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Zooplankton-Mediated Changes of Bacterial Community Structure

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Abstract. Enclosure experiments in the mesotrophic Schöhsee in northern Germany were designed to study the impact of metazooplankton on components of the microbial food web (bacteria, flagellates, ciliates). Zooplankton was manipulated in 500-liter epilimnetic mesocosms so that either *Daphnia* or copepods were dominating, or metazooplankton was virtually absent. The bacterial community responded immediately to changes in zooplankton composition. Biomass, productivity, and especially the morphology of the bacteria changed drastically in the different treatments. Cascading predation effects on the bacterioplankton were transmitted mainly by phagotrophic protozoans which had changed in species composition and biomass. When *Daphnia* dominated, protozoans were largely suppressed and the original morphological structure of the bacteria (mainly small rods and cocci) remained throughout the experiment. Dominance of copepods or the absence of metazoan predators resulted in a mass appearance of bacterivorous protists (flagellates and ciliates). They promoted a fast decline of bacterial abundance and a shift to the predominance of morphologically inedible forms, mainly long filaments. After 3 days they formed 80–90% of the bacterial biomass. The results indicate that metazooplankton predation on phagotrophic protozoans is a key mechanism for the regulation of bacterioplankton density and community structure.

Introduction

Free-living heterotrophic bacteria have been shown to form a substantial part of the suspended particulate organic matter in marine and freshwater habitats and in oligotrophic systems, even dominating over phytoplankton carbon [8, 46]. Together with phototrophic picoplankton they form the base of a complex microbial food web, which can account for a large proportion of planktonic respiration, productivity, and nutrient recycling [1, 5, 11]. The regulation of bacterioplankton

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biomass occurs by the supply of algal derived organic substrates [11] and predation by phagotrophic protozoans, especially heterotrophic nanoflagellates (HNF) [12, 35, 41].

Most studies on pelagic food web structures have focused either on the traditional food web (phytoplankton, zooplankton, fish) or on components of the microbial loop. There are few studies that have examined the impact of higher trophic levels on the bacterioplankton. Metazoan grazing on bacterioplankton is generally less important than predation by protozoans [35, 41]. The main pathway by which metazooplankton populations affect planktonic bacteria is thought to be indirect, through the impact on the phytoplankton and thereby the supply of substrates [2, 11]. When bacterial community parameters like biomass and productivity were examined, effects after food web manipulations could be detected in whole lake experiments [30], long-term observations [23], and short-term enclosure experiments [9, 15]. However, the available information is still scarce and often contradictory, and bacteria cannot be included convincingly in scenarios about trophic interactions of plankton communities.

The trophic cascade hypothesis suggests that top level piscivores determine the abundance of planktivorous fish, which have a strong impact upon the structure and productivity of the zooplankton and phytoplankton [6]. This concept could also explain the cascading predation effects on heterotrophic protozoans, which have been seen in enclosure experiments [9, 31, 37].

Changes in the bacterioplankton community structure are more difficult to assess, and, unless molecular methods are employed, the analysis is restricted to the community level. However, the phenotypic, morphological picture of natural bacteria and activity measurements yield certain indications for changes in the controlling mechanisms. Considering these aspects we present direct experimental evidence from mesocosm experiments that demonstrate that predatory interactions can have a strong impact on the bacterioplankton. When two contrasting zooplankton communities were examined (copepods versus daphnids), major changes occurred at the bacterial level. Alterations of the bacterial community structure, as judged from the morphological appearance, were more important than changes of bacterial biomass.

Materials and Methods

From 19 May to 26 May 1992, six 500-liter polyethylene enclosures were exposed in the epilimnion of the mesotrophic Schöhsee (54 10′N, 10 27′E, 82 ha, 30 m maximum depth). The lake was thermally stratified and at the beginning of the spring clear-water phase, had high optical transparency and low chlorophyll concentration. The zooplankton consisted mainly of daphnids (*Daphnia hyalina, D. galeata*, and *D. hyalina* × *D. galeata* hybrids) and cyclopoid and calanoid copepods.

Parallel enclosures with daphnids (D1, D2), with copepods (C1, C2), and without metazoans (N1, N2) were used to study the metazooplankton impact on components of the microbial food web. In order to remove most of the metazooplankton, the bags were first filled with epilimnetic lake water that was gently filtered (without using a pump) through a 50 μm plankton net. To simulate and examine the effects of copepods and daphnids, respectively, these zooplankters were added to different enclosures in biomass quantities comparable to the in situ populations. Daphnids (*D. galeata*) were grown in the laboratory from stock cultures of a lake clone and added at approximately the same densities as in the lake (25 individuals l^{-1}). Copepod-dominated enclosures were achieved by filling in zooplankton from epilimnetic net hauls. These were taken during midday when most of the diurnally migrating daphnids stayed in the hypolimnion.

One of each zooplankton treatment (N2, C2, and D2) received a daily addition of glucose (15 mg l^{-1}) to enhance bacterial production. The experiment revealed that this bottom-up stimulus had only a minor effect on the general development of the microorganisms in the enclosures compared to the effects mediated by the metazoan composition. In most aspects the bags from series 1 and 2 were almost identical, and the results will be discussed as parallel treatments with respect to the zooplankton manipulation (N, C, D).

