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Regulation of bacterial biomass and community structure by metazoan and protozoan predation

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Abstract

We performed food web manipulation experiments in three eutrophic *Daphnia*-dominated ponds, to compare the predation impact on planktonic bacteria exerted by metazoan and protozoan bacterial consumers. We analyzed the bacterial morphological composition by image analysis and the taxonomic composition by fluorescent in situ hybridization with group-specific oligonucleotide probes. The removal of *Daphnia* always resulted in a microbial succession in which first free-living bacteria and then heterotrophic nanoflagellates (HNFs) increased. Distinct stages of bacterial grazing either exclusively by *Daphnia* or by HNFs allowed a quantitative and qualitative comparison of their predation impact on the bacterial assemblage. Both bacterial consumers showed a strong size-selective impact that shifted the free-living bacterial community structure toward small cells. Suppression of bacterial biomass below the carrying capacity was similar with *Daphnia* or HNFs in one experiment and was significantly stronger with *Daphnia* as bacterial consumer in two experiments. This was due to the fact that bacteria that were resistant to protozoan predation partially compensated grazing mortality. Bacteria attached to aggregates and detrital particles were more important as grazing-resistant forms than bacterial filaments and constituted up to 50% of total bacterial biomass at the end of the experiments. Changes in predation pressure were also associated with shifts in bacterial community composition. Bacteria belonging to the beta subclass of the class Proteobacteria and to the Cytophaga/Flavobacterium group dominated originally. The latter were most strongly reduced by HNF grazing, whereas other groups, in two experiments the alpha sublass (ALF) of Proteobacteria and in one experiment bacteria hybridizing with the probe for *Archaea*, even increased during HNF grazing. The composition also differed between bacteria associated with aggregates and freely suspended bacteria, the most obvious being the dominance of ALF growing on detrital aggregates. The experiments demonstrated that predation is a major structuring force for planktonic bacterial communities and that changes in predation regime probably have a much stronger impact on the structure of the bacterial community than on bacterial abundance and biomass.

Predation is a major structuring force in aquatic ecosystems that influences food web organization, size structure, and species composition of the different trophic levels (Brooks and Dodson 1965; Hairston and Hairston 1993). Direct and indirect effects of predation have been especially well studied in the classical grazer food chain of freshwater lakes (fish-zooplankton-phytoplankton). Many phenotypic adaptations toward predation have been found in nearly all kinds of organisms (Tollrian and Harvell 1999), and strong impacts of grazers on the taxonomic composition of their prey communities are well known for zooplankton-phytoplankton interactions (Sterner 1989).

Although bacteria are now generally considered to be a significant component of planktonic food webs, which mediate key processes in biogeochemical cycles (e.g., Cole 1999), the mechanisms that regulate their biomass and community structure are still poorly understood. In experimental field studies, resources (organic carbon and inorganic nutrients) and predation have been identified as the main limiting factors of planktonic bacteria (Elser et al. 1995; Pace and Cole 1996; Simon et al. 1998). There is increasing evidence that bacterial production is regulated by resource supply and standing stock of bacterial biomass by predation (del Giorgio et al. 1996; Pace and Cole 1996).

The microbial food web and the classical grazer food chain are coupled by many direct and indirect links—for example, metazoan predation on protozoa (Carrick et al. 1991; Sanders and Wickham 1993) and consumption of bacteria by various trophic levels (Vaqué et al. 1992; Porter 1996). One of the major differences in freshwater compared with marine systems is that the presence of large filter-feeding zooplankton, mainly the genus *Daphnia*, frequently results in the suppression of the microbial food web because of strong predatory control on heterotrophic and autotrophic nanoplankton (Güde 1988; Jürgens 1994). When *Daphnia* biomass is sufficiently high (e.g., in meso- to eutrophic lakes), they can become the major bacterial consumer, and bacterial production is directly linked to the trophic level of metazooplankton (Pace et al. 1990; Jürgens 1994). The planktonic community structure of a particular system can change during the course of the season, which has implications for the fate of bacterial production, e.g., from bacterial consumption by daphnids to protozoans as major bacterial grazers (Pace et al. 1990; Jürgens and Stolpe 1995).

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The absence or presence of *Daphnia* might be also a key factor deciding whether predation effects may cascade down to the bacterial level. Trophic cascades that affect planktonic bacteria can be mediated via zooplankton regulation of phytoplankton (as a substrate source) (Pace 1993) or by predatory effects via bacterivorous protozoans (Jürgens et al. 1994). The evidence for cascading effects on bacterioplankton is controversial. In several zooplankton manipulation experiments, the predation impact seemed to be truncated at the level of protozoans (Pace and Funke 1991; Pace 1993; Wickham 1998), whereas other mesocosm studies found clear top-down effects on bacteria (Riemann 1985; Christoffersen et al. 1993). One underlying reason might be the differing trophy of the system, i.e., higher zooplankton biomass and predation effects in more productive systems (Riemann and Christoffersen 1993). The other reason might be compensatory mechanisms (e.g., development of grazing-resistance) within the bacterial community, which can stabilize bacterial biomass (Jürgens and Güde 1994).

Bacteria have generally a high phenotypic plasticity, and there is compelling evidence from laboratory studies that show that certain bacterial strains can adapt to protozoan predation by the development of resistant morphotypes such as aggregates and filaments (Güde 1979; Jürgens and Güde 1994; Hahn and Höfle 1999). The appearance of grazing-resistant bacterial morphotypes, such as filaments, has also been observed in eutrophic lakes and was generally correlated to an increase in abundance of heterotrophic nanoflagellates (HNFs) (Güde 1989; Jürgens and Stolpe 1995; Sommaruga and Psenner 1995). In chemostat studies (Pernthaler et al. 1997; Šimek et al. 1997; Hahn and Höfle 1999) and in mesocosm experiments (Jürgens et al. 1999; Šimek et al. 1999), it has been shown that this predation-mediated shift toward grazing-resistant bacteria can be accompanied by a shift in the bacterial community composition (BCC). Although an increasing spectrum of molecular tools for analyzing bacterial diversity is now available, these techniques are just beginning to be combined with traditional experimental field studies designed to examine the regulating factors for bacterioplankton (Jürgens et al. 1999; Šimek et al. 1999).

Previous food web manipulation experiments revealed that *Daphnia*-dominated lakes and ponds are an ideal system to study the bacterial grazing impact of metazoans and protozoans and to analyze morphological and taxonomical shifts in bacterial communities triggered by enhanced protozoan grazing (Jürgens et al. 1994, 1999). This is because of the fact that during *Daphnia* dominance, algae and heterotrophic protozoans are virtually eliminated, and the only organisms still present in significant quantities are heterotrophic bacteria (Jürgens 1994). In productive systems, the removal of mesozooplankton initiates a microbial succession in which first bacteria increase, followed by HNFs, and, later, grazing-resistant bacteria and larger protozoans (Jürgens et al. 1999). This sequence of *Daphnia*- and HNF-dominated bacterial consumption offers the chance to study and compare the impact of these two important bacterial grazers on biomass, community composition, and phenotypic properties of planktonic bacteria.

In a recent comparable study, this experimental design was used to demonstrate that HNF grazing can shift the BCC toward the dominance of a formerly rare bacterial group (Jürgens et al. 1999). Here we present results from similar types of food web manipulation experiments, performed in different *Daphnia*-dominated ponds. BCC was analyzed by fluorescent in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes, which is a suitable technique for obtaining combined information on phenotypic properties and taxonomic affiliation of bacterial cells (Amann et al. 1995). The goals were to compare qualitative and quantitative aspects of the grazing impact exerted by *Daphnia* and by HNFs on planktonic bacteria and to analyze whether there is a consistent pattern in predation-mediated shifts in BCC. Our experiments demonstrated that grazing by *Daphnia* and HNFs can be an equally important factor in regulating bacterial abundance and biomass. Concerning qualitative aspects of their grazing impact, some similarities, as well as important differences, became visible.

