its biological

structure are described in Jeppesen *et al.* (Jeppesen *et al.*, 1990, 1996, 1997a, 1998), Hansen and Jeppesen (Hansen and Jeppesen, 1992) and Jürgens *et al.* (Jürgens *et al.*, 1999b).

The mesocosm experiments in Lake Søbygård were conducted during two consecutive stages in the plankton succession, from 6–11 July (Exp. 1) and from 19–24 July 1994 (Exp. 2). Data on the development of the plankton community in the lake were obtained by sampling at a mid-lake station (1.5 m depth) from early July to early August. Details on the experimental design are given in Jürgens *et al.* (Jürgens *et al.*, 1999b). Briefly, six 500 l polyethylene mesocosms, closed at the bottom and fixed to a wooden frame, were installed close to the shore of the lake. All mesocosms were first gently filled with water filtered through a 50 µm mesh in order to remove mesozooplankton. In three of the mesocosms (in the following referred to as +ZP) zooplankton was reintroduced with a 50 µm plankton net at approximately natural densities. Three of the mesocosms were left without additional zooplankton (referred to as <50). Additionally, three 10 l glass bottles were filled with lake water, filtered through a 10 µm mesh to remove micro- and mesozooplankton, and incubated near the other mesocosms in the lake (referred to as <10).

#### *Sampling and biological analysis*

Zooplankton abundance and composition were determined at the beginning and at the end of the experiments, in the mesocosms and in the lake. Samples of 2 l were filtered through a 40 µm mesh net and subsequently fixed in Lugol. Zooplankton were enumerated and sized with a half-automated image analysis system (SIS GmbH, Münster) equipped with a stereomicroscope. The biomass of the different groups of organisms (copepods, cladocerans, rotifers) was calculated from empirical length–weight relationships (Bottrell *et al.*, 1976; E.Jeppesen, unpublished).

Sampling of the mesocosms for microbial components (picoplankton, protozoans) was done daily using a core sampler after gently mixing the water. For the analysis of the response of all organism groups, we chose two dates for each experiment: one in the middle (after 2–3 days) and one towards the end of the experiment (after 4–5 days). This sampling scheme covered the initial responses as well as the main shift of the on-going succession. Ciliates were counted and sized from Lugol-fixed samples using settling chambers and an inverted microscope. Bacteria, APP and HNF were enumerated from formalin-fixed samples (1.5%) after DAPI staining (Porter and Feig, 1980) in an epifluorescence microscope (Zeiss Axiophot). Autotrophic flagellates were distinguished from heterotrophs by Chl *a* autofluorescence. APP were counted under green light excitation and differentiated into prokaryotic APP (pAPP) (*Synechococcus*-like cells) and eukaryotic APP (eAPP) (mainly *Chlorella*-like cells) according to size, shape and presence of a nucleus. Whereas the size of pAPP (1–2 µm) fell into the formally defined picoplankton size range [0.2–2 µm, according to (Sieburth and Estep, 1985)], eAPP were slightly larger (2–3.5 µm) and partly belonged to the nanoplankton. However, as these cells overlap in size with pAPP and are generally

accounted for as picoplankton, we included them here in APP. For some selected samples, cell sizes of heterotrophic and autotrophic picoplankton were measured with an automated image analysis system (SIS GmbH, Münster, Germany). Filamentous bacteria, which were defined as bacterial cells (or chains of cells) exceeding 5  $\mu\text{m}$  in maximal length, were measured from the same DAPI preparations by screening transects across the filter and measuring the length of the filaments with the help of an ocular grid. For cell volume calculations, bacteria were assumed to represent cylinders with hemispherical ends, and filamentous bacteria were assumed to have the same mean width as the single-celled bacteria measured with the image analysis system.

The incorporation of methyl- $^3\text{H}$ -thymidine into bacterial DNA was measured at the start and end of each experiment as an estimate of bacterial production. [ $^3\text{H}$ ]thymidine was added to water samples (two replicates per mesocosm) and to a formalin-killed control at a final concentration of 20 nM and incubated *in situ* for 20 min. Incubations were terminated by adding formalin (1.5% final concentration), followed by filtration of the samples on a 0.2  $\mu\text{m}$  cellulose acetate filter, and rinsed eight times with 1 ml ice-cold 5% TCA. Radioactivity of the filters was assayed by liquid scintillation counting; quench correction was made using the external channels ratio method.

#### *Enumeration of filamentous bacteria in old Lugol-fixed samples*

To obtain more information on the relationship between the appearance of the major zooplankton taxa and filamentous bacteria in Lake Søbygård, we tried to determine the abundance and biomass of filamentous bacteria in acid Lugol-fixed samples from previous years for which data on zooplankton were available (back until 1984). We tried to develop a procedure for visualizing bacterial filaments in these samples and tested different fluorochromes and procedures for staining, post-fixation and embedding. The optimum protocol found was as follows. A subsample of the Lugol sample is decolourized by the addition of a few drops of thio-sulphate. To a 1 ml sample, either sodium or potassium citrate (25 mM final concentration) and the fluorochrome SYTO 9 (10  $\mu\text{M}$  final concentration; SYTO 9 is available in the LIVE/DEAD kit from Molecular Probes) are added. The sample is filtered, similar to the DAPI staining, on a black 0.2  $\mu\text{m}$  polycarbonate membrane filter (Millipore), and the wet filter is placed on a glass slide and covered with a drop of melting point bath oil (Sigma) and a cover slip. Bacterial filaments (and all other organisms) can be viewed under green excitation. Although fading was very rapid, it was possible to apply the same counting procedure for bacterial filaments as in normal DAPI preparations.

For the assessment of bacterial filaments in old Lugol samples, we chose sampling dates at which one of the three major zooplankton groups in Lake Søbygård, daphnids, cyclopoid copepods or rotifers, dominated total zooplankton biomass by more than 80%, and when total zooplankton biomass exceeded 1 mg dry wt  $\text{l}^{-1}$ .

### *Other parameters and statistical analysis*

In a parallel study (Jürgens *et al.*, 1999b) we described physico-chemical data on the lake and the mesocosms during the study period: water temperature, inorganic nutrients (N, P), particulate organic carbon (POC) and Chl *a*. Furthermore, we provided data on the identification and species composition of the ciliate assemblage and of the metazooplankton in the lake and in the experiments, and the results will therefore only be repeated briefly here.

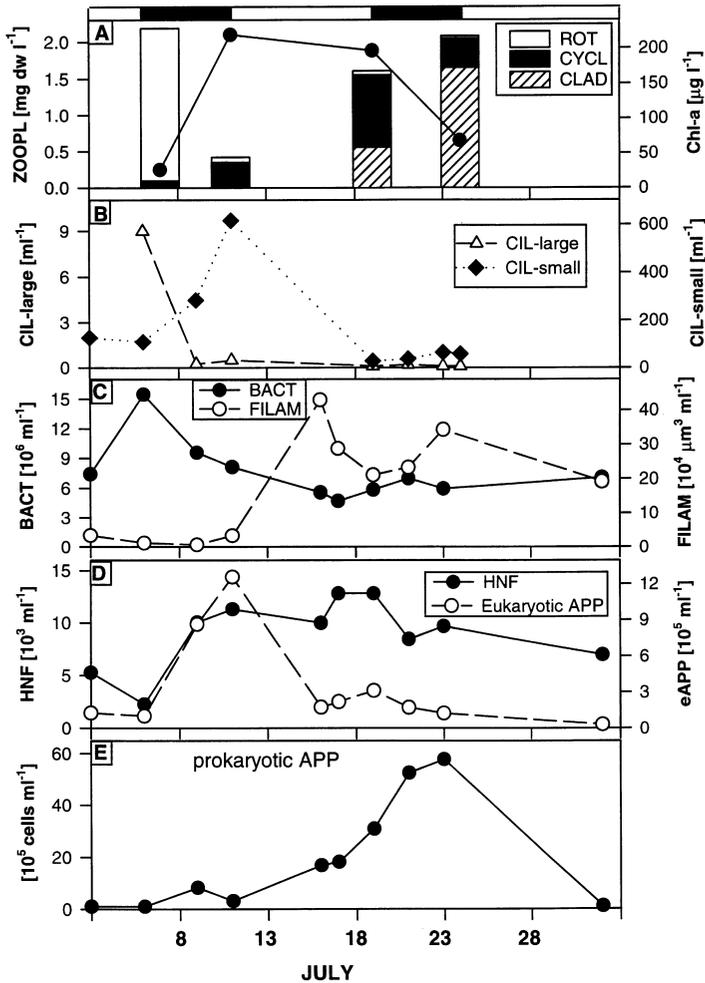
For all statistical analysis of the data, we used the software package STATISTICA. Differences in development of the various groups of organisms in the mesocosms, and differences in filament biovolume in old Lugol samples, were tested by a one-way analysis of variance (ANOVA). Data were log-transformed to stabilize variances. In the case of significant differences, we compared the mean values of the different treatments with post-hoc tests (Tukey's HSD). In order to reveal potential relationships in the development of the different organisms, a correlation analysis (Pearson) was performed on log-transformed data.