Sampling of the enclosures was done twice daily (9 a.m. and 8 p.m.) during the first 3 days and then once per day (9 a.m.) for the remainder of the experiment. Before taking samples, the enclosures were thoroughly mixed with a secchi disk. Chemical analyses were performed according to standard methods [49]. Zooplankton abundance in the enclosures was determined at the beginning and after 5 days from 4-liter samples that were filtered through a 40 μm net, then fixed in sugar formalin, and counted entirely. Sizing of the animals was done with a dissecting microscope equipped with a half-automated image analyser. Biovolumes were converted to dry weight using the regressions in Bottrell et al. [3], or, for *Daphnia*, regressions obtained for the lake species [Stibor, unpublished]. Animals were identified to species and development stage except for cyclopoid copepods. Copepodites were pooled with adults of the different cyclopoid species. A subsample was used for detailed identification of the dominant species. Nauplii were pooled from both cyclopoid and calanoid copepods.

Bacteria and HNF were enumerated by epifluorescent microscopy [34]. Formalin fixed samples were stained with 2,4-diamidino-phenyl-indol (DAPI) on black 0.2 μm (bacteria) and 0.8 μm (HNF) Nuclepore filters. Autotrophic and mixotrophic flagellates were distinguished by the presence of chlorophyll autofluorescence. For sizing of DAPI-stained bacteria, an automated image analysis system was used [40]. Between 600 and 1,000 cells of a sample were measured to calculate the mean bacterial volume. Size estimations of nanoflagellates and large filamentous and aggregated bacteria were done directly in the epifluorescence microscope. Large flagellates and ciliates were counted and sized from Lugol-fixed samples in settling chambers with an inverted microscope. Ciliate identification was verified with protargol impregnations in bright field optics [13] from selected samples. Assessment of chloroplasts was confirmed with DAPI-stained preparations in the epifluorescence microscope.

[^{3}H]thymidine incorporation into bacteria was measured in 30-ml bottles incubated in situ, and essentially followed the standard protocol [14]. A concentration of 15 nM was used which was sufficient for saturated uptake [Jürgens, unpublished]. After 1 h of incubation, [^{3}H]thymidine uptake was stopped by the addition of formaldehyde (final concentration 1.5%). Formalin-killed samples were used as blanks. At day 6, the distribution of thymidine incorporation into different size fractions was measured. After fixation the samples were fractionated through 0.2, 1, and 3 μm Nuclepore filters [19].

After 4 days we performed a bottle experiment for verification of the interactions between bacterial community structure and zooplankton. Daphnids from enclosure D1 (enriched to about 100 individuals l^{-1}) were incubated in 1-liter bottles (two replicates) with unfiltered water from enclosure N1 for 20 h in situ. Samples were fixed at the beginning and end of the incubation, and the changes in bacteria (edible and inedible) and protozoans were compared to a control without *Daphnia*.

Results

Physical-Chemical Parameters

Stable, warm weather conditions resulted in a constant temperature in the lake and in the enclosures throughout the study period (between 18 and 20°C). Dissolved P was rather depleted, whereas dissolved N (NH_4 and NO_3) was present in higher concentrations (Table 1). Major differences in nitrate concentrations in the enclosures were visible after 5 days. The high levels remained only in the *Daphnia* treatments, whereas in the other four enclosures nitrate was strongly reduced. Chlorophyll *a* was consistently below 2 μg l^{-1} in the lake as well as in the enclosures. This indicates that the almost twofold increase of particulate organic carbon (POC) in the enclosures without *Daphnia* was due mainly to heterotrophic biomass.

Table 1. Physicochemical data on Schöhsee and the enclosures. N, without metazooplankton; C, with copepods; D, with *Daphnia*. Series 1, without enrichment; series 2, with glucose addition

	SRP[a]	TP[b]	POC[c]	NH_4-N	NO_3-N	Chl *a*
	($\mu g\ l^{-1}$)					
Schöhsee 19 May	0.0	18.9	490	14.2	29.8	1.1
Enclosures 20 May						
N1	1.6	10.0	450	6.5	22.1	1.1
N2	1.6	10.0	550	5.9	20.2	1.3
C1	2.4	12.4	510	12.9	20.0	1.6
C2	2.8	12.0	510	9.7	20.0	1.3
D1	2.0	9.6	450	6.5	20.7	1.6
D2	2.4	10.4	510	7.4	23.5	1.1
Enclosures 25 May						
N1	0.0	3.2	880	17.4	4.5	1.8
N2	4.4	8.8	950	10.0	1.4	1.3
C1	4.8	8.0	900	11.0	1.3	1.4
C2	0.0	3.2	1100	6.7	1.0	1.2
D1	4.0	5.6	600	23.0	27.0	1.2
D2	4.4	6.0	570	4.8	23.1	1.1
Schöhsee 26 May	1.6	8.5	420	14.9	21.7	1.0

[a] Soluble reactive P
[b] Total P
[c] Particulate organic carbon

Zooplankton

The intended manipulation of the zooplankton communities was achieved successfully. In the enclosures, which were designed to be without metazoans (N1, N2), more than 90% of the zooplankton biomass was removed by filtration. The amount of daphnids in the copepod dominated enclosures (C1, C2) and the number of copepods in the *Daphnia* enclosure (D1, D2) were 10–20% of the total zooplankton, in terms of biomass (Table 2).