Materials and methods

Study sites and experimental design—Three small (<0.1 ha) fishless ponds in northern Germany, named Rixdorf, Hasselburg, and Freudenholm, were selected for the experiments. They were all characterized by high densities of *Daphnia magna* and long-lasting clear water states. Water from these ponds was used to fill 4.8- (Rixdorf) or 24.6-liter (Hasselburg, Freudenholm) polycarbonate bottles (Nalgene). For a more representative sampling, water was first taken from various spots of the pond and mixed in a 60-liter container from which the bottles were filled. There were two treatments, both of them with three replicates each. Bottles were either filled with unaltered water to maintain natural zooplankton densities [referred to as ''DAPH(+)''] or filtered through a 200-μm mesh to remove mesozooplankton [referred to as ''DAPH(−)'']. The bottles were incubated in situ for a period of 4–5 d in 0.5 m depth at temperatures varying between 18.4°C and 20.5°C and sampled twice a day (morning and evening). To characterize the initial situation in the ponds, samples for the determination of inorganic nutrients and chlorophyll *a* were taken and analyzed by use of standard procedures (Parsons et al. 1984).

Enumeration of organisms—Samples for enumeration of bacteria and HNFs were preserved in formalin (final concentration 2%) and stored at 4°C until further processing (usually within the next 24 h). One-milliliter subsamples were filtered on black polycarbonate filters (25 mm, pore size 0.2 μm, Millipore) and stained with 4,6-diamidino-2-phenylindol (DAPI, final concentration 100 μg ml^{-1}) (Porter and Feig 1980). The filters were cut into halves, of which one was immediately embedded on a slide and used to count bacteria and HNFs under an epifluorescence microscope (Axiophot II; Zeiss). The other half was stored at −20°C for later analysis of bacterial cell volumes by use of automated image analysis.

Between 400 and 500 bacteria per sample were counted in randomly chosen filter sections at 1,250× magnification. HNFs were counted by screening transects (5–25 mm) across the filter and sized by use of an ocular grid. Heterotrophic

flagellates were distinguished from autotrophic ones by checking for Chl *a* autofluorescence under blue light excitation. Bacteria were operationally divided into different morphological groups, according to their presumed accessibility toward HNF grazing (Jürgens et al. 1999). Freely suspended bacteria <5 μm were defined as protozoan-edible bacteria (BACT), whereas filamentous bacteria (FIL) and bacterial aggregates (AGG) were considered to be grazing resistant. FIL included elongated bacterial cells and diverse types of threads and filaments >5 μm in length. For AGG we considered not only bacterial aggregates but also bacteria attached to or embedded in detritus particles. AGG were quantified by ultrasonication of fixed samples (Branson Sonifier) at an intensity that disrupted aggregates but did not destroy bacterial cells. AGG cell numbers were determined by counting total bacteria after sonication and subtracting the number of free-living bacteria counted in DAPI preparations from untreated samples.

For the enumeration of ciliates, samples were preserved with acid Lugol's solution (final concentration 1%). Subsamples of 10–50 ml were counted in sedimentation chambers with an inverted microscope. At the end of the experiments, zooplankton >50 μm was preserved in sucrose-formaldehyde (final concentration 4%) and counted and sized with a dissecting microscope equipped with a semiautomated image analyzer (SIS). The dry weight of *D. magna* in the experimental bottles was calculated by use of length-weight regressions from the literature (Dumont et al. 1975).

Bacterial biovolume determination—Biovolumes of the different bacterial morphotypes (BACT, FIL, and AGG) were estimated by multiplying the abundance with the mean cell volume. For cell volume calculation of filaments, these were assumed to be cylinders with hemispherical ends, and length and width were measured directly under the microscope by use of an ocular grid.

For measuring cell-size distribution and mean volume of small, freely dispersed cells (BACT), an automated image analysis system was used (SIS). Briefly, the procedure consisted of recording images of DAPI-stained cells (at least 400 cells per sample, magnification of 1000×) with a SONY 3CCD camera and measuring the cell dimensions (pixel area and perimeter) after edge detection with a second derivative filter, manual thresholding, and binarization (e.g., Massana et al. 1997).

Because there were abundant DAPI-stained particles that we considered to be too small to be bacteria (virus-like particles or bacterial fragments released by grazers), we set a lower threshold for the analysis of digitized pictures (area of 10 pixels or 0.12 μm^2). Cell volumes were calculated from area and perimeter by use of the formula derived by Björnsen (1986). To estimate the biovolume of bacteria in aggregates, the same procedure was run with sonicated samples, and the resulting mean cell volume was used to calculate the biovolume of attached bacteria. This estimate of AGG biovolume was biased, however, by the inclusion of freely suspended bacteria.

FISH—For whole cell in situ hybridization, the membrane filter method described by Glöckner et al. (1996) was used. Therefore, 10–20 ml samples were taken from the bottles every 24 h, preserved with formaldehyde (final concentration 2%), filtered on polycarbonate membrane filters (Millipore; 47-mm diameter), and stored at −70°C until further processing. Oligonucleotide probes for the following bacterial groups were used for all sample times of all experiments: EUB338 for the kingdom Bacteria (EUB), BET42a for the beta subdivision of the Proteobacteria (BET), ALF1b for the alpha subdivision of the Proteobacteria (ALF), GAM42a for the gamma subdivision of the Proteobacteria (GAM), CF319a for the Cytophaga-Flavobacterium cluster of the Cytophaga-Flavobacterium-Bacteroides phylum (C/F), and ARCH915 for Archaea (ARCH). For selected time points, the following probes were also used: Pla5a for Planctomycetales and HGC69a for gram-positive bacteria with high G + C content (here the filters were treated with lysozyme and/or 50% ethanol before hybridization). All probes were synthesized with the fluorochrome Cy3 at the 5′ end (Interactiva Biotechnologie). Probe sequences, target positions, and the formamide concentration of the hybridization buffer corresponded to those used by Snaidr et al. (1997). After hybridization, the filter sections were stained with DAPI for 1 min (final concentration 1 $\mu g\ ml^{-1}$), rinsed with 70% ethanol, washed with Milli-Q, and dried. Subsequently, they were mounted on glass slides with Citifluor AF 1 (Citifluor).

The slides were inspected with an Axiophot II epifluorescence microscope (Zeiss) equipped with the Zeiss filter set 01 for UV excitation (DAPI) and a Chroma HQ 41007 set (AF Analysentechnik) for green excitation (Cy3). At least 300 Cy3-stained cells in 10 fields were counted per filter section at a magnification of 1,250×. The fraction of DAPI-stained cells that was detected with the EUB probe was examined by switching between the two filter sets and counting the same microscopic field. Rare groups and filamentous bacteria were counted from transects (5–20 mm) across the filter. For one sampling date at the end of each experiment, the number of bacteria of the different groups attached to aggregates was estimated by counting hybridized bacteria directly in aggregates on the filter.

Grazing experiments—In order to compare the bacterial grazing impact of *Daphnia* and HNFs in the experimental containers, we measured the disappearance of fluorescently labelled bacteria (FLB) over an extended incubation period (~48 h) (Marrasé et al. 1992). Bacteria for the preparation of FLB were obtained from a glucose-limited mixed chemostat culture and stained with the dye DTAF (5-[(4,6-Dichlorotriazin-2-yl)amino]fluorescein; Sigma) according to the method of Sherr et al. (1987). The mean volume of the FLB was ~0.15 μm^3. Bacterial grazing losses were measured at two sampling dates: (1) at the beginning of the experiments, by comparison of *Daphnia* grazing in DAPH(+) to bottles virtually without grazers [DAPH(−)]; and (2) during the exponential growth phase of HNF in DAPH(−), by comparison of HNF- with *Daphnia*-dominated grazing in DAPH(+). Subsamples (300–600 ml) were taken from the containers, spiked with FLB, (final concentration $1.5–2.5 \times 10^6\ ml^{-1}$) and incubated in 1-liter glass bottles under in situ temperatures for 48 h (2–3 replicates per treatment). Samples were taken every 9–13 h, preserved with formalin (final concen-

tration 2%), and stained with DAPI. We counted FLB, natural bacteria, and HNFs. The zooplankton from the DAPH(+) treatments was counted and sized when the experiments were terminated.