## **Results**

### *Plankton development in Lake Søbygård*

The period during which our mesocosm experiments were performed was characterized by warm and stable weather conditions, with water temperatures of 20–25°C. The plankton community showed a pronounced dynamic development with strong shifts in population sizes of the different functional groups. At the beginning of our observation period, the metazooplankton was dominated by rotifers, mainly *Brachionus calyciflorus* and *Keratella quadrata* [for a detailed zooplankton species list see (Jürgens *et al.*, 1999b)], and abundance of cyclopoid copepods was low (Figure 1). Phytoplankton had, with a Chl *a* concentration of 26 µg l<sup>-1</sup>, the lowest biomass during the 1994 growing season and mainly consisted of cryptomonads and green algae (*Actinastrum* sp., *Monoraphidium* sp., *Scenedesmus* sp., *Pediastrum* sp.). This corresponds to a 'clear water' state for this hypertrophic lake (Jeppesen *et al.*, 1990). For most of the remaining time from April to November, the Chl *a* concentrations fluctuated between 200 and 400 µg l<sup>-1</sup> (E.Jeppesen, unpublished data). Besides rotifers, the abundance of large (>30 µm), mainly raptorial ciliates (e.g. *Monodinium* sp., *Lagynophria* sp.) was also high, up to 9 cells ml<sup>-1</sup>. Small ciliates (<30 µm) were at a medium level (118 ml<sup>-1</sup>) and mainly consisted of small oligotrichous species (*Halteria grandinella*, *Strobilidium* spp.) and small prostomatids (Jürgens *et al.*, 1999b). Maximum bacterial levels were >15 × 10<sup>6</sup> ml<sup>-1</sup>, consisting nearly entirely of freely suspended single cells (Figure 2). Filamentous bacteria constituted only an insignificant part of total bacterial biomass (<10<sup>4</sup> cells ml<sup>-1</sup>); pAPP and eAPP were also at their minimal levels during our study period.

During the next week (until July 12), corresponding to the duration of Experiment 1, significant changes occurred within the plankton community, starting with a collapse of rotifers which decreased to less than 5% of the previous level. Only



**Fig. 1.** Development in the planktonic community of Lake Søbygård during the study period (mid-lake station); black, horizontal bars correspond to the time of the enclosure experiments. **A:** zooplankton biomass of the three groups (cladocerans, cyclopoids and rotifers), estimated for the dates when enclosure experiments 1 and 2 started and finished; chlorophyll-*a* concentrations (closed circles); **B:** small and large (>30 µm) ciliates; **C:** bacterial abundance (freely suspended rods and cocci) and biovolume of filamentous bacteria; **D:** eukaryotic (mainly *Chlorella*-type) autotrophic picoplankton (eAPP) and HNF; **E:** prokaryotic (*Synechococcus*-type) autotrophic picoplankton (pAPP).

the predatory *Asplanchna* spp. remained, at around 600 ind. l<sup>-1</sup>. The reason for this rotifer collapse was probably a combination of higher predation losses by *Asplanchna* spp. and by the increasing numbers of cyclopoids (mainly *Cyclops vicinus*) and, in the case of *Brachionus* spp., strong infection by parasites (microsporidians). The decline in rotifers and the overall low metazooplankton biomass were paralleled by a strong increase in Chl *a* to 219 µg l<sup>-1</sup>, caused by a

bloom of *Scenedesmus* spp. and picoalgae, and also by pronounced shifts within the microbial food web.

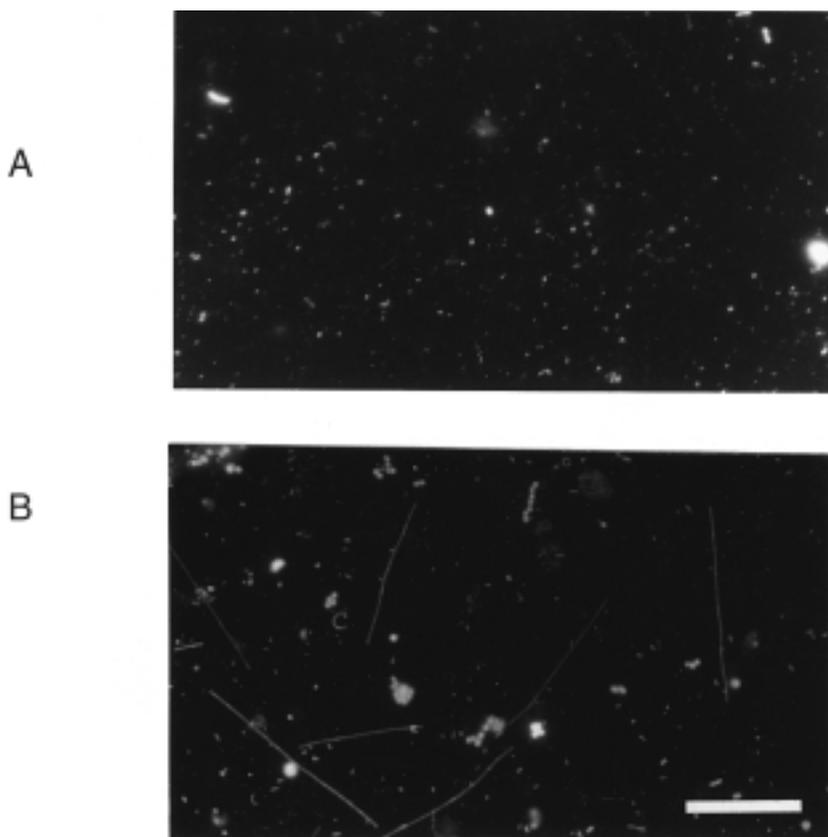
Whereas large ciliates declined, similar to rotifers, small ciliates (prostomatids and oligotrichous species) increased exponentially [for details on ciliate composition see (Jürgens *et al.*, 1999b)]. Besides small ciliates, HNF and eukaryotic APP also increased to a maximum value during the observation period. Both functional groups developed relatively synchronously during the study period and overlapped also in size; HNF consisted mainly of naked forms (probably chrysoomonads) in the size range 3–5  $\mu\text{m}$ , and picoeukaryotes resembled mainly *Chlorella* and *Monoraphidium*, in the size range 1.5–3.5  $\mu\text{m}$ .

One week later, at the start of the second experiment, the cycloids had reached their population maximum and cladocerans (mainly *Daphnia* sp.) had appeared as the second crustacean zooplankton group. The fact that Chl *a* was still at around 200  $\mu\text{g l}^{-1}$  indicated that daphnids were not yet exerting a substantial grazing pressure. Again, strong alterations in the functional groups within the microbial food web occurred. The peak in *Cyclops vicinus* (>300 ind.  $\text{l}^{-1}$ , copepodites + adults) was accompanied by a decline of the ciliate community to about 10% of the previous level (30–60 cells  $\text{ml}^{-1}$ ), whereas HNF remained fairly high (7–13  $\times 10^3$  cells  $\text{ml}^{-1}$ ). Another pronounced change compared with the week before was the appearance of large numbers of filamentous bacteria. The concentration of filaments, which were mostly long, straight threads (Figure 2), increased to >10<sup>5</sup>  $\text{ml}^{-1}$ , with mean cell lengths of 15–30  $\mu\text{m}$ , and remained at those high concentrations until the end of the study period. Prokaryotic APP (*Synechococcus*-type), in the form of single cells and small loose aggregates, increased continuously during the second experiment and reached maximum numbers of >5  $\times 10^6$  cells  $\text{ml}^{-1}$ . Although *Daphnia* spp. became the dominant zooplankton taxa until the end of the second experiment (>200 ind.  $\text{l}^{-1}$ , mean size 1 mm), no more drastic changes occurred within the microbial food web. An increase in total grazing pressure was indicated by a decrease in Chl *a* to 68  $\mu\text{g l}^{-1}$  and a further decline in protists. On the last sampling date at the beginning of August, picocyanobacteria had declined to comparatively low numbers (but still around 10<sup>5</sup> cells  $\text{ml}^{-1}$ ) and the levels of heterotrophic protists remained low (ciliates) or intermediate (HNF) compared with the previous weeks.