Copepods consisted of calanoids (mainly *Eudiaptomus gracilis*) and cyclopoids (mainly *Thermocyclops oithonoides, Cyclops kolensis*, and *Cyclops abyssorum*) in approximately equal numbers. Other metazoans (*Bosmina longirostris*, rotifers), due to their low numbers (in the enclosures C and D between 0.1 and 10.7% of the biomass), probably had lttle or no impact on plankton development. Despite some changes in total biomass, the overall composition of the zooplankton community remained constant until the end of the experiment (Table 2).

Bacteria

The bacterial assemblage in the lake and also in the enclosures in the beginning consisted of more than 90% freely dispersed small rods and cocci (Table 3), which represents the typical picture of freshwater bacterioplankton [20]. Bacterial concentration ($4.6 \times 10^6\ ml^{-1}$) and mean biovolume ($0.047\ \mu m^3$) were within the range as reported from other mesotrophic lakes during spring [44].

To illustrate the main development of the bacterioplankton in the enclosures, we used morphological criteria to distinguish two functional categories of bacteria that might be related to the food web structure. Small rods and cocci were assumed to be the main prey items for phagotrophic protozoans and were therefore counted as "protozoan-edible bacteria." Aggregated and attached bacteria, as well as larger filamentous forms, are known to be less accessible to protozoan grazers [20] and were examined separately as "protozoan-inedible bacteria."

Table 2. Zooplankton biomass and composition in the enclosures after 1 and 5 days. For abbreviations see Table 1

	After 1 day				After 5 days			
	Total biomass	Composition (% of biomass)			Total biomass	Composition (% of biomass)		
Enclosure	(μg dry wt l^{-1})	Daphnids	Copepods	Others	(μg dry wt l^{-1})	Daphnids	Copepods	Others
N1	8.7	10.7	53.5	35.8	16.1	0.0	73.3	26.7
N2	18.6	0.0	99.5	0.5	25.5	5.0	89.6	5.4
C1	258.1	9.7	82.8	7.6	191.0	14.1	85.6	0.4
C2	183.1	10.9	78.4	10.7	241.9	13.6	86.1	0.3
D1	135.5	89.0	10.5	0.5	367.7	92.7	7.1	0.2
D2	190.0	78.3	21.6	0.1	161.1	79.0	20.6	0.4

Table 3. Bacteria and protozoa at the beginning (mean ± SD from all enclosures) and in the different enclosures after 96 h. For enclosure abbreviations see Table 1

		Bacterial abundance	Bacterial biomass[a]	Protozoan biovolume	Bacterial composition (%)		
	Enclosure	($\times 10^6$ ml^{-1})	(μg C l^{-1})	(10^3 μm^3 ml^{-1})	Rods, cocci	filaments	aggregates
After 12 h		4.57 ± 0.21	73.5 ± 3.4	357.2 ± 60.5	94.2 ± 1.6	5.3 ± 1.5	0.5 ± 0.2
After 84 h	N1	0.36	39.0	2146.1	12.5	85.1	2.4
	N2	0.33	47.0	2169.0	8.7	91.0	0.3
	C1	0.27	64.9	1872.3	4.5	92.6	2.9
	C2	0.33	42.4	2141.9	9.7	89.8	0.6
	D1	1.62	29.4	475.4	83.1	15.6	1.3
	D2	1.55	28.9	569.0	81.1	17.8	1.1

[a] Calculated with a conversion factor of 380 fg C μm^3 [27]