Community grazing rates were calculated under the assumption of an exponential model (Salat and Marrasé 1994) that considers losses due to grazing (decrease of FLB over time) as well as the net growth of the natural bacterial community (considered as the net change of the abundance of natural bacteria). Grazing rates (g) and instantaneous growth rates (μ) were calculated and then used to estimate the bacterial consumption rate per hour (G), according to the method of Salat and Marrasé (1994):

$$G = \frac{(N_t - N_0)g/\mu}{t}$$

where N_0 and N_t are the concentrations of bacteria at the beginning and end of the time interval t, respectively.

Ingestion rates (bacteria individual^{-1} h^{-1}) were calculated by dividing G through the grazer concentration. The mean concentration of HNF (N_m) was calculated for the time interval of exponential increase, according to the method of Heinbokel (1978):

$$N_m = \frac{N_t - N_0}{\ln N_t - \ln N_0} \quad (\text{ml}^{-1})$$

where N_0 and N_t refer to the HNF concentrations at the beginning and at the end of the time interval, respectively.

Growth rates of bacteria and HNFs—Net growth rates of bacteria and HNFs could be determined from the microbial succession in which first bacteria and later HNFs increased nearly without predation (Jürgens et al. 1999). For both groups, growth rates (μ) were calculated from the exponential increase of cell concentration after the removal of *Daphnia* in the DAPH(−) treatment:

$$\mu = \frac{\ln N_t - \ln N_0}{\Delta t} \quad (\text{h}^{-1})$$

where N_0 and N_t refer to the concentrations of bacteria (or HNFs) at the beginning and at the end of the time interval Δt of exponential increase.

Additionally, we measured bacterial production in the three ponds at the beginning of the experiments by the incorporation of ^{3}H-thymidine into bacterial DNA (Fuhrman and Azam 1980). Samples of 5 ml were incubated with 20 nM ^{3}H-thymidine (Amersham; specific activity 75–86 Ci mol^{-1}) for 30 min at in situ temperatures (four replicates and two formalin-killed controls) and then fixed with formalin (final concentration 2%). Samples were filtered on 0.2-μm membrane filters (Nucleopore) and rinsed three times with 5 ml of ice-cold 5% TCA. Filters were dissolved in scintillation cocktail (Quickscint 454; Zinsser Analytik), and incorporated radioactivity was measured with a scintillation counter with external standard. We used an empirical conversion factor of 2×10^{18} cells mol^{-1} thymidine (e.g., Smits and Riemann 1988) to convert thymidine incorporation into cell production rate.

Statistical analysis—All values for the different parameters are given as means of the three parallel enclosures for each treatment (±1 SD). Differences between DAPH(+) and DAPH(−) treatments over time were tested with repeated measures ANOVAs. Mean bacterial cell volumes were compared by use of a one-way design. Differences in total bacterial biovolume were tested using a two-way design after the data set was transformed following $\log(x + 1)$ to normalize variances. The relative BCC (percent of DAPI counts detected with the probes BET42a, GAM42a, ALF1b, C/F319a, and ARCH915) was tested for differences in time and zooplankton abundance with a one-way repeated measures ANOVA after the data were transformed with $\sqrt{x}/100$.

Results

General characteristics—The situations in all three ponds represented a typical clear-water state caused by a high biomass of filter-feeding zooplankton (e.g., Lampert et al. 1986). The mean biomass (mg dw L^{-1}) of *D. magna* in the ponds and in the DAPH(+) enclosures was 2.8 ± 0.2 in Rixdorf, 2.6 ± 0.3 in Hasselburg, and 3.7 ± 0.5 in Freudenholm. The total *Daphnia* concentrations (ind L^{-1}) were 88 ± 18, 53 ± 12, and 213 ± 74, respectively. These concentrations were maintained until the end of the experiment. From the DAPH(−) enclosures, mesozooplankton was virtually excluded by the 200-μm filtration. Other zooplankton taxa were rare in all treatments, rotifers were not found at all, and the concentration of copepods (cyclopoid nauplii, copepodids, and adults) was always <10 ind L^{-1}, except in the DAPH(−) bottles of the Rixdorf experiment, where cyclopoid nauplii, which passed the 200-μm mesh, achieved a density of 51 ± 0.3 ind L^{-1}. Phytoplankton was very low, with Chl *a* concentrations of 0.8 ± 0.5 μg L^{-1} in Rixdorf, 8.5 ± 0.8 μg L^{-1} in Hasselburg, and 2.8 ± 0.4 μg L^{-1} in Freudenholm. With respect to inorganic nutrients, all ponds were highly eutrophic, with PO_4^{3-} ranging from 13 to 46 μM L^{-1}, NH_4^+ from 34 to 374 μM L^{-1}, NO_2^- from 4 to 31 μM L^{-1}, and NO_3^- from 34 to 6276 μM L^{-1}. We also measured nutrient concentrations at the end of each experiment in one DAPH(−) and DAPH(+) replicate each. Although there was a slight decrease in nutrient concentrations, in no case did a depletion of any inorganic nutrient occur (data not shown).

Microbial succession after the elimination of Daphnia—The initial situation in all experiments was characterized by very low protozoan densities (<1.5 ± 10^3 HNFs ml^{-1}, <0.62 ciliates ml^{-1}), and moderate bacterial concentrations: 3.5 ± 0.2 × 10^6 cells ml^{-1} (mean cell volume 0.08 ± 0.01 μm^3) in Rixdorf, 3.5 ± 0.1 × 10^6 cells ml^{-1} (mean cell volume 0.09 ± 0.005 μm^3) in Hasselburg, and 2.3 ± 0.2 × 10^6 cells ml^{-1} (mean cell volume 0.07 ± 0.006 μm^3) in Freudenholm. The development of the different groups of organisms differed substantially between the DAPH(+) and DAPH(−) treatments (Fig. 1). A distinct microbial succession took place in the bottles without *Daphnia*, whereas the situation in the DAPH(+) enclosures remained relatively constant.

The succession in DAPH(−) was characterized by an ini-

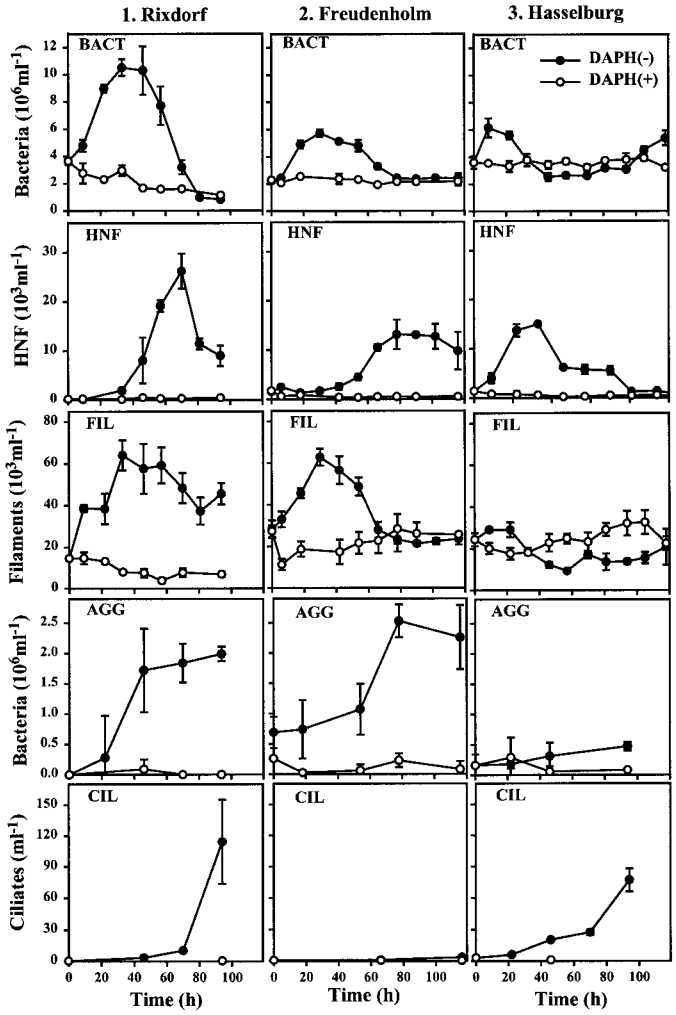

Fig. 1. Development of freely dispersed edible bacteria (BACT), filaments (FIL), bacteria in aggregates (AGG), HNF, and ciliates (CIL) in treatments with and without *Daphnia.* Values are means ±1 SD from three replicate enclosures.