Inorganic nutrients also changed from the first to the second experiment (Jürgens *et al.*, 1999b). Whereas dissolved P and N were present in high concentrations during the first experiment (0.8 and 0.9 mg  $\text{l}^{-1}$ , respectively), ammonium and nitrate were reduced to undetectable levels due to the phytoplankton bloom during the second experiment. Concurrently, total sestonic organic carbon increased in parallel from ~3 to >10 mg C  $\text{l}^{-1}$  [data in (Jürgens *et al.*, 1999b)].

### *Mesocosm experiments*

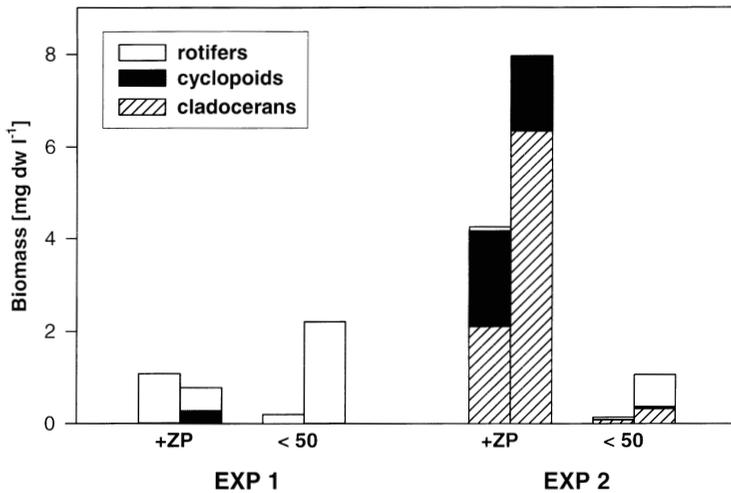
The manipulation of the zooplankton community by our size-fractionated filling of the mesocosms proved to be successful and in both experiments, we obtained treatments virtually without any metazooplankton (<10  $\mu\text{m}$ ), without crustacean zooplankton and brachionids (<50  $\mu\text{m}$ ), and with a zooplankton composition



**Fig. 2.** DAPI-stained preparations (1 ml sample on 0.2  $\mu\text{m}$  filters) of bacterioplankton from Lake Søbygård. **A:** start of Experiment 1; **B:** start of Experiment 2. Bar in panel B corresponds to 20  $\mu\text{m}$  and applies to both panels.

comparable with that of the lake (+ZP). However, differences between +ZP and the lake were recorded with respect to total zooplankton biomass. In the first experiment, the +ZP mesocosms received only about half and in the second experiment, nearly twice the biomass compared with the actual lake community (Figure 3).

The strong dynamics in zooplankton biomass and composition occurring in the lake (Figure 1) also appeared in the mesocosms. The *Brachionus* spp. population collapsed in +ZP, and in <50 a large population of small rotifers, mainly *Filinia* spp., developed. This resulted in a reversed situation by the end of the experiment; zooplankton biomass in <50 was higher than in +ZP. In the second experiment, zooplankton biomass of +ZP and <50 remained clearly different throughout. As in the lake, a shift from *Cyclops* to *Daphnia* dominance occurred in +ZP, whereas in <50, crustacean zooplankton remained at very low levels and only rotifer abundance increased significantly (Figure 3).

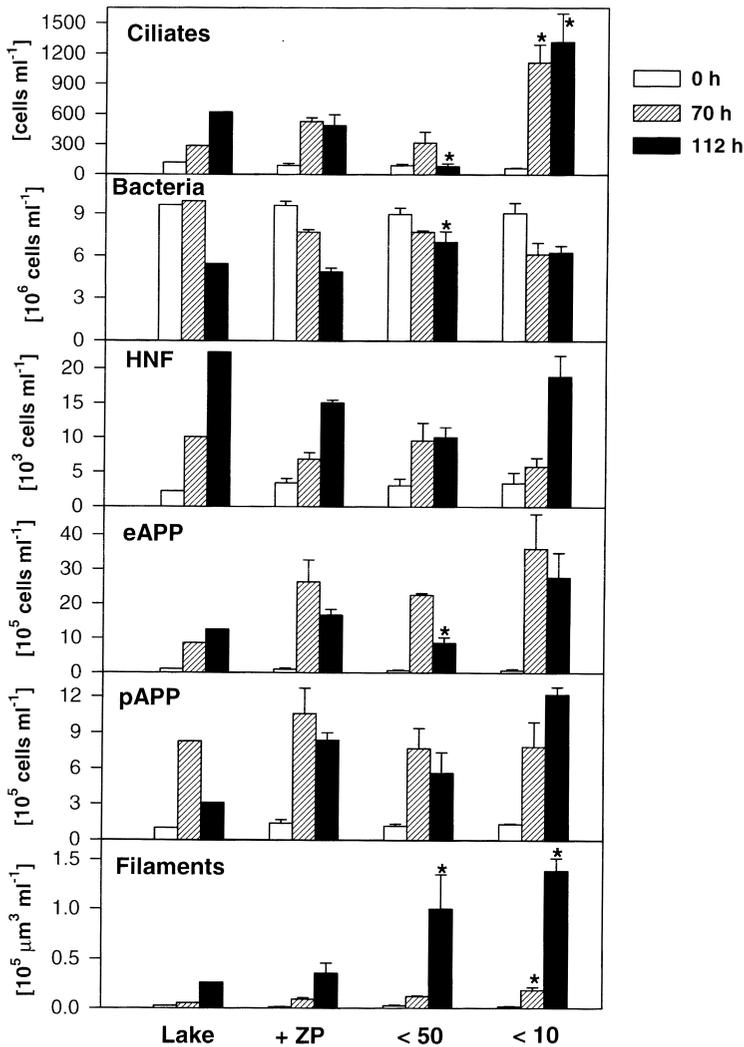


**Fig. 3.** Biomass and composition of metazooplankton in the enclosure experiments. Left bars: start of experiment; right bars: end of experiment. Mean values of three replicate enclosures. Mean coefficient of variation (CV) in the biomass of the three zooplankton taxa was 31%.

For the analysis of corresponding changes in the microbial food web of the different zooplankton treatments, we focused on the development of the functional groups ciliates, HNF, total bacteria, filamentous bacteria, pAPP and eAPP. The response of these groups in the mesocosms as well as in the lake is shown in Figure 4 (Exp. 1) and Figure 5 (Exp. 2). As the zooplankton community of +ZP was similar to that of the lake, we compared the data of <50 and <10 with the +ZP mesocosms. Significant differences ( $P < 0.05$ ) in the mean values compared with +ZP are indicated by asterisks in Figures 4 and 5.

Experiment 1, in which the zooplankton community was dominated by rotifers with a slight increase of cycloids, revealed a relatively similar pattern in the response of the microbial components in all treatments compared with the lake (Figure 4). All groups of organisms increased in abundance during the course of the experiment except bacteria, which continuously decreased, and ciliates, which declined in <50. Compared with +ZP, the mesocosms with an altered (<50) or eliminated (<10) metazooplankton community showed the most significant differences in the development of ciliates and filamentous bacteria. Small ciliates increased in <10 and decreased in <50. The high ciliate concentrations (>1000 cells ml<sup>-1</sup>) in <10 were mainly composed of the algivorous *Urotricha* spp. The decrease in small ciliates in <50 was paralleled by an increase in small rotifers (Figure 2) and large, predatory ciliates (Jürgens *et al.*, 1999b). The other variable which developed significantly differently was filamentous bacteria. Although there was a general trend towards an increase of filaments in the lake and in +ZP, this increase was more pronounced in <50 and especially in <10.