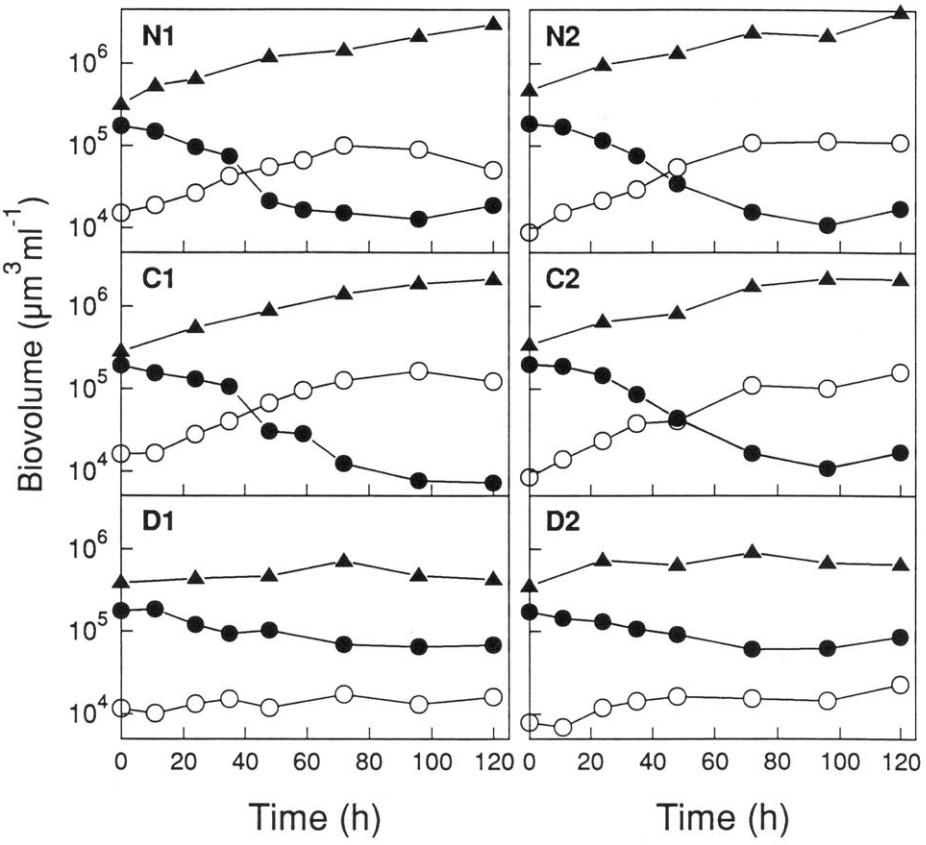

Fig. 1. Development of bacterial and protozoan biomass in the enclosures. N, Without metazoans; C, with copepods; D, with *Daphnia*. Series 1 (left side), without glucose addition; series 2 (right side), with glucose addition. Protozoan biomass (▲) includes heterotrophic and autotrophic flagellates and ciliates. Protozoan-edible cells (●) were defined as small cocci and rods up to a length of about 6 µm. Protozoan-inedible bacteria (○) include longer filaments and aggregates.

Considering these categories, the development of the bacterioplankton differed strikingly in the enclosures. After approximately 1 day, began a dramatic change in the bacterial community structure in all enclosures without *Daphnia* (N1, N2 and C1, C2) (Fig. 1). Small, edible bacteria declined continuously, with the strongest drop after about 40 h. Concomitantly protozoan-inedible cells, mainly filamentous bacteria, increased exponentially and replaced the original bacterial assemblage. The majority of the filaments were between 15 and 30 µm in length, which makes them unsuitable food even for most of the larger protozoans.

Due to the disappearance of the protozoan-edible bacterial fraction, the total bacterial abundance dropped to less than 0.4×10^6 ml^{-1} (Table 3), a number which is found in lakes of similar trophy only during the winter [44]. The data on bacterial biomass (Table 3) have to be seen as rough estimates, because the volume to carbon ratio can change with the size of the bacteria [45] and might be different for the filamentous forms. However, it became evident that the decline in cell number was not reflected in terms of biomass, which decreased only about 40%. The large filaments could compensate partly for the decline in other bacteria, and the distribution of biomass between edible and inedible bacteria was reversed after 3 days (Fig. 1, Table 3).

Table 4. Distribution of bacterial biomass in protozoan edible and inedible forms in the enclosures with and without *Daphnia*

	Proportion of bacterial biomass after 96 h (%) (mean ± SD)	
	Inedible	Edible
With *Daphnia* (n = 2)	18.1 ± 1.1	82.1 ± 1.0
Without *Daphnia* (n = 4)	88.6 ± 4.0	11.4 ± 4.0

In contrast, the situation in the *Daphnia* enclosures (D1, D2) changed only slightly. Daphnids, which are known to ingest planktonic bacteria with variable efficiency [4, 33], probably caused a reduction in bacterial abundance and mean cell volume (Table 3). But small protozoan-edible cells remained dominant in the bacterial community (80% of the biomass), whereas filaments increased only slightly (Fig. 1). Bacterial biomass was more strongly reduced than in the other enclosures (Table 3). The main outcome for the bacterial community is summarized in Table 4 in which data from the enclosures with and without *Daphnia* were pooled, and the proportion of protozoan-edible and -inedible bacteria compared.

The experiment with daphnids exposed to a microbial community, which has developed without metazoans during 4 days in enclosure N1, revealed that the bacterial community structure can be reversed rapidly when *Daphnia* are present (Fig. 2). Within 20 h single bacterial cells reappeared and exceeded the biomass of the protozoan-grazing resistant forms. This was accompanied with a suppression of the protozoans (Fig. 2).

Although the shift from small, freely dispersed bacterial cells to large filaments was the most pronounced change in the bacterial community, the remaining edible bacterial fraction also changed in morphological composition. The volume distribution after 12 and 84 h is shown for enclosures N1, C1, and D1 in Fig. 3. The bacterial assemblage shifted to the smallest size classes in all enclosures, with more than 50% of the bacteria less than 0.02 $\mu m.^3$ This seemed to be independent of the type of bacterial grazers because it occurred when protozoans were the main bacterivores (N1 and C1), and when daphnids were bacterial consumers (D1).

Differences in the bacterioplankton in the enclosures were also obvious from the thymidine uptake measurements which are an indication of bacterial productivity and activity. Total and specific incorporation increased in all treatments throughout the experiment (Fig. 4). The higher thymidine incorporation in the enclosure of series 2 was the only indication that glucose addition affected the bacteria. This was not the case for the *Daphnia* enclosures, which had rather similar values for both treatments (Fig. 4). In Lake Schöhsee, as well as in other mesotrophic lakes, generally 80–95% of the bacterial activity is found in the <1 μm fraction [19]. This was still the case in the *Daphnia* treatment (unenriched) after 6 days, whereas in enclosures N and C the activity was then concentrated in the >3 μm fraction, which reflected the dominance of the long filaments (Table 5).

Protozoans

Besides typical obligate bacterivores, e.g., colorless chrysomonads and small scuticociliates, a number of mixotrophic flagellates and ciliates as well as algivorous ciliates were important during the experiment. Although the strongest grazing pressure on the bacteria was probably exerted by HNF, most of the other protists potentially ingest bacteria as well and impact the bacterial community. Therefore the overall protozoan biomass, as plotted in Fig. 1, should be a good indicator of the degree of bacterivory in the enclosures.