tial bacterial growth phase and a subsequent grazing phase, when HNF concentrations started to increase. Directly after removal of zooplankton, BACT started to increase without a visible lag phase and reached maximum concentrations that were approximately three times (Rixdorf and Freudenholm) or two times (Hasselburg) higher than initial numbers. The net bacterial growth rates calculated from the increase in cell numbers were quite similar to the rates calculated from ^{3}H-thymidine incorporation (Table 1). Hasselburg was the most productive pond, with a bacterial doubling time of ~10 h. In Rixdorf and Freudenholm, BACT seemed to reach a stationary phase before HNFs developed. With increasing HNF numbers, bacterial concentrations were reduced to (Freudenholm) or below (Rixdorf and Hasselburg) the initial levels (Fig. 1).

The bacterial size distribution at three stages of the microbial succession in DAPH(−) (start, stationary phase, and HNF grazing) revealed a comparable successional pattern, shown only for the Rixdorf experiment (Fig. 2) but with a similar trend and with highly significant changes of mean

Table 1. Growth rate (μ) and doubling time (TD) for the total bacterial community (DAPI-stained bacteria) and HNFs.

Experiment	Group	Δt (h)	μ (hr^1)	TD (hr^{-1})
		Exponential Increase		
Rixdorf	Bacteria	0–22	0.04±0.01	20.7±3.1
	HNF	46–70	0.07±0.03	11.1±4.4
Freudenholm	Bacteria	6–30	0.04±0.003	19.9±1.7
	HNF	54–116	0.04±0.02	23.5±12.1
Hasselburg	Bacteria	0–9	0.06±0.01	11.3±2.0
	HNF	0–22	0.09±0.04	9.1±4.9
		Thymidine Incorporation		
Rixdorf	Bacteria	—	0.03±0.01	24.5±13.6
Freudenholm	Bacteria	—	0.07±0.03	11.8±4.8
Hasselburg	Bacteria	—	0.08±0.01	8.6±0.9

* Growth rates were calculated from time intervals with exponential increase Δt.

† Growth rates were calculated from ^{3}H-thymidine incorporation. All values show mean ± 1 SD from three replicates (exponential increase) and four replicates (^{3}H-thymidine), respectively.

cell volume in all three experiments (Table 2). There was a clear shift into larger size classes directly after the removal of *Daphnia.* The mean cell volume of BACT increased and doubled from the start of the experiment to the end of the bacterial growth phase. When HNFs developed, this trend reversed, and BACT shifted again into smaller size classes. In the DAPH(+) enclosures of the different experiments, no

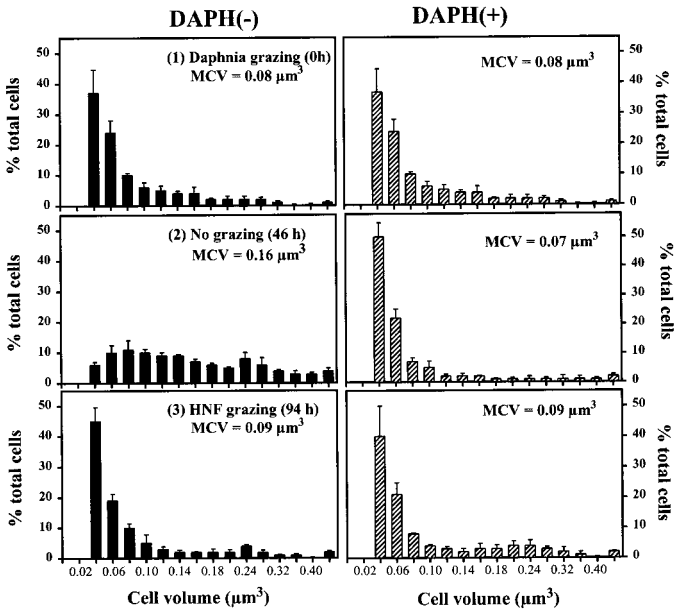

Fig. 2. Size distribution of the edible part of the bacterial community in Rixdorf in enclosures without *Daphnia* [DAPH(−)] and with *Daphnia* [DAPH(+)]. Shown is the size distribution at three stages of the microbial succession that occurred in DAPH(−): (1) at the beginning of the experiment, when grazing by *Daphnia* has shaped the bacterial community; (2) in the bacterial growth phase before the appearance of HNFs, and (3) in a stage with intense HNF grazing pressure. Graphs show the relative composition of bacteria in different volume classes (0.02–0.4 μm^3). The mean cell volume (MCV) of the total bacterial community is indicated for each graph.

Table 2. Effects of zooplankton and time on different properties of the bacterial communities in Rixdorf, Freudenholm, and Hasselburg (repeated-measures design). For each analysis, sample points were considered for which complete data were available, resulting in differences in degrees of freedom (df) as they are observable below. (1) temporal variation in mean bacterial cell volume (no independent variable); (2) effect of zooplankton on total bacterial biovolume; and (3) effect of zooplankton on the relative abundance of ALF, BET, C/F, GAM, and ARCH (expressed as percentage of total number of DAPI counts). Df (treatment/error) and *F* values are shown. *$p<0.05$, **$p<0.001$, ***$p<0.0001$. ND = not determined.

	Rixdorf		Freudenholm		Hasselburg	
Factors	df	*F*	df	*F*	df	*F*
Mean cell volume						
Time DAPH(+)	2,4	8.60*	4,8	3.77	3,6	3.23
Time DAPH(−)	4,8	48.62***	4,8	17.87**	5,10	16.92**
Biovolume						
Zooplankton	1,4	482.0***	1,4	589.3***	1,4	1.39
Time	2,8	48.02***	3,12	25.41***	2,8	12.59*
Zooplankton × time	2,8	42.28***	3,12	43.38***	2,8	36.66***
Community composition						
BET						
Zooplankton	1,4	358.4***	1,4	47.76*	1,4	0.51
Time	2,8	3.21	4,16	1.76	2,8	3.57
Zooplankton × time	2,8	16.00*	4,16	6.48*	2,8	0.21
ALF						
Zooplankton	1,4	64.02*	1,4	14.32*	1,4	50.44*
Time	2,8	41.19***	4,16	7.17*	2,8	64.37***
Zooplankton × time	2,8	33.40**	4,16	6.92*	2,8	32.06**
C/F						
Zooplankton	1,4	32.36*	1,4	2.95	1,4	14.48*
Time	2,8	1.73	4,16	24.94***	2,8	5.86*
Zooplankton × time	2,8	71.16***	4,16	50.62***	2,8	2.69
GAM						
Zooplankton	1,4	0.36	1,4	26.50*	1,4	12.90*
Time	2,8	1.46	4,16	3.07*	2,8	71.14***
Zooplankton × time	2,8	10.06*	4,16	6.41*	2,8	3.07
ARCH						
Zooplankton	1,4	ND	1,4	3.58	1,4	2.95
Time	2,8	ND	4,16	40.18***	2,8	4.80*
Zooplankton × time	2,8	ND	4,16	22.93***	2,8	0.60

significant changes of mean bacterial cell volume occurred over time (Table 2).