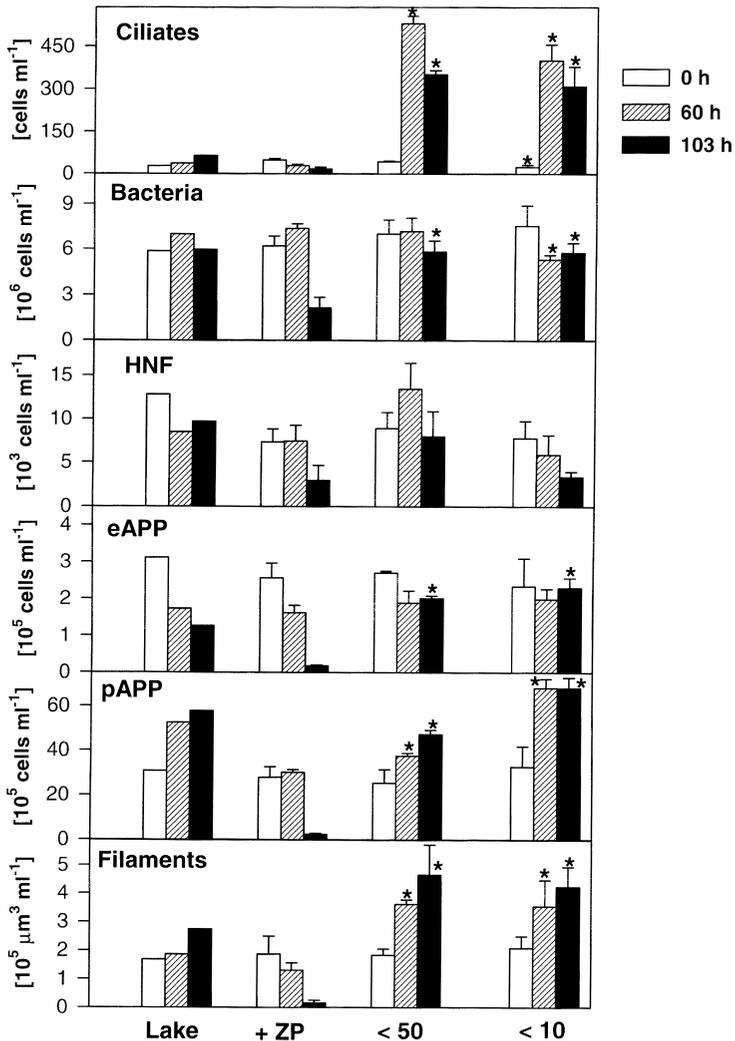
The initial situation of the second experiment was characterized by a high phytoplankton biomass and a relatively low ciliate concentration (28 cells ml<sup>-1</sup>),



**Fig. 4.** Development in the different functional groups of the microbial food web in Lake Søbygård and in the enclosures of Experiment 1. Mean values of three enclosures  $\pm$  1 SD. Asterisks denote significant differences ( $P < 0.05$ ) compared with the +ZP enclosures (Tukeys's HSD).

mainly small-sized (13–18  $\mu\text{m}$ ) *Urotricha* spp. (Jürgens *et al.*, 1999b). Drastic changes, compared with the first experiment, were that eAPP abundance was about one order of magnitude lower, whereas pAPP abundance and biovolume of filamentous bacteria were about one order of magnitude higher (Figures 4 and 5).

The zooplankton in +ZP was composed of cyclopooids and daphnids (Figure 3). This was comparable with the lake except that population growth of *Daphnia* spp. was much higher in the mesocosms than in the lake, and the situation shifted from



**Fig. 5.** Development in the different functional groups of the microbial food web in Lake Søbygård and in the enclosures of Experiment 2. Mean values of three enclosures  $\pm$  1 SD. Asterisks denote significant differences ( $P < 0.05$ ) compared with the +ZP enclosures (Tukeys's HSD).

dominance of *Cyclops* to dominance of *Daphnia* within the experimental period. The response of the microbial components in +ZP was relatively similar to that of the lake at the first sampling date (60 h), but deviated strongly towards the last sampling (103 h). Then, all components decreased, with an overall reduction of sestonic biomass to about 10% of the initial level, indicating the beginning of a *Daphnia*-induced clear water phase.

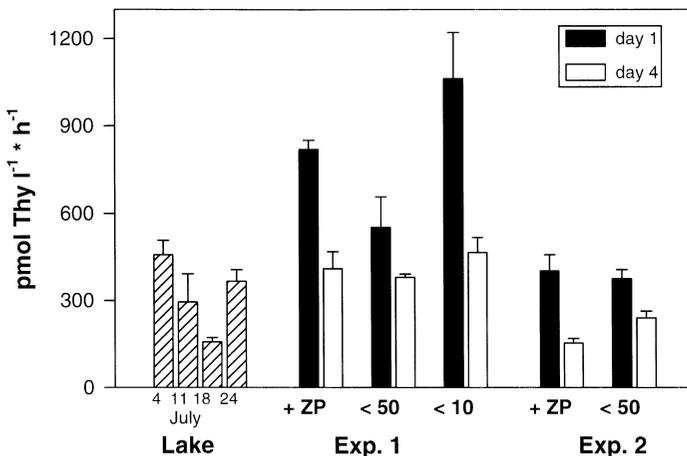
Consequently, the comparison of the zooplankton manipulated treatments <10 and <50 with the +ZP mesocosms revealed significant differences ( $P < 0.05$ ) for

all components except HNF at the second sampling date (Figure 5). For the first sampling date, when *Cyclops* still dominated the metazooplankton, the increase in ciliates was the most obvious effect after zooplankton removal, but also, pAPP increased significantly in the <50 and <10 mesocosms.

A correlation analysis of all available data points from both experiments and from the lake ( $n = 60$ ) revealed some relatively weak, though significant ( $P < 0.05$ ), correlations (all positive) between some of the functional groups: ciliates were correlated to HNF ( $r = 0.37$ ) and to eAPP ( $r = 0.67$ ), HNF also to pAPP ( $r = 0.40$ ), and filamentous bacteria to HNF ( $r = 0.46$ ). The only strong positive correlation was between filamentous bacteria and pAPP ( $r = 0.90$ ).

### Bacterial productivity

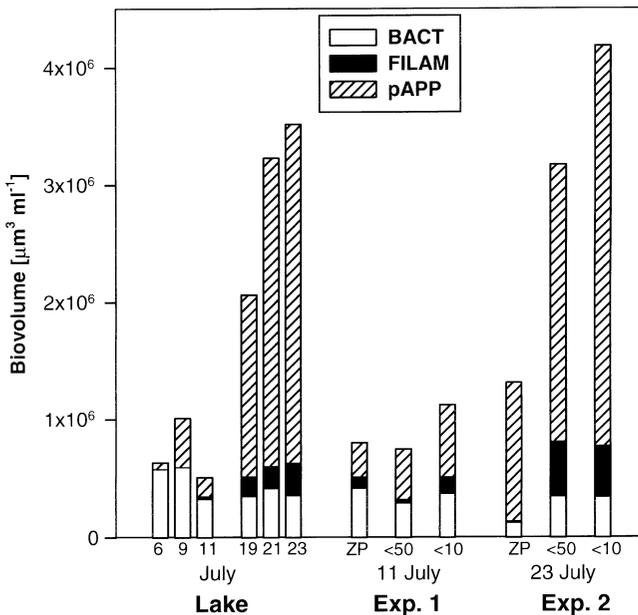
The data on thymidine incorporation give an estimate of the bacterial production in the lake and the mesocosms, and also some indication of possible shifts in bacterial activity during enclosure. Thymidine incorporation into lake bacterioplankton was in the range 150–450 pM Thy  $\text{h}^{-1}$  during the study period (Figure 6). Taking a standard conversion factor of  $2 \times 10^9$  cells (nmol thymidine) $^{-1}$  [e.g. (Bell, 1988)] and the actual bacterial concentrations, the doubling times of the bacterial community were in the range of 7–14 h. Bacterial productivity in the mesocosms of Exp. 1 (1 day after filling) was significantly ( $P < 0.05$ ) enhanced compared with the lake (Figure 6). However, parallel to the decline in bacterial abundance during the course of the experiment, thymidine incorporation also decreased and reached values more similar to those of the lake. Also in the second experiment, thymidine values declined, parallel to the decrease in bacterial abundance.



**Fig. 6.** Incorporation of [ $^3\text{H}$ ]-thymidine into the macromolecular fraction of bacterioplankton in the two enclosure experiments and in Lake Søbygård. Mean values of three enclosures  $\pm 1$  SD.

*Contribution of bacteria, APP and filamentous bacteria to picoplankton biomass*

The three different groups of prokaryotic picoplankton, heterotrophic single-cell bacteria, filamentous bacteria and picocyanobacteria (pAPP), were important functional groups of the microbial food web and contributed substantially to overall biomass as well as to the plankton dynamics in the lake and in the mesocosms. To illustrate the importance of these groups in terms of biomass, and with respect to their changes during shifts in zooplankton composition, we estimated, from measurements of cell dimensions, the total biovolume of pAPP, bacteria and filaments. As mean cell volumes we used  $0.06 \mu\text{m}^3$  for heterotrophic bacteria and  $0.5 \mu\text{m}^3$  for pAPP, which were mean values from measurements on several dates. For filaments, we used the actually measured cell volumes (range from  $0.8$  to  $2.8 \mu\text{m}^3$ ). Major changes occurred in the contribution of the three different prokaryotic groups from the first to the second experimental period in the lake and in the mesocosms (Figure 7). Filamentous bacteria reached 44–77% of the biovolume of normal heterotrophic bacteria, whereas pAPP markedly exceeded the biomass of heterotrophic bacteria and made up more than 80% of total prokaryotic picoplankton biomass. In the experiments, pAPP and filamentous bacteria developed most strongly in <10, but differences were also visible between +ZP and <50 (Figure 7).