Protozoan biomass increased steadily in the enclosures with copepods (C) and without metazoans (N). After 1 week they reached a level that was about one order of magnitude higher than at the beginning or in the *Daphnia* enclosures (Fig. 1). A succession of protozoans occurred, which was especially pronounced in the enclosures without *Daphnia* and is exemplified for enclosures N1, C1,

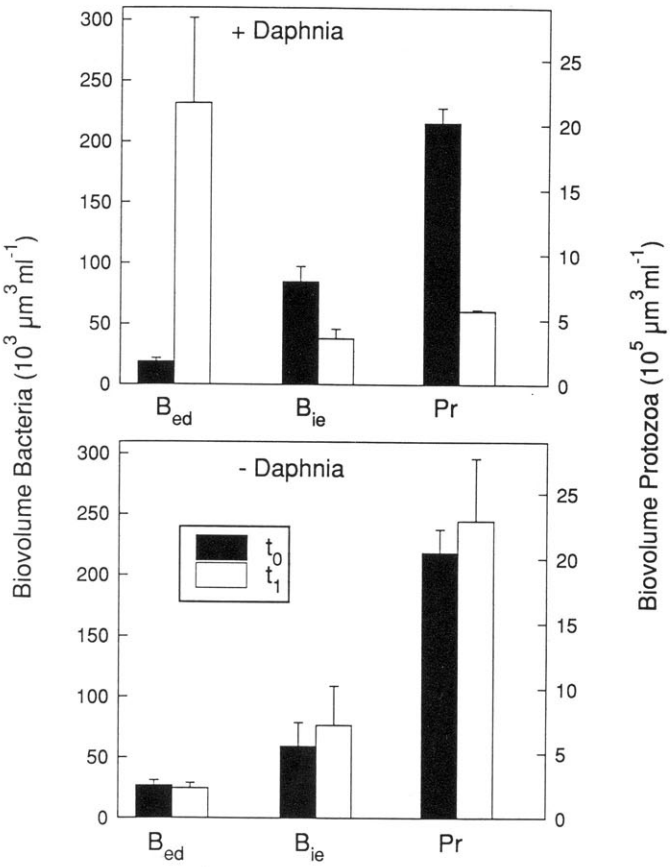

Fig. 2. Short-term changes (20 h) in the biomass of edible bacteria (B_{ed}), inedible bacteria (B_{ie}) and protozoans (Pr) in the presence and absence of *Daphnia*. Daphnids were taken from enclosure D1 and incubated with water from enclosure N1 at day 4 of the mesocosm experiment.

and D1 in Table 6. In N and C, bacterivorous nanoflagellates developed rapidly (peak after 36 h), and later autotrophic and larger heterotrophic flagellates and ciliates increased. The increase of ciliates was accompanied by a change from mostly mixotrophic (mainly *Pelagohalteria viridis*) to heterotrophic species (mainly *Pseudobalanion planctonicum*). The ciliate composition resembled the one described for Lake Constance [29], a lake of similar trophy. Because most of the ciliates and larger flagellates can feed on nano-sized cells (e.g., HNF) [29, 51], predation among the protozoans increased during the course of the experiment, but the overall pattern was set by the metazoan community.

Top-down control of the copepods on the protozoan assemblage was relatively small and affected only the ciliates (about 50% reduction compared to enclosures without metazoans). Stronger predation control was exerted in the *Daphnia* enclosures (D1, D2) where protozoan development was essentially suppressed and overall protozoan biomass stayed more or less constant or increased only slightly. Flagellates declined to very low numbers, and the only ciliate that increased significantly was the mixotrophic *Pelagohalteria viridis*. This is a species that has been observed to increase towards the end of the clear-water phase [29], and might be one of the few protozoans that is not under predation control by the cladocerans. Top-down effects were also visible in the separate *Daphnia* experiment; total protozoan biomass decreased more than 70% within 20 h of *Daphnia* grazing (Fig. 2).