Although the general pattern was similar in the three experiments, obvious differences in the dynamics of the microbial succession occurred, which proceeded most rapidly in the Hasselburg experiment. Here both BACT and HNFs increased within the first 20 h of the experiment, whereas in the other two experiments HNF increased later (>40 h). The time lag between the peaks of BACT and HNFs was 24 h in Hasselburg, 36 h in Rixdorf, and 48 h in Freudenholm. HNFs consisted mainly of small, colorless chrysomonades (2–5 μm in diameter); later during the course of the experiments, larger heterotrophic and autotrophic (or mixotrophic) forms also became more abundant (up to 4×10^3 ml^{-1}).

Ciliates developed only in higher numbers in the Rixdorf and Hasselburg experiments but remained at a low concentration in Freudenholm. Their increase was paralleled by a simultaneous decrease of HNFs (Fig. 1). Dominant taxa were *Halteria* spp. and *Urotricha* spp. in both experiments, but other taxa (mainly small oligotrichs) were also represented. In Hasselburg, *Halteria* spp. had developed already between 22 and 46 h, with a maximum growth rate of 0.04 ± 0.01 h^{-1}, reaching a concentration of 16.5 ± 3.5 ml^{-1} at the end of this time interval.

FIL showed an approximately threefold increase in abundance after removal of zooplankton in two experiments (Rixdorf and Freudenholm) but no increase in the Hasselburg experiment (Fig. 1). Although FIL were considered to be grazing resistant, there was a clear decrease (up to 50% of the maximum) of FIL when HNFs developed. In Hasselburg and Freudenholm, the mean filament length increased in all experiments parallel to the increase of HNFs, from 6.8 ± 0.3 to 8.7 ± 0.8 μm in Hasselburg and from 9.7 ± 2.7 to 15.5 ± 0.3 μm in Freudenholm. In Rixdorf, the mean filament length increased only slightly, from 15.5 ± 0.2 to 16.2 ± 0.5 μm after the exponential growth phase. However, at least in the Hasselburg and Freudenholm experiments, it seemed that short filaments in particular were negatively affected by HNFs. In the DAPH(+) enclosures, FIL stayed relatively constant, with some decrease (Rixdorf) or increase

Table 3. Results of grazing experiments. Grazing parameters were calculated from the decline of fluorescently labeled bacteria within 48 h after their addition to subsamples taken from the enclosures. Δt (h): the time interval from which the grazing parameters were calculated; C_{Daph} (liter^{-1}): concentration of *Daphnia*; N_m (10^3 ml^{-1}): mean HNF concentration in the time interval with exponential increase; G (bacteria $\times$ 10^6 ml^{-1} h^{-1}): community mortality rate of bacteria; I (bacteria ind^{-1} h^{-1}): ingestion rate. Values are mean $\pm$ 1 SD from two replicates in Rixdorf and Hasselburg and three replicates in Freudenholm, respectively.

Grazer type	Δt	C_{Daph}	N_m	G	I
Rixdorf					
D. magna	0–31	245±83	—	0.40±0.04	1.7±0.4 × 10^6
D. magna	47–78	94±19	—	0.18	2.1±0.5 × 10^6
HNF	47–57	—	22.0±2.0	1.38±0.14	55.4±0.99
Freudenholm					
D. magna	0–30	189±99	—	0.27±0.10	1.4±1.0 × 10^6
D. magna	54–91	142±12	—	0.14±0.01	0.9±0.2 × 10^6
HNF	54–91	—	9.9±0.6	0.18±0.03	19.9±3.3
Hasselburg					
D. magna	0–31	152±26	—	0.26±0.03	1.8±0.5 × 10^6
D. magna	47–78	59±9.2	—	0.21±0.01	3.9±2.5 × 10^6
HNF	8–31	—	10.9±4.0	0.11±0.04	11.1±7.4

(Hasselburg and Freudenholm) toward the end of the experiments (Fig. 1).

The other type of grazing-resistant bacteria were AGG, which consisted mainly of bacterial cells embedded in a visible organic matrix and cells attached to detrital particles. AGG increased in the Rixdorf and Freudenholm experiments simultaneously with increasing HNF numbers and reached maximum concentrations of 2.0 $\pm$ 0.1 $\times$ 10^6 ml^{-1} and 2.5 $\pm$ 0.3 $\times$ 10^6 ml^{-1}, respectively. At the end of the experiments, AGG accounted for 50%–60% of total bacterial biomass (Fig. 1). AGG did not increase as strongly in the Hasselburg experiment and accounted for at most 16% $\pm$ 3% of total bacterial biomass. In the treatments with *Daphnia*, AGG remained low in all three experiments (average for all sampling dates and experiments [n = 39] 4.2% $\pm$ 6.7% of total bacterial biomass).

Grazing rates of HNFs and Daphnia *on natural bacteria*—The results for the community grazing rates are shown in Table 3. The different loss rates were not tested for statistical differences, because the results indicated that the grazing rates in particular were highly dependent on the grazer concentration in the bottles, which was quite variable between the different experiments because of the difficulty in obtaining subsamples from the bottles with representative zooplankton densities. However, these results indicate that HNFs and *Daphnia* potentially contribute equally to the mortality of natural bacteria. Most community grazing rates ranged between 0.1 and 0.4 $\times$ 10^6 bacteria ml^{-1}; the only exception was found in the DAPH(−) treatments in Rixdorf during the exponential increase of HNFs, where community grazing mortality rates were much higher and achieved 1.38 $\pm$ 0.14 $\times$ 10^6 bacteria ml^{-1} h^{-1}. Calculated individual ingestion rates were between 0.75 and 6.4 $\times$ 10^6 bacteria h^{-1} for *Daphnia* and between 5.9 and 56.1 bacteria h^{-1} for HNF (Table 3).

The estimated daily turnover rates of the bacterial communities (total mortality divided by bacterial concentration) did not differ much between the three ponds when *Daphnia* was the main bacterial consumer (Rixdorf 2.57 $\pm$ 0.16 d^{-1}, Freudenholm 2.20 $\pm$ 0.21 d^{-1}, and Hasselburg 1.70 $\pm$ 0.20 d^{-1}). Variation in turnover rates was much higher during the period of intense grazing by HNFs (Rixdorf 10.50 $\pm$ 1.68 d^{-1}, Freudenholm 1.86 $\pm$ 0.12 d^{-1}, and Hasselburg 0.70 $\pm$ 0.08 d^{-1}).

Development and distribution of bacterial biovolume—The total bacterial biovolume increased strongly in all three experiments directly after the removal of *D. magna*: nearly sixfold in Rixdorf and approximately twofold in Hasselburg and Freudenholm. During this growth phase, the bacterial community was dominated by freely suspended edible bacteria, which make up >80% of bacterial biomass (Fig. 3). Simultaneous with increasing HNF numbers, the total bacterial biovolume dropped again. In Rixdorf and Freudenholm it remained, however, on a higher level, compared with the initial stage, because of the high proportions of protozoan-inedible bacteria (mainly AGG). In the DAPH(+) treatments, the total bacterial biovolume remained relatively constant, except in Rixdorf, where it gradually declined. In general, the variability between the replicate treatments was quite low, with a coefficient of variation (CV) for the total bacterial biovolume of 3%–21%.