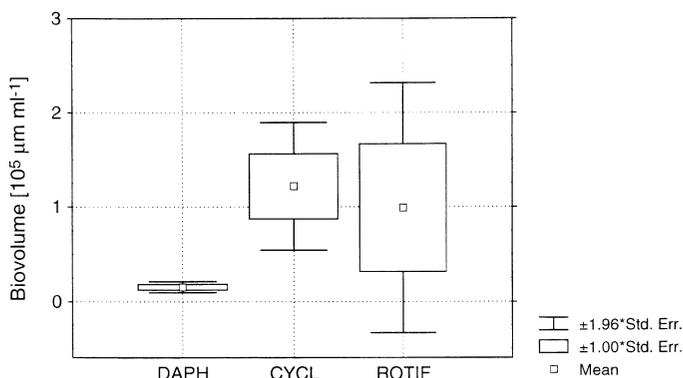


**Fig. 7.** Contribution of picocyanobacteria (pAPP), heterotrophic single-celled bacteria and filamentous bacteria to picoplankton biovolume in the lake and at the end of the experiments in the enclosures (mean values of three replicate enclosures).

*Filamentous bacteria during zooplankton maxima in previous years*

With our staining protocol and the fluorochrome SYTO 9 we were able to visualize filamentous bacteria and phytoplankton in samples fixed with acidic Lugol and stored for more than 15 years in the dark at room temperature. For several samples, which were about 5 years old at the time of the staining, we had numbers and mean length of filamentous bacteria determined from DAPI-preparations of formalin-fixed samples. In this case, the comparison of SYTO 9 staining with DAPI-preparations revealed that SYTO 9 yielded approximately 20–30% lower values.

For all three zooplankton groups, *Daphnia* spp., *Cyclops* spp. (mainly *C. vicinus*) and rotifers (mainly *Brachionus* spp., E. Jeppesen unpublished), samples from dates when they dominated the total biomass of the zooplankton assemblage were available. The examination of 37 Lugol samples, collected from 1984–1992, revealed significant differences in filament biovolume in relation to dominant zooplankton taxa ( $P < 0.01$ ). Filamentous bacteria had much lower abundance and biovolume when *Daphnia* spp. dominated than in communities dominated by rotifers or cyclopoids (Figure 8). Although *Cyclops*-dominated situations had the highest mean number and biovolume of filaments, this was not significantly different from the rotifer dates due to the high variability of the data, especially of the rotifer-dominated dates. The mean filament biovolume determined from the old Lugol samples was within the range of data obtained from the mesocosm experiments in which we also found low values for the *Daphnia*-dominated situation (end of Exp. 2 in +ZP), but high concentrations of filaments when *Cyclops* dominated (beginning of Exp. 2). Filament concentrations were substantially lower in the rotifer-dominated situation in Exp. 1 than in older samples of rotifer dominance. However, the rotifer-dominated situations of previous years showed the highest variation in filament numbers, ranging from



**Fig. 8.** Whisker box-plots of filamentous bacterial biovolume in Lugol-fixed samples from Lake Søbygård from the years 1984–1992, differentiated for dates when zooplankton composition was dominated either by rotifers, daphnids or cyclopoids to more than 80% (of biomass dry weight) and when total zooplankton biomass was  $>1 \text{ mg dry wt l}^{-1}$ .

very low to very high filament abundance, and therefore have the highest uncertainty regarding the influence of the zooplankton composition.

## Discussion

The field situation during our study resembled the typical summer succession known from previous years (Jeppesen *et al.*, 1990), with a shift from rotifers to copepods and cladocerans, and an alternating sequence of phytoplankton blooms and collapses. Our experimental study covered the following successional sequences: (i) final stage of a rotifer-induced phytoplankton depression; (ii) collapse of the rotifer population probably due to predation and parasitism, strong population growth of *Cyclops vicinus*; (iii) phytoplankton bloom (mainly *Scenedesmus*) and population maxima of cyclopoid copepods; (iv) population increase of *Daphnia* spp. and probably the beginning of the next phytoplankton collapse.

The differently filled mesocosms had clearly contrasting zooplankton assemblages although rapid population growth, enabled by high food concentrations and high temperatures, resulted in shifts during the experimental period. We assumed that changes in the microbial food web structure which occurred parallel to a shift in metazooplankton composition were caused mainly by changes in predation impact. Together with the plankton dynamics in the lake, we used the responses in the mesocosms to infer some conclusions on the major trophic interactions and the coupling of microbial components to higher trophic levels.

Resource supply as the controlling mechanism (bottom-up) seemed improbable for the phagotrophic protozoans as pico- and nanoplankton concentrations were high and responses of the respective micro-organisms were generally rapid after changes in metazooplankton. Inorganic nutrients (P, N) were not limiting during the first experiment, but N-limitation might have had some influence on the phytoplankton succession during the second experiment (nitrate and ammonium were close to the detection limit).

### *Experiment 1: Impact of a rotifer-dominated zooplankton community*

The period of the first experiment (July 6–12) was characterized by a collapse of the large rotifer community, resulting in an overall low metazooplankton biomass (Figure 1). This seemed to have released all phototrophic and phagotrophic organisms from predation control during that week, and only bacterial numbers decreased, probably due to increased protozoan grazing. The large *Brachionus* population had produced not only a phytoplankton depression as in previous years (Jeppesen *et al.*, 1990), but had also exerted some grazing pressure on small protozoans, indicated by the rapid response of ciliates and HNF after the rotifer collapse. The dominant ciliate species during our experimental period were small-sized forms (15–25  $\mu\text{m}$ ), which are susceptible to microfiltration, and grasping rotifer species (Arndt, 1993; Gilbert and Jack, 1993; Thouvenot *et al.*, 1999) as are HNF (Sanders *et al.*, 1994; Jürgens *et al.*, 1996; Thouvenot *et al.*, 1999). The very high ciliate net growth rates in the range 1.5–2.3  $\text{day}^{-1}$  (Jürgens *et al.*, 1999b)

were, however, sufficient to compensate for these grazing losses and could establish moderately high ciliate densities ( $100\text{--}130\text{ ml}^{-1}$ ) even during the rotifer peak. Large, predatory ciliates showed the opposite pattern and declined, similarly to the rotifers. This could be due to predation by *Asplanchna* and the increasing *Cyclops* population, as large ciliates are most susceptible to grasping, selectively feeding rotifers and cyclopoid copepods (Gilbert and Jack, 1993; Wickham, 1995). Besides protozoans, APP also profited from the low metazooplankton biomass after the rotifer collapse and increased in numbers. Bacterivory by some of the occurring rotifer species (e.g. *Filinia longiseta*, *Brachionus angularis*) has been demonstrated (Starkweather *et al.*, 1979; Ooms-Wilms *et al.*, 1995; Hwang and Heath, 1999) and the grazing efficiency on APP, which are  $1\text{--}3\ \mu\text{m}$  in length, is probably higher than on heterotrophic bacteria.

The analysis of the responses in the experimental mesocosms was complicated by the fact that in +ZP, as in the lake, a collapse of the rotifers occurred, whereas the <50 mesocosms enabled a rapid population increase in small rotifer species (*Filinia* spp.) and in large, predatory ciliates. This resulted in a reversed situation, contrary to our expectations, with more suppression of nanoplankton (ciliates, eAPP) in <50 than in +ZP. The <10 mesocosms, in which rotifers were absent, showed the strongest increase in all phagotrophic and phototrophic organisms, indicating relief from grazing pressure.

The functional groups of the microbial food web, which showed the most significant differences between <50 and +ZP treatments, were ciliates and filamentous bacteria. Ciliates were probably suppressed in <50 because of the strong microzooplankton development (small rotifers, large, predatory ciliates). Filamentous bacteria, which are inedible for protozoans, have been shown to develop in eutrophic lakes in parallel with HNF (Jürgens and Stolpe, 1995; Sommaruga and Psenner, 1995) but might be promoted as grazing-resistant morphotypes, also from grazing pressure by ciliates (Shikano *et al.*, 1990). For the higher filament numbers in <50, the underlying reasons are less obvious. Potential bacterial grazers (HNF, ciliates), which might have triggered the feedback response in the bacterial assemblage, were even less abundant than in +ZP. Only in <10 was the appearance of grazing-resistant filaments paralleled by a strong increase in protozoan numbers.