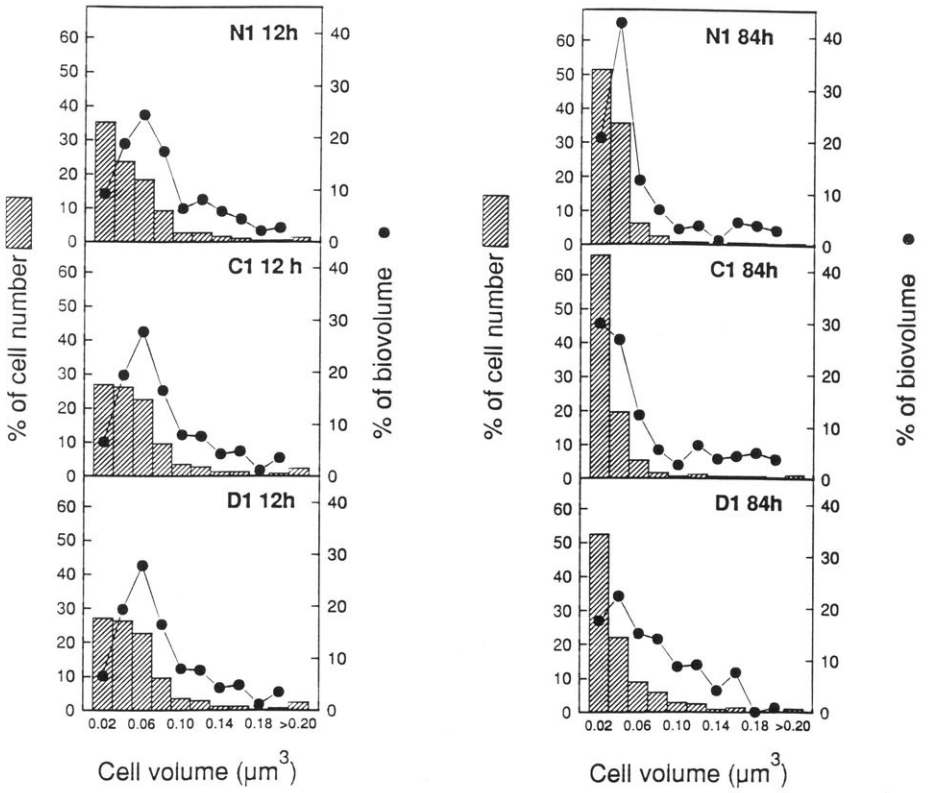

Fig. 3. Bacterial size distribution in the enclosures N1, C1, and D1 after 12 and 84 h. Filamentous and aggregated bacteria were not included. For enclosure abbreviations see Fig. 1.

Discussion

Bacterial losses due to grazing, mainly by nanoprotists, are well studied in marine and freshwater systems. Comparatively little is known about how grazing can impact and alter the bacterial assemblage. One reason is that the development and application of appropriate techniques to study species composition and diversity of microorganisms in nature is still in a very early stage [32]. More obvious and easier to assess features are the morphology and activity of planktonic bacteria which were used in this study to follow the changes of the microbial community. The overall morphological structure is a good indicator of changes in community structure that are caused by the underlying regulating mechanisms, substrate supply and grazing [26, 36, 50].

In this respect, a drastic and rapid change in enclosures N and C occurred after the zooplankton manipulation. The original bacterial community was replaced by protozoan grazing-resistant forms within less than three days. The development of the filamentous bacteria was clearly correlated to the appearance of protozoan grazers (Fig. 5). In culture experiments it has been shown that protozoan grazing pressure can shift bacterial populations towards morphologically inedible forms

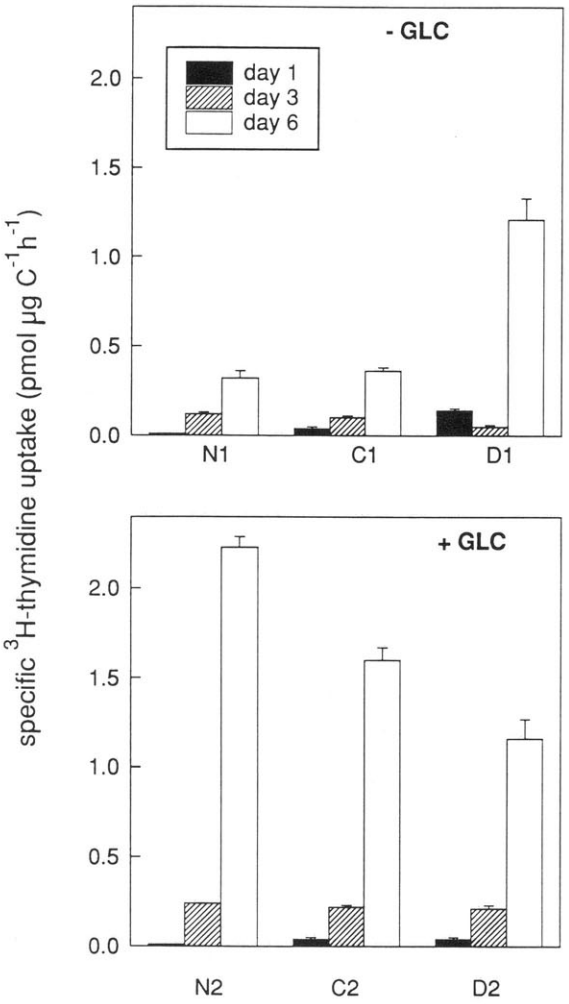

Fig. 4. [^{3}H]thymidine uptake in the enclosures after 1, 3, and 6 days. For enclosure abbreviations see Figure 1.

such as filaments and aggregates and eliminate other nonresistant species [17]. This shift can be caused by changes in species [17] or by a phenotypic change in morphology of certain strains [42]. The occurrence of filamentous or aggregated planktonic bacteria during the presence of nanoprotists has been observed also in situ [18, 43]. At least in more eutrophic systems and during certain situations, these can comprise a significant part of the bacterial biomass. For example, in Grosser Binnensee, a shallow, eutrophic lake in northern Germany, it was estimated that peaks of filamentous bacteria, which occurred during the mass development of HNF, comprised up to 40% of the total bacterial biomass [Jürgens and Stolpe, in preparation].