In Rixdorf and Freudenholm, the total bacterial biovolume was on all occasions significantly lower under grazing impact of *Daphnia* [DAPH(+) enclosures], compared with HNF grazing [DAPH(−) enclosures] (two-way repeated measures ANOVA: F_{Rixdorf} (1,4) = 482.0, p < 0.0001; $F_{\text{Freudenholm}}$ (1,4) = 589.3; p < 0.0001). At the end of the experiments, total bacterial biovolume was two times (Freudenholm) and five times (Rixdorf) higher in DAPH(−) than in DAPH(+), and this was mainly due to the appearance of grazing-resistant filaments and aggregates (Fig. 3). In the Hasselburg experiment, the presence or absence of *Daphnia* alone did not effect the bacterial biovolume [F (1,4) = 1.39, p = 0.30]. In Rixdorf and Freudenholm, the absolute and, especially the relative, proportions of grazing-resistant morphotypes increased during the course

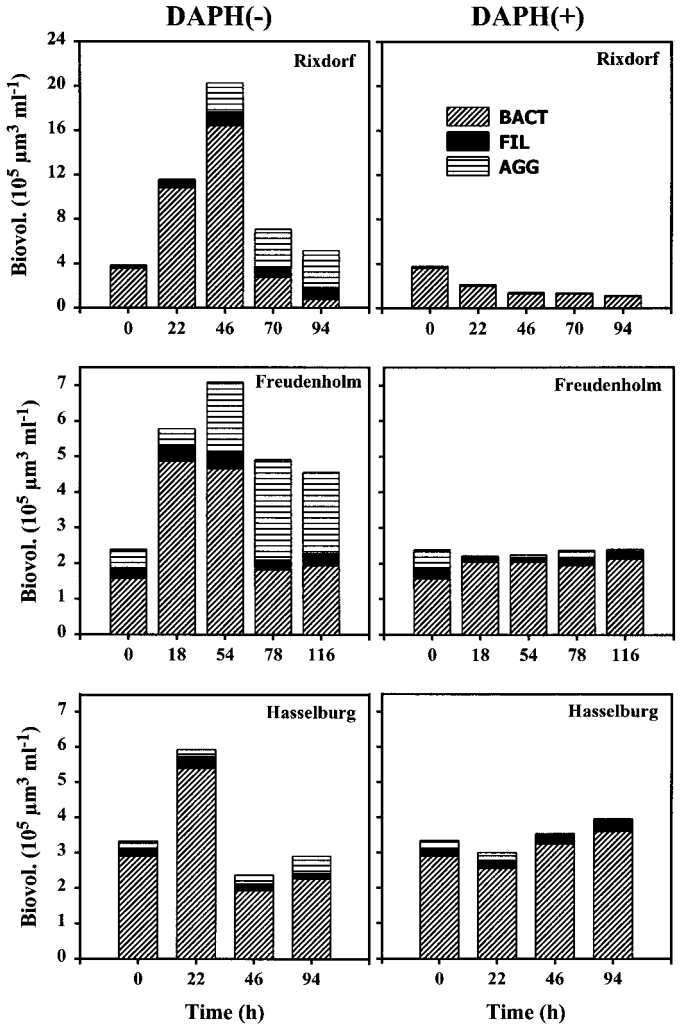

Fig. 3. Development of total bacterial biovolume and biovolume distribution in DAPH(−) and DAPH(+). The contribution of freely suspended edible bacteria (BACT), filamentous bacteria (FIL), and bacteria in aggregates (AGG) is shown for different sample points. All values show means from three replicates (see text for coefficients of variation).

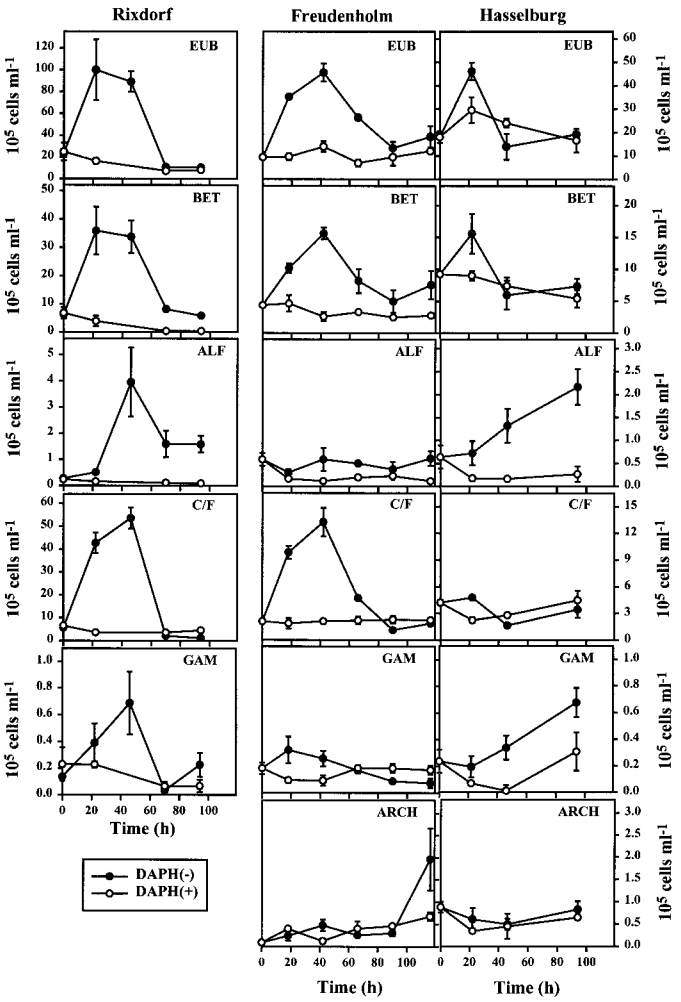

Fig. 4. Development of different bacterial groups in the treatments with and without Daphnia, as determined by in situ hybridization with probes specific for Eubacteria (EUB), the beta subgroup of the Proteobacteria (BET), the alpha subgroup of the Proteobacteria (ALF), the gamma subgroup of the Proteobacteria (GAM), the Cytophaga-Flavobacterium cluster (C/F), and Archaea (ARCH). Values are means for three replicate treatments ±1 SD. Note the different scales of the *y* axes.

of the experiment (Fig. 3), and AGG constituted 58% ± 11% and 54% ± 2%, respectively, of the total bacterial biovolume after HNFs had developed. Filaments, in contrast, were generally of lower importance, with a maximum contribution in the Rixdorf experiment, where they accounted for 23.6% ± 6.6% of total biovolume at the end of the experiment (94 h).

Changes in bacterial community composition—Analysis of BCC by FISH with group-specific oligonucleotide probes was performed at different stages of the microbial succession in the DAPH(−) enclosures (1) at the beginning, when the bacterial community was still influenced by the presence of *D. magna*, (2) in the bacterial growth phase after removal of zooplankton, (3) in the stationary phase before HNFs increased, and (4) after HNFs had increased and reduced the number of BACT.

Autofluorescent picoalgae, which could interfere with the identification of Cy3-fluorescending bacteria, were generally absent in the experiments and did not contribute to unspecific background values. Mean hybridization efficiencies (defined as the proportion of DAPI-stained bacteria that were detectable with the EUB probe) calculated for all sample dates were in general slightly higher in the DAPH(−) enclosures (Rixdorf 75% ± 11%, Hasselburg 68% ± 12%, and Freudenholm 75% ± 9%), compared with the DAPH(+) enclosures (Rixdorf 71% ± 4%, Hasselburg 69% ± 14%, and Freudenholm 61% ± 11%).

The development of most bacterial groups detected by FISH followed the general successional pattern of total bacterial biomass and abundance (Fig. 4)—i.e., they increased directly after the elimination of zooplankton, reached a maximum, and afterwards declined simultaneously with the ap-

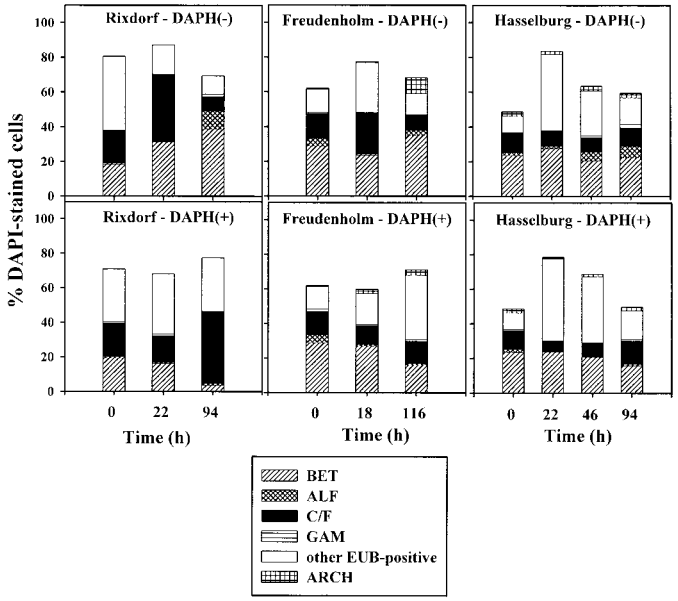

Fig. 5. Bacterial community composition relative to the total number of DAPI-stained cells at different sample points in DAPH(+) and DAPH(−) treatments. For Rixdorf and Freudenholm, the three bars refer to the following situations in the DAPH(−) treatments: (1) the start of experiment, (2) the bacterial growth phase (no grazing), and (3) high protozoan grazing impact. The total length of each bar represents the fraction of the count of DAPI-stained cells that was detectable with the probe EUB338. Values are means for three replicate treatments.