In summary, the rotifer-dominated metazooplankton seemed to have exerted only moderate, although significant, top-down control on the microbial food web, especially on small ciliates, HNF and APP. The decline in rotifers produced some cascading effects on the bacterial level, with a decreasing abundance of single cells and the appearance of grazing-resistant filaments. Furthermore, there was no indication that APP (prokaryotic or eukaryotic) were subject to significant grazing by protozoans as their concentrations increased parallel to those of ciliates and HNF (for example in <10). This is in contrast to observations in mesoeutrophic systems in which APP can be controlled by ciliates and HNF (Pernthaler *et al.*, 1996; Šimek *et al.*, 1997). Complex interactions within the micro- and nanozooplankton seem to be typical when larger metazooplankton are absent; then ciliates, which comprise different trophic levels, and rotifers often co-occur in high numbers (Sommaruga, 1995; E. Jeppesen, unpublished data).

*Experiment 2: Impact of a Cyclops- and Daphnia-dominated zooplankton community*

The impact of metazooplankton on the microbial community (and the difference between +ZP and <50) was much more pronounced in the second experiment, but shifts here also occurred in the zooplankton composition, from dominance of cyclopoids to dominance of daphnids (Figure 1). The most obvious effect of the initial dominance of *Cyclops vicinus* was the very low level of ciliates (<30 ml<sup>-1</sup>) despite high phytoplankton biomass. The very high grazing efficiency of *C. vicinus* and related cyclopoids on planktonic ciliates has been recently demonstrated (Wiackowski *et al.*, 1994; Wickham, 1995; Rabette *et al.*, 1998). Experiment 2 (Figure 5) clearly demonstrated the strong *Cyclops* predation control of ciliates, as these showed a rapid increase after zooplankton removal in <50 (Figure 5). Only the smallest ciliates remained in the lake during the copepod peak, consisting nearly exclusively of *Urotricha* spp. 12–15 µm in length. HNF, which are not efficiently captured by cyclopoids (Jürgens *et al.*, 1996), were at a relatively high level. Another major difference compared with the first experimental period was the high abundance of pAPP and filamentous bacteria, constituting the major portion of picoplankton biomass (Figure 7). Both groups appeared concomitantly with the increase in cyclopoids and the strong decline in ciliates. We can, however, only speculate about the underlying trophic links which triggered this shift. One possibility is that the strong predation control of ciliates by *C. vicinus* enabled higher HNF numbers and thereby, higher grazing pressure on heterotrophic, free-living bacteria.

Towards the end of Exp. 2, the zooplankton was already dominated by *Daphnia*. This was reflected in the well-known top-down control on all components of the microbial food web (Jürgens, 1994): all functional groups examined showed a strong decline from the second to the last sampling date in the +ZP mesocosms. We suspect that fish predation in the lake prevented a high *Daphnia* biomass similar to that in +ZP and therefore, the effects on lower trophic levels were less pronounced in the lake at that time. The contribution of *Daphnia* spp. to zooplankton biomass in Lake Søbygård is highly linked to the abundance of planktivorous fish (roach, rudd) (Jeppesen *et al.*, 1998), and fish density was at an intermediate level in 1994 (E.Jeppesen, unpublished data).

*Role of autotrophic and heterotrophic picoplankton in Lake Søbygård*

Heterotrophic 'normal' bacteria, i.e., small, freely-dispersed cells, did not, in terms of cell number and biomass, constitute an important component in our mesocosm experiments. During the second half of the experimental period, pAPP alone significantly exceeded bacterial biomass (Figure 7). From an estimation of total phytoplankton (based on Chl *a*) and bacterial (based on biovolume) biomass, we estimated the bacteria:phytoplankton biomass ratio. It was 0.2–0.3 during the phytoplankton depression at the beginning of the first experiment, and ~0.02 during the phytoplankton bloom of the second experimental period.

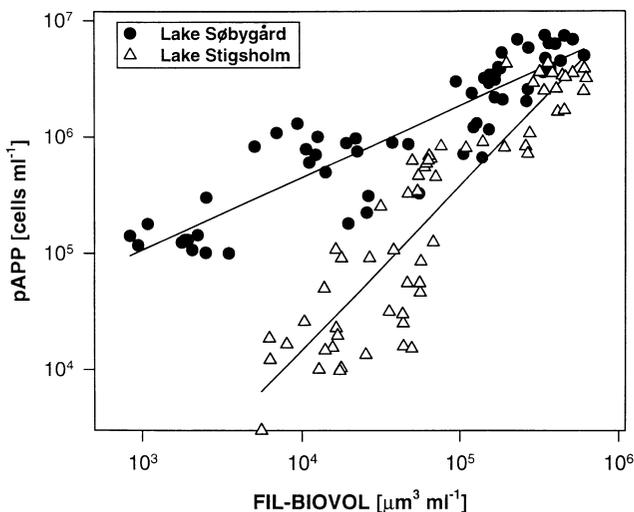
From 8 years of seasonal studies in Lake Søbygård, it has become evident that only a small fraction of phytoplankton is channelled through bacterioplankton,

with a bacterial to phytoplankton production ratio of only 2–8% during summer (Jeppesen *et al.*, 1997a). It seems to be a general trend that the bacteria:phytoplankton ratios decrease with increasing trophicity of the system, and that bacterial biomass is generally small compared with phytoplankton biomass in hypertrophic systems (Robarts *et al.*, 1994; Sommaruga and Robarts, 1997). The underlying reasons for this pattern are not known. In our study, we suspect that predation was a major controlling factor for bacterial abundance as bacterial productivity was very high (Figure 6). TdR incorporation was mostly  $>300 \text{ pmol l}^{-1} \text{ h}^{-1}$ , which corresponds to a production of  $>15 \text{ mg C l}^{-1} \text{ h}^{-1}$ , and is thus among the highest values measured for hypertrophic lakes (Sommaruga and Robarts, 1997). Despite this high productivity, bacterial abundance remained relatively constant throughout the study period and showed much fewer fluctuations than the other components of the microbial food web. This confirms previous studies in Lake Søbygård which showed that a significant variation in phytoplankton biomass and production is not reflected by similar changes for bacterioplankton (Jeppesen *et al.*, 1997).

Filamentous bacteria constituted an important fraction of the bacterioplankton, reaching 50 to  $>70\%$  of the biomass of normal, single bacterial cells in the lake which they even exceeded in the mesocosms ( $<50$ ,  $<10$ ) of Exp. 2. High biomass of filamentous bacteria has been found in other eutrophic lakes (Jürgens and Stolpe, 1995; Sommaruga and Psenner, 1995) and was generally correlated with increased grazing pressure by bacterivorous protists. The appearance of filamentous bacteria can be caused by the phenotypic plasticity of certain strains already present (Hahn *et al.*, 1999) but can also involve a strong alteration of the bacterial community structure (Jürgens *et al.*, 1999a). Filament formation is a very effective defence mechanism against phagotrophic protists (Hahn *et al.*, 1999) but not against daphnids (Jürgens *et al.*, 1994). Evidence of a predation-triggered shift towards filamentous bacteria comes from the peaks in HNF and ciliates which preceded the filament bloom. The significant, but relatively weak, correlation of filamentous bacteria with HNF ( $r = 0.46$ ) indicates that the functional relationship is not as clear as in previous studies (Jürgens *et al.*, 1994; Jürgens and Stolpe, 1995). Bacterivory by some of the ciliate species might contribute as well to the selection pressure towards grazing-resistant forms (Shikano *et al.*, 1990). The differential responses of filaments in our manipulated mesocosms implies, however, that filamentous bacteria might be a sensitive indicator of certain food web constellations and of changes in food web structure. This is also corroborated by the enumeration of filamentous bacteria in Lugol samples from previous years (Figure 8). The fact that filaments occur predominantly during population maxima of cyclopoid copepods might be interpreted as a trophic cascade in the following sense: *Cyclops* dominance results in efficient predation control of ciliates but not of HNF, which can develop maximum concentrations. This in turn enhances the grazing pressure on bacteria, and filamentous bacteria appear as grazing-resistant forms. We need to know more about the ecological role and underlying regulating mechanisms of filamentous bacteria, as they are an important component and probably stabilize a high bacterial biomass, even under enhanced grazing pressure in productive systems.

The abundance of APP, first eukaryotic and later prokaryotic cells, in Lake Søbygård is among the highest reported in the literature (Sommaruga and Robarts, 1997). APP such as *Synechococcus*-like cells are known to be important primary producers in more oligotrophic systems [see reviews by (Stockner and Antia, 1986; Weisse, 1993)], but less is known about their ecological role in highly eutrophic systems. Several investigations have shown that the relative contribution of APP to total phytoplankton biomass decreases with increasing trophicity (Søndergaard, 1991). However, as Sommaruga and Robarts (Sommaruga and Robarts, 1997) pointed out in their review, we presently have only few data on picophytoplankton in hypertrophic lakes and there is no consistent trend, as hypertrophic systems seem to have either very low or very high APP concentrations (Carrick and Schelske, 1997; Vörös *et al.*, 1998). One difference compared with many other hypertrophic lakes is that most of the Danish, shallow, eutrophied lakes are dominated by green algae instead of blue-greens (Jensen *et al.*, 1994).