Table 5. Incorporation of [^{3}H]thymidine at the end of the experiments (after 6 days), and distribution in different size fractions. Values are means of triplicate measurements (±1 SD)

	Thymidine incorporation				
	Total	Per bacterial biomass	Size distribution (%)		
Enclosure	(pmol l^{-1} h^{-1})	(pmol μg C^{-1} h^{-1})	0.2–1.0 μm	1.0–3.0 μm	>3.0 μm
N1	8.3 ± 1.1	0.32 ± 0.04	21.8	3.6	76.4
N2	108.1 ± 2.9	2.23 ± 0.06	14.0	18.0	68.0
C1	17.7 ± 1.1	0.36 ± 0.02	25.4	19.5	55.1
C2	107.7 ± 4.4	1.60 ± 0.07	2.2	12.6	85.8
D1	38.7 ± 3.9	1.21 ± 0.12	82.8	7.9	9.3
D2	53.2 ± 5.1	1.16 ± 0.11	40.0	12.1	47.9

Table 6. Protozoan composition in the enclosures N1, C1, and D1 after 36 and 108 h. For enclosure abbreviations see Table 1

	After 36 h			After 108 h		
Enclosure	N	C	D	N	C	D
Heterotrophic flagellates (ml^{-1})						
2–5 μm	10955	7766	3973	482	1445	883
5–10 μm	1247	1264	722	2950	4454	401
>10 μm	2.0	3.9	3.5	26.5	11.7	3.6
Autotrophic/mixotrophic flagellates (ml^{-1})[1]	4382	3491	1866	15713	20589	2087
Ciliates						
Total number (ml^{-1})	11.8	9.5	12.8	92.3	53.7	24.9
With chloroplasts (%)	80.9	82.1	83.6	24.8	43.7	87.6
Dominant taxa (ml^{-1}):						
Strobilidium sp.	0.3	0.1	0.6	12.8	6.5	2.4
Pelagohalteria viridis	8.3	6.7	8.9	9.4	10.4	23.1
Strombidium viride	1.1	0.9	0.7	11.2	12.4	1.1
Pseudobalanion planctonicum	1.9	1.6	2.4	52.3	23.2	0.7

[1]More than 90% *Rhodomonas minuta* and *Chrysochromulina* sp.

Our enclosure experiments offer direct experimental evidence that these changes in bacterial community structure can occur on a short time scale and are linked to the food web structure. As elucidated by Güde [20], free single cells profit when competition for substrates is the only selection factor, whereas more complex growth forms are selected under protozoan grazing pressure. The immediate response of the bacterial assemblages in enclosures C and N was probably forced by a rapid change from substrate to grazing control. The protozoans, which had changed quantitatively and qualitatively in the different enclosures (Table 6), seemed to act as a transmitter for the metazoan impact on the bacterioplankton. Formation of grazing-resistant, complex bacterial growth forms can occur only when the substrate supply is sufficient [20]. Although thymidine incorporation cannot always be directly related to new cell production [21] and has to be interpreted carefully, it

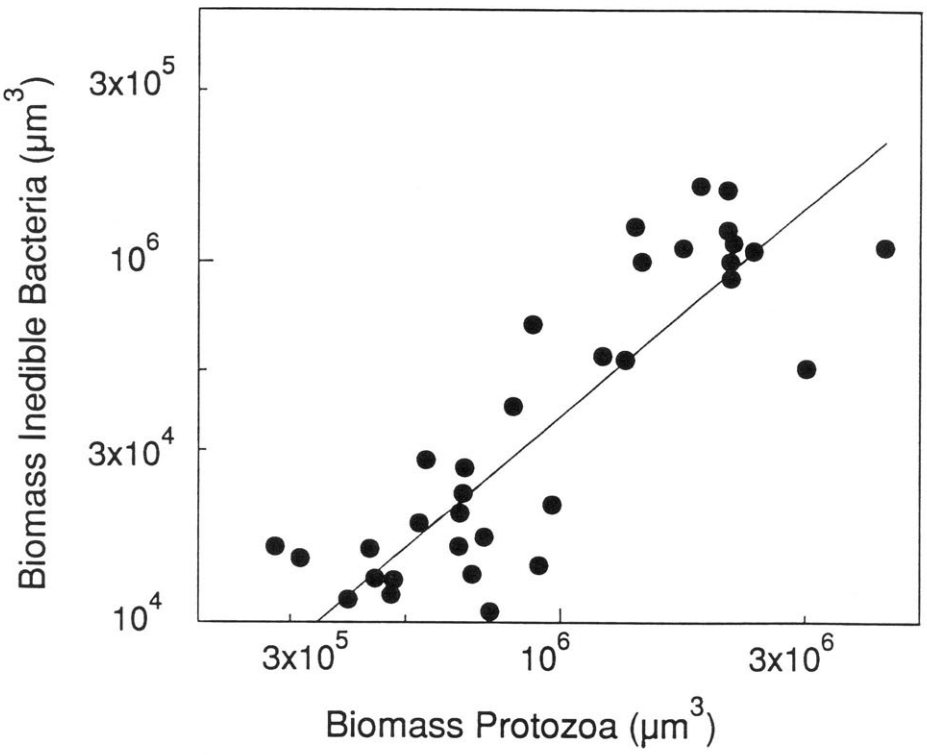

Fig. 5. Relationship of protozoan biomass to biomass of protozoan-inedible bacteria (filaments and aggregates) and regression line ($r = 0.87$). Data are pooled from all enclosures.

does indicate relative changes in bacterial activity. According to the increase in thymidine uptake during the experiment (Fig. 3), substrate supply seemed to be enhanced, possibly due to the enclosure and permanent high temperatures. Due to the new selection conditions, bacterial production in enclosures N and C was channeled to the filamentous bacteria which formed a considerable biomass (Table 3). Such a dynamic system as observed in our enclosure experiment is probably limited to situations of higher productivity. In comparable enclosure experiments, Pace and Funke [31] did not find a response of the bacteria in the presence of *Daphnia* and concluded that top-down effects are truncated at the protozoan level. One reason for their different results might be that their experiment was performed in an oligotrophic system, where all microbial components may be severely bottom-up limited. Further, changes on the bacterial level might be invisible when focusing only on bacterial abundance and not taking into account changes in the community structure.