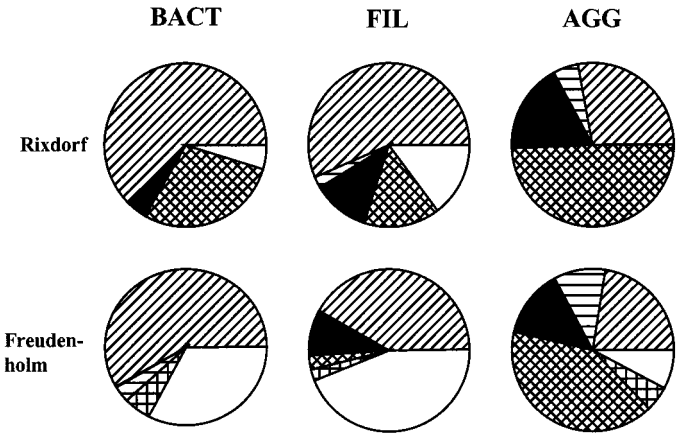

Fig. 6. Relative composition (in % of EUB-positive cells) of freely dispersed edible bacteria (BACT), filaments (FIL), and attached bacteria (AGG) in the treatments without *Daphnia* at the end of the Rixdorf (94 h) and Freudenholm (90 h) experiments. Means for three replicates are shown (mean CV was 25% for freely dispersed bacteria [BACT + FIL] and 40% for AGG). See Fig. 5 for symbol definitions.

pearance of HNFs. However, some differences between the bacterial groups could be found as well, which resulted in an altered community composition. In particular, ALF did not consistently follow the general trend: in Rixdorf they had a delayed increase, in Hasselburg they increased slowly but continuously during the whole experiment, and only in Freudenholm did they remain at a constant low level (Fig. 4). A continuous increase was also observed for GAM in Hasselburg, although their number in general was quite low ($<1 \times 10^5$ ml^{-1}). ARCH915-detectable cells, which were found in Hasselburg and Freudenholm, also showed a deviating development. In Freudenholm, their numbers increased threefold during the last 24 h of the experiment (Fig. 4). ARCH915-positive cells were short, thick rods (3–5 μm), and because of their characteristic cell form, we could see that these cell also hybridized with the EUB probe, indicating that these cells did not in fact belong to the Archaea. However, because they could be assigned to a distinct bacterial group because of their characteristic morphology, we called them ARCH915-positive cells. No positive signals were detected with the probes Pla5a and HGC69a.

The relative taxonomic bacterial community composition was calculated for stages 1, 2, and 4 of the microbial succession in DAPH(−) and (as a control) also in DAPH(+) (Fig. 5). In Hasselburg, the separation into the distinct successional stages was less clear, and therefore all investigated sample points were included (Fig. 5).

In Rixdorf and Freudenholm, the presence of zooplankton had a significant effect on the relative proportions of all bacterial groups during the course of the experiments (Table 2). The bacterial community was initially dominated by BET and C/F, whereas other groups were of minor importance (ALF <5%, GAM <1.5% of abundance). During the bacterial growth phase in DAPH(−), this situation did not change much, except that C/F increased slightly in relative abundance (Fig. 5). Stronger changes occurred after the development of HNFs. The abundance of C/F especially decreased, whereas BET remained almost unchanged and other groups increased (ALF in Rixdorf and ARCH in Freudenholm). In Hasselburg, the BCC changed only slightly in DAPH(−), which was mainly attributable to an increase of ALF. The BCC in the DAPH(+) enclosures was relatively constant, and slight changes took place only toward the end of the experiments, e.g., C/F increased in Rixdorf and Hasselburg.

In Rixdorf and Freudenholm, there was a clear biomass shift into aggregates when HNFs became abundant (Fig. 3). In order to get at least a rough estimate concerning the taxonomic composition of the attached bacterial community, we counted separately hybridized cells that were located at or within the aggregates. This was possible because the total number of bacteria at the aggregates labeled with the group-specific probes was generally moderate. Most of the aggregates were small and therefore easy to focus (mean diameter in Rixdorf 26 $\pm$ 5 μm, mean diameter in Freudenholm 23 $\pm$ 2 μm). The total number of attached bacteria was considerably underestimated by this approach, because calculated cell concentrations gave only ~10% of the total number of attached bacteria that had been obtained from sonicated DAPI-stained samples. However, if we assume that these 10% that were taxonomically identified are representative for the total aggregate-associated bacterial community, this assemblage can be judged as clearly different in composition, compared with free-living bacteria (Fig. 6). ALF was the dominating group in aggregates (35%–49% of total abundance), whereas most free-living bacteria belonged to BET

(38%–57% of total abundance). Filamentous bacteria could be found in all investigated taxonomic groups, and relative abundance and temporal changes very often followed the development of the corresponding edible bacteria.

The proportion of bacteria that hybridized with the EUB probe but with none of the group-specific probes (so-called other EUB-positive cells) was generally high in the DAPH(+) enclosures (Rixdorf 30%–40%, Hasselburg 12%–47%, and Freudenholm 8%–44%). The same was the case for many sample dates in the DAPH(−) enclosures in Freudenholm (12%–39%) and Hasselburg (26%–44%)—only in Rixdorf did this group of unknown bacteria contribute <20% to the total bacterial community at all sampling occasions.

Discussion

Daphnia-*dominated systems as ''tools'' to study microbial interactions*—Because of the simplicity of the food web structure, *Daphnia*-dominated systems are ideal for examining grazing-mediated changes in BCC, and the same experimental design as in this study has been successfully applied elsewhere (Jürgens et al. 1994, 1999). The removal of mesozooplankton >200 μm initiated a clear microbial succession from bacteria to HNFs and larger protozoans. Therefore, we were able to investigate bacterial community composition in three distinct stages: (1) grazing by *Daphnia*, (2) exponential growth of bacteria (no grazing impact), and (3) grazing by HNFs. The stage with intense HNF grazing could be used to examine differential grazing impact on the bacterial community and feedback effects in the bacterial community—e.g., the development of grazing resistance and changes in bacterial community composition.

Even though the size fractionation constitutes a manipulation of the original water sample, we consider this to be a minor disturbance, since essentially all organisms except mesozooplankton can easily pass this mesh size. Phytoplankton concentration was low, despite high inorganic nutrient load, and therefore phytoplankton-bacteria interactions were probably not important for the regulation of bacteria. All systems were heterotrophically dominated, with *Daphnia* and bacteria as the main organisms. The *Daphnia* treatments [DAPH(+)] served as controls, in which the bacterial community remained essentially constant, for the comparison with the protozoan-grazing-induced changes in DAPH(−).