The strongest correlation of microbial components found during our study was the positive correlation between pAPP and filamentous bacteria. As a comparable relationship was found in another mesocosm study from nearby eutrophic Lake Stigsholm (Jürgens and Jeppesen, 1998), we plotted both relationships in Figure 9. The data from Lake Stigsholm were obtained from large littoral mesocosms with and without macrophytes (Jeppesen *et al.*, in preparation) and also included *Daphnia*-dominated situations with very low pAPP abundance. Thus, they comprised a wider range of pAPP concentrations than Lake Søbygård and the log-log relationship had a much steeper increase. However, both relationships



**Fig. 9.** Relationship between log-transformed data of filamentous bacteria (biovolume) and prokaryotic autotrophic picoplankton (pAPP) for Lake Søbygård ( $\log_{10} \text{pAPP} = 3.17 + 0.62 \log_{10} \text{FIL}$ ,  $r^2 = 0.81$ ,  $P < 0.05$ ) and Lake Stigsholm ( $\log_{10} \text{pAPP} = -1.46 + 1.41 \log_{10} \text{FIL}$ ,  $r^2 = 0.81$ ,  $P < 0.05$ ).

have a high correlation coefficient, are highly significant and merge at comparable maximum values of APP and filamentous bacterial biovolume.

Picocyanobacteria have been reported to be ingested efficiently by phagotrophic protozoans (Pernthaler *et al.*, 1996; Šimek *et al.*, 1997). However, from laboratory experiments, there is also evidence of inferior food quality of *Synechococcus* for protozoans (Caron *et al.*, 1991) and from feeding studies with mesozooplankton, the poor nutritional value (DeMott and Müller-Navarra, 1997) and sometimes even toxic effects of picocyanobacteria, is well known. The strong correlation of pAPP and filamentous bacteria, and the fact that pAPP abundance was not affected by increased ciliate or HNF numbers in the experiments (in contrast to heterotrophic bacteria), suggests that they are not efficiently grazed by protists. Only when *Daphnia* developed in larger numbers in Experiment 2 did pAPP strongly decline (Figure 5). *Synechococcus* and related genera comprise a high genetic and ecological diversity (Postius *et al.*, 1996) and it is too early to generalize from a single study on their general ecological role and their fate in the planktonic food web.

Predation has been shown to be the most important structuring force in shallow eutrophic lakes, including Lake Søbygård (Jeppesen *et al.*, 1997b, 1998), and predatory interactions between fish, zooplankton and phytoplankton determine the structure of the classical grazing food chain. The microbial food web is connected mainly via the different grazing pattern of the metazooplankton. This study illustrates that the importance of cascading effects on the microbial community depends on which components of the microbial food web we are considering. Although our knowledge is still preliminary, it seems to be evident that phagotrophic protozoans, auto- and heterotrophic picoplankton differ strongly in their response to changes at higher trophic levels. Whereas the cascading effects of the different zooplankton communities on bacterial abundance or productivity seem to be relatively modest and much less significant than on phytoplankton (Jeppesen *et al.*, 1997a), effects on bacterial community structure (indicated by filamentous bacteria), on autotrophic picoplankton and on protozoans are most likely fairly strong. As for zooplankton, the predation impact and cascading effects would probably be even more visible if we were able to consider more of the heterogeneity and the species composition of pico- and nanoplankton in food web studies.

### Acknowledgements

The assistance of the technical staff of the National Environmental Research Institute, Silkeborg, is gratefully acknowledged. KJ was supported by a DANVIS grant from the Danish Research Academy and EJ by the Danish Natural Science Research Council (grant 9601711).

### References

- Arndt, H. (1993) Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates)—a review. *Hydrobiologia*, **255/256**, 231–246.

- Bell,R. (1988) Thymidine incorporation and estimates of bacterioplankton production: Are the conversion factors valid? *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, **31**, 163–171.
- Bottrell,H.H., Duncan,A., Gliwicz,Z.M., Grygierek,E., Herzig,A., Hillbricht-Ilkowska,A., Kurasawa,H., Larsson,P. and Weglenska,T. (1976) A review of some problems in zooplankton production studies. *Norw. J. Zool.*, **24**, 419–456.
- Brett,M.T., Wiackowski,K., Lubnow,F.S., Müller-Solger,A., Elser,J.J. and Goldman,C.R. (1994) Species-dependent effects of zooplankton on planktonic ecosystem processes in Castle Lake, California. *Ecology*, **75**, 2243–2254.
- Burns,C.W. and Gilbert,J.J. (1993) Predation on ciliates by freshwater calanoid copepods: Rates of predation and relative vulnerabilities of prey. *Freshwater Biol.*, **30**, 377–393.
- Burns,C.W. and Schallenberg,M. (1996) Relative impacts of copepods, cladocerans and nutrients on the microbial food web of a mesotrophic lake. *J. Plankton Res.*, **18**, 683–714.
- Caron,D.A., Lim,E.L., Miceli,G., Waterbury,J.B. and Valois,F.W. (1991) Grazing and utilization of chroococcoid cyanobacteria and heterotrophic bacteria by protozoa in laboratory cultures and a coastal plankton community. *Mar. Ecol. Prog. Ser.*, **76**, 205–217.
- Carrick,H.J. and Schelske,C.L. (1997) Have we overlooked the importance of small phytoplankton in productive waters? *Limnol. Oceanogr.*, **42**, 1613–1621.
- Christoffersen,K., Riemann,B., Klynsner,A. and Søndergaard,M. (1993) Potential role of fish predation and natural populations of zooplankton in structuring a plankton community in eutrophic lake water. *Limnol. Oceanogr.*, **38**, 561–573.
- DeMott,W.R. and Müller-Navarra,D.C. (1997) The importance of highly unsaturated fatty-acids in zooplankton nutrition—evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. *Freshwater Biol.*, **38**, 649–664.
- Gilbert,J.J. and Jack,J.D. (1993) Rotifers as predators on small ciliates. *Hydrobiologia*, **255/256**, 247–254.
- Hahn,M.W., Moore,E.R.B. and Höfle,M.G. (1999) Bacterial defence mechanism of filament formation against flagellate grazing is growth rate-controlled in bacteria of different phyla. *Appl. Environ. Microbiol.*, **65**, 25–35.
- Hansen,A.M. and Jeppesen,E. (1992) Life cycle of *Cyclops vicinus* in relation to food availability, predation, diapause and temperature. *J. Plankton Res.*, **14**, 591–605.
- Hwang,S.J. and Heath,R.T. (1999) Zooplankton bacterivory at coastal and offshore sites of Lake Erie. *J. Plankton Res.*, **21**, 699–719.
- Jensen,J.P., Jeppesen,E., Olrik,K. and Kristensen,P. (1994) Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in shallow Danish lakes. *Can. J. Fish. Aquat. Sci.*, **51**, 1692–1699.
- Jeppesen,E., Søndergaard,M., Sortkjaer,O., Mortensen,E. and Kristensen,P. (1990) Interactions between phytoplankton, zooplankton and fish in a shallow, hypertrophic lake: a study of phytoplankton collapses in Lake Søbygård, Denmark. *Hydrobiologia*, **191**, 149–164.
- Jeppesen,E., Søndergaard,M., Jensen,J.P., Mortensen,E. and Sortkjaer,O. (1996) Fish-induced changes in zooplankton grazing on phytoplankton and bacterioplankton—a long-term study in shallow hypertrophic Lake Søbygård. *J. Plankton Res.*, **18**, 1605–1625.
- Jeppesen,E., Erlandsen,M. and Søndergaard,M. (1997a) Can simple empirical equations describe the seasonal dynamics of bacterioplankton in lakes: An eight-year study in shallow hypertrophic and biologically highly dynamic Lake Søbygård, Denmark. *Microb. Ecol.*, **34**, 11–26.
- Jeppesen,E., Jensen,J.P., Søndergaard,M., Lauridsen,T., Pedersen,L.J. and Jensen,L. (1997b) Top-down control in freshwater lakes: The role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia*, **342/343**, 151–164.
- Jeppesen,E., Søndergaard,M., Jensen,J.P., Mortensen,E., Hansen,A.-M. and Jørgensen,T. (1998) Cascading trophic interactions from fish to bacteria and nutrients after reduced sewage loading: a 18 year study of a shallow hypertrophic lake. *Ecosystems*, **1**, 250–267.
- Jumars,P.A., Penry,D.L., Baross,J.A., Perry,M.J. and Frost,B.W. (1989) Closing the microbial loop: Dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion and absorption in animals. *Deep-Sea Res.*, **36**, 483–495.
- Jürgens,K. (1994) Impact of *Daphnia* on planktonic microbial food webs—A review. *Mar. Microb. Food Webs*, **8**, 295–324.
- Jürgens,K. and Jeppesen,E. (1998) Cascading effects on microbial food web structure in a dense macrophyte bed. In Jeppesen,E., Søndergaard,M., Søndergaard,M. and Christoffersen,K. (eds), *The Structuring Role Of Submerged Macrophytes In Lakes*. Springer-Verlag, New York, pp. 262–273.
- Jürgens,K. and Stolpe,G. (1995) Seasonal dynamics of crustacean zooplankton, heterotrophic nanoflagellates and bacteria in a shallow, eutrophic lake. *Freshwater Biol.*, **33**, 27–38.