The highly dynamic development of the bacterial community in our enclosures probably reflects a transient state after the perturbation of the metazooplankton community; on a longer time scale the initial situation might revolve. Nevertheless, these results allow some interesting insights into the interactions between bacteria, protozoans, and metazoans. Bacterial densities in pelagic habitats are correlated to

the trophy of the system [2, 11] and generally show rather small fluctuations during the growing season despite large fluctuations in their grazers [24, 25]. Estimates of bacterial resources for nanoflagellates in meso- and eutrophic lakes revealed that HNF are probably neither food-limited nor intensively exploiting the bacterioplankton [24]. Also, empirical studies from fresh- and saltwater indicate that HNF generally do not control bacterial numbers below a threshold limit. Instead, both flagellates and bacteria increase in abundance with the trophy of the system [38]. Two underlying mechanisms are possible. First, a considerable fraction of the bacteria might possess some kind of grazing resistance which would buffer seasonal fluctuations and maintain high densities (depending on the substrate supply). Second, HNF as the main bacterivores might be permanently top-down controlled. Both mechanisms can act together and are probably responsible for the observed results in the enclosure experiments.

Presently almost no information exists regarding to what extent planktonic bacteria are edible and support the growth of HNF. More than 90% of the initial bacterial assemblage had vanished in the enclosures without daphnids (C and N) after protozoans had developed. This indicates that the bacteria in this situation were indeed edible for protozoans but could be exploited only when protozoans were released from grazing by *Daphnia*. The remaining approximately 10% of the single-celled bacteria in the enclosures belonged to the smallest size classes (Fig. 3). Reduced cell size can also reduce vulnerability to grazers [10, 16] and may constitute another type of refuge.

Due to the rapid compensation of bacterial grazing losses by the development of resistant forms, the total bacterial biomass was less affected and was maintained on a relatively high level. Morphologically inedible bacteria are the most obvious types of grazing resistance, but we must assume that other strategies exist that are less obvious, for example chemically mediated resistance. If the observed shift toward inedible bacteria occurs frequently in nature, it implies that grazers are more important in structuring bacterial communities than in controlling bacterial biomass.

Top-down control of zooplankton on nanoflagellates was included by Wright [52] in a steady-state model of bacterial dynamics, and enabled rather stable bacterial densities. Metazoan predation on protozoans is probably an important link between the microbial food web and higher trophic levels [7, 48]. However, whether nanoprotists are strongly top-down controlled depends very much on the species composition of the metazooplankton community. Our experiments showed that a copepod-dominated community exerts, in contrast to *Daphnia*, only minor top-down effects. It has to be verified for situations when other metazoans (or ciliates) are the dominant grazers of nano-sized cells to what extent bacterivorous protists are predation controlled.

Daphnia is known to be a keystone species for the cascading trophic interactions in freshwater ecosystems [6], and their impact on microbial food webs is more complex and profound compared to other zooplankters [18]. Daphnids can eliminate nanoprotists and shortcut the carbon flow from bacteria to higher trophic levels [37, 39, 47]. For our study lake, it has been shown that *Daphnia* is able to consume the bacterioplankton, but only the larger cells with high efficiency [4]. Their impact on nanoprotists is much stronger and results in extremely low HNF concentrations during the clear-water phase [18, 24].

The influence of *Daphnia* on the bacterial community structure might be more important than direct consumption. They promote the prevalence of a rather homogenous assemblage of free, single-celled bacteria, as observed for the clear-water phase in Lake Constance [18] and also in our *Daphnia* enclosures. This is exerted directly and indirectly. First, predation pressure of HNF on bacteria is kept low, and no selection pressure toward morphologically complex growth forms occurs. Second, daphnids probably directly remove larger bacteria and aggregates [18].

These interactions between metazoans, protozoans, and bacteria, as described here, cannot necessarily be generalized to other systems or stages of the plankton succession but are representative of the situation during the spring clear-water phase in a mesotrophic lake. It is still to be verified which mechanisms and interactions are responsible for high bacterial densities in eutrophic systems without *Daphnia* [24]. The importance of grazing-resistant bacteria and of predation control of bacterivorous protozoans probably changes seasonally.

Although we have no information about the genetic diversity of the bacterioplankton in the enclosures, our results show that trophic cascades can have a significant impact and rapidly alter the microbial community structure. Molecular approaches also showed remarkable changes in natural bacterial communities in comparable time ranges [22, 28].

For aquatic ecosystems it must be assumed that changes in the highest trophic levels, e.g., piscivorous fish, should be reflected in taxonomic and metabolic changes of the bacterial assemblage. Short-term cascading predation effects, as forces that shape bacterioplankton community structure, might be of similar importance as the effects mediated by the phytoplankton via substrate supply. Trophic interactions from fish to picoplankton must be considered for a better understanding of microbial food webs and their pivotal role in carbon and energy flow.

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