Although the growth phases of bacteria and HNFs occurred in the DAPH(−) treatments in all experiments, some differences between the ponds were obvious in the succession of other microbial components. In Freudenholm, ciliates did not occur in significant numbers during the experiment, and in Hasselburg, the development of bacterial aggregates and filaments was lacking. The Hasselburg experiment deviated from the other two ponds as well as from previous experiments with a similar design (Jürgens et al. 1994, 1999), in the sense that the microbial succession proceeded more rapidly in this productive pond. Larger heterotrophic protozoans (ciliates and large heterotrophic flagellates) increased early during the course of the experiment, and HNFs themselves were probably subject to predatory control. This fast-running microbial succession might have been a reason why feedback effects at the bacterial level—i.e., changes in morphological and taxonomic composition—were much less pronounced than those in the other two experiments. The oligotrichous ciliate *Halteria grandinella,* which has been shown to be an efficient consumer of bacteria and of HNFs (e.g., Šimek et al. 2000) was the main ciliate species that developed rapidly in Hasselburg. The grazing impact of this ciliate was also indicated by the second FLB grazing experiment, in which we observed remarkable grazing pressure on bacteria despite low HNF numbers. However, size-independent defense mechanisms in bacteria, related to physicochemical surface structures or the biochemical composition of bacteria (Jürgens and Güde 1994), could also have played a role in the lack of complex bacterial morphologies in Hasselburg.

Grazing impact of Daphnia *and HNFs on planktonic bacteria*—Although HNFs are generally considered to be the most efficient planktonic bacterial consumers, they did not exhibit a stronger control of bacteria than the general filter-feeder *D. magna.* On the contrary, because of an increase in morphologically grazing-resistant forms, the total bacterial biovolume was even substantially higher in two of the experiments when bacteria were grazed by HNFs, rather than by *Daphnia.*

In general, the grazing experiments revealed no consistent difference in rates of bacterial grazing mortality caused either by *Daphnia* or HNFs. Instead, differences in grazing rates occurred between and within treatments, which was partly due to varying grazer concentrations in the replicates. The calculated bacterial community turnover rates in the presence of *Daphnia* indicate that grazing losses were approximately balanced by bacterial growth, resulting in a constant standing stock at a certain equilibrium state. Only in the Rixdorf experiment did HNFs exhibit an extremely high mortality on the bacterial community, resulting in very high individual ingestion rates, compared with literature values for small chrysomonad flagellates (e.g., Caron 1990) or HNFs in general (Vaqué et al. 1994). Calculated ingestion rates for HNFs in Hasselburg and Freudenholm were generally in the range of literature values (Vaqué et al. 1994).

The bacterial community under *Daphnia* grazing was generally characterized by a relatively homogeneous structure with a dominance of small cells, which is typical for the clear water state in lakes (Güde 1988). However, filamentous bacteria, belonging to different phylogenetic groups, which should theoretically be efficiently removed by *Daphnia* (Güde 1989; Jürgens et al. 1994), were also frequently found in two experiments. Calculated growth rates showed that bacterial productivity was high, which might result in the appearance of filamentous bacteria with a high turnover. Not much is known about resistance of bacteria toward *Daphnia* grazing, except that very small cells cannot be retained by the filtering structures (Brendelberger 1991). The only study that has examined the impact of *Daphnia* grazing on BCC, by analysis of extracted 5S-rRNA, found a reduction of overall diversity but no development of new species (Höfle et al. 1999). This is consistent with the view of an unselective removal of bacteria above a certain size threshold. However,

there is some evidence that not all bacteria are digested within zooplankton (King et al. 1991), and viable gut passage might be another mechanism to survive *Daphnia* grazing.

After removal of zooplankton, we observed not only an increase in bacterial concentration but also a shift toward larger bacteria. This confirms the conclusion that *Daphnia* grazing preferentially eliminates the fast-growing part of the bacterial community, which consists of relatively large cells (Jürgens 1994). Thus, despite very different feeding modes of cladocerans and interception-feeding flagellates, both groups seem to have a comparable impact by removing the active, growing, and larger-sized portion of a bacterial community, leaving smaller and more slowly or nongrowing cells (Sherr et al. 1992; del Giorgio et al. 1996). It can therefore be summarized that grazing by *Daphnia* as well as by HNFs forces the bacterial community toward the lower end of the size spectrum but that HNF grazing additionally promotes the growth of complex grazing-resistant growth forms. These observations agree with the model proposed by Güde (1989) and that partially found in other field studies (Pernthaler et al. 1996).

Filaments and aggregates as grazing refuges for planktonic bacteria—Attachment of bacteria to particles is a common phenomenon in aquatic systems, especially in those that are rich in particulate matter like, e.g., eutrophic (Middelboe et al. 1995) or humic lakes (Tranvik and Sieburth 1989) and estuaries (Crump et al. 1999). The same is true for our study ponds, which are shallow and well mixed and receive considerable input of detrital organic matter.

It has been shown that bacterial aggregates develop during high protozoan grazing pressure (Jürgens et al. 1997; Hahn and Höfle 1999) but that they become eliminated by filter-feeding zooplankton (Jürgens et al. 1997), which was also the case in this study. The attachment to detrital particles might serve several functions, and attachment to particles promotes higher growth rates and activity because of enhanced nutrient concentrations (Grossart and Ploug 2000). Although the exact underlying mechanisms of the formation of bacteriodetrital aggregates remain unclear, it was obvious that attached bacteria were selected in comparison with free-living bacteria when HNFs became abundant. However, reduced grazing pressure on attached bacteria is probably only temporarily the case when chrysomonads dominate the HNF assemblage. Flagellates adapted to feed on bacteria in aggregates, such as bodonids, might develop in a later stage of the microbial succession (Caron 1987; Artolozaga et al. 1997).

In contrast to previous studies (Jürgens et al. 1994, 1999), filamentous bacteria were only of minor importance with regard to their contribution to total biomass. Filaments developed in all groups and therefore did not seem to be restricted to certain phylogenetic divisions. The situation in the ponds, with a relatively high input of allochthonous particles, might favor attached bacteria as predation-resistant growth forms. The dynamics of filamentous bacteria cannot clearly be explained by trophic interactions. In most previous experiments or field observations, the development of HNFs and filamentous bacteria was positively correlated (Jürgens et al. 1994; Jürgens and Stolpe 1995; Sommaruga and Psenner 1995), suggesting that filaments benefit from high grazing pressure. This was not the case here, and filaments increased during the exponential growth phase (without predation) in Rixdorf and Freudenholm and decreased parallel to the increase in HNFs. We therefore assume that, at least in some parts of the experiments, factors other than grazing were more important in controlling filament abundance. Changes in supply or qualitative composition of dissolved organic substrates might have influenced the development of filamentous bacteria. There is some evidence that larger bacterial cell morphologies, which are resistant to protozoan predation, develop mainly under sufficient substrate supply and when high growth rates are achieved (Hahn and Höfle 1999). In order to be able to elucidate the underlying mechanisms for the development of grazing-resistant bacteria and to reveal the contribution of phenotypic plasticity and of genetic changes, we need species-specific probes for the important bacterial taxa.

Morphological and taxonomic changes within the bacterial community due to grazing by HNFs—Community analysis by FISH revealed that the bacterial assemblages in the ponds were mainly dominated by representatives of BET and C/F, which seems to be a general characteristic of freshwater plankton (Methe et al. 1998; Glöckner et al. 1999). This situation did not essentially change throughout the experiment in the DAPH(+) treatments, except in Rixdorf, where the C/F group increased later during the experiment. The treatments without zooplankton were designed to study shifts in bacterial community structure in response to increased grazing pressure by HNFs. During the exponential and stationary growth phase after removal of daphnids, the BCC did not change substantially in comparison with the initial situation and was still dominated by freely suspended, single cells of C/F and BET.

Changes in the BCC became obvious when protozoan populations increased. C/F seemed to be most susceptible toward protozoan grazing, whereas ALF (Rixdorf and Hasselburg) or ARCH915-positive cells (Freudenholm) increased in relative abundance simultaneously with HNFs, thus obviously succeeding in keeping their grazing losses relatively moderate in comparison with other groups. Neither group developed long filamentous forms but did develop elongated cells and dividing stages, mainly in the size range 2–5 μm. Our threshhold of 5-μm cell length was probably an underestimation of the grazing-