- Jürgens,K., Arndt,H. and Rothhaupt,K.O. (1994) Zooplankton-mediated changes of bacterial community structure. *Microb. Ecol.*, **27**, 27–42.
- Jürgens,K., Wickham,S.A., Rothhaupt,K.O. and Santer,B. (1996) Feeding rates of macro- and microzooplankton on heterotrophic nanoflagellates. *Limnol. Oceanogr.*, **41**, 1833–1839.
- Jürgens,K., Pernthaler,J., Schalla,S. and Amann,R. (1999a) Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. *Appl. Environ. Microbiol.*, **65**, 1241–1250.
- Jürgens,K., Skibbe,O. and Jeppesen,E. (1999b) Impact of metazooplankton on the composition and population dynamics of planktonic ciliates in a shallow, hypertrophic lake. *Aquat. Microb. Ecol.*, **17**, 61–75.
- Ooms-Wilms,A.L., Postema,G. and Gulati,R.D. (1995) Evaluation of bacterivory of *Rotifera* based on measurements of *in situ* ingestion of fluorescent particles, including some comparisons with *Cladocera*. *J. Plankton Res.*, **17**, 1057–1077.
- Pace,M.L. and Funke,E. (1991) Regulation of planktonic microbial communities by nutrients and herbivores. *Ecology*, **72**, 904–914.
- Pace,M.L., McManus,G.B. and Findlay,S.E.G. (1990) Planktonic community structure determines the fate of bacterial production in a temperate lake. *Limnol. Oceanogr.*, **35**, 795–808.
- Pace,M.L., Cole,J.J. and Carpenter,S.R. (1998) Trophic cascades and compensation—differential responses of microzooplankton in whole-lake experiments. *Ecology*, **79**, 138–152.
- Pernthaler,J., Šimek,K., Sattler,B., Schwarzenbacher,A., Bobkova,J. and Psenner,R. (1996) Short-term changes of protozoan control on autotrophic picoplankton in an oligo-mesotrophic lake. *J. Plankton Res.*, **18**, 443–462.
- Porter,K.G. and Feig,Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, **25**, 943–947.
- Porter,K.G., Paerl,H., Hodson,R., Pace,M., Priscu,J., Riemann,B., Scavia,D. and Stockner,J. (1988) Microbial interactions in lake food webs. In Carpenter,S.R. (ed.), *Complex Interactions in Lake Communities*. Springer-Verlag, New York, pp. 209–227.
- Postius,C., Ernst,A., Kenter,U. and Böger,P. (1996) Persistence and genetic diversity among strains of phycoerythrin-rich cyanobacteria from the picoplankton of Lake Constance. *J. Plankton Res.*, **18**, 1159–1166.
- Rabette,C., Thouvenot,A. and Lair,N. (1998) Laboratory experiments on trophic relationships and remote detection between two ciliates and *Cyclops vicinus*. *Hydrobiologia*, **374**, 157–167.
- Riemann,B. (1985) Potential importance of fish predation and zooplankton grazing on natural populations of freshwater bacteria. *Appl. Environ. Microbiol.*, **50**, 187–193.
- Riemann,B. and Christoffersen,K. (1993) Microbial trophodynamics in temperate lakes. *Mar. Microb. Food Webs*, **7**, 69–100.
- Robarts,R.D., Arts,M.T., Evans,M.S. and Waiser,M.J. (1994) The coupling of heterotrophic bacterial and phytoplankton production in a hypertrophic, shallow prairie lake. *Can. J. Fish. Aquat. Sci.*, **51**, 2219–2226.
- Sanders,R.W. and Wickham,S.A. (1993) Planktonic protozoa and metazoa: Predation, food quality and population control. *Mar. Microb. Food Webs*, **7**, 197–223.
- Sanders,R.W., Caron,D.A. and Berninger,U.-G. (1992) Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. *Mar. Ecol. Prog. Ser.*, **86**, 1–14.
- Sanders,R.W., Leeper,D.A., King,C.H. and Porter,K.G. (1994) Grazing by rotifers and crustacean zooplankton on nanoplanktonic protists. *Hydrobiologia*, **288**, 167–181.
- Shikano,S., Luckinbill,L.S. and Kurihara,Y. (1990) Changes of traits in a bacterial population associated with protozoan predation. *Microb. Ecol.*, **20**, 75–84.
- Sieburth,J.M. and Estep,K.W. (1985) Precise and meaningful terminology in marine microbial ecology. *Mar. Microb. Food Webs*, **1**, 1–16.
- Šimek,K., Hartman,P., Nedoma,J., Pernthaler,J., Springmann,D., Vrba,J. and Psenner,R. (1997) Community structure, picoplankton grazing and zooplankton control of heterotrophic nanoflagellates in a eutrophic reservoir during the summer phytoplankton maximum. *Aquat. Microb. Ecol.*, **12**, 49–63.
- Sommaruga,R. (1995) Microbial and classical food webs: A visit to a hypertrophic lake. *FEMS Microb. Ecol.*, **17**, 257–270.
- Sommaruga,R. and Psenner,R. (1995) Permanent presence of grazing-resistant bacteria in a hypertrophic lake. *Appl. Environ. Microbiol.*, **61**, 3457–3459.
- Sommaruga,R. and Robarts,R.D. (1997) The significance of autotrophic and heterotrophic picoplankton in hypertrophic ecosystems. *FEMS Microb. Ecol.*, **24**, 187–200.

- Søndergaard, M. (1991) Phototrophic picoplankton in temperate lakes: seasonal abundance and importance along a trophic gradient. *Int. Rev. Ges. Hydrobiol.*, **76**, 505–522.
- Starkweather, P.L., Gilbert, J.J. and Frost, T.M. (1979) Bacterial feeding by the rotifer *Brachionus calyciflorus*: clearance and ingestion rates, behaviour and population dynamics. *Oecologia*, **44**, 26–30.
- Sternér, R.W. (1989). The role of grazers in phytoplankton succession. In Sommer, U. (ed.), *Plankton Ecology—Succession in Plankton Communities*. Springer-Verlag, Berlin, pp. 107–169.
- Stockner, J.G. and Antia, N.J. (1986) Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. *Can. J. Fish. Aquat. Sci.*, **43**, 2472–2503.
- Thouvenot, A., Debroas, D., Richardot, M. and Devaux, J. (1999) Impact of natural metazooplankton assemblage on planktonic microbial communities in a newly flooded reservoir. *J. Plankton Res.*, **21**, 179–199.
- Vaqué, D. and Pace, M.L. (1992) Grazing on bacteria by flagellates and cladocerans in lakes of contrasting food-web structure. *J. Plankton Res.*, **14**, 307–321.
- Vörös, L., Callieri, C., V-Balogh, K. and Bertoni, R. (1998) Freshwater picocyanobacteria along a trophic gradient and light quality range. *Hydrobiologia*, **369/370**, 117–125.
- Weinbauer, M.G. and Höfle, M.G. (1998) Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. *Appl. Environ. Microbiol.*, **64**, 431–438.
- Weisse, T. (1993) Dynamics of autotrophic picoplankton in marine and freshwater ecosystems. *Adv. Microb. Ecol.*, **13**, 327–370.
- Wiackowski, K., Brett, M.T. and Goldman, C.R. (1994) Differential effects of zooplankton species on ciliate community structure. *Limnol. Oceanogr.*, **39**, 486–492.
- Wickham, S.A. (1995) *Cyclops* predation on ciliates: Species-specific differences and functional responses. *J. Plankton Res.*, **17**, 1633–1646.
- Wickham, S.A. (1998) The direct and indirect impact of *Daphnia* and *Cyclops* on a freshwater microbial food web. *J. Plankton Res.*, **20**, 739–755.

*Received on June 4, 1999; accepted on November 29, 1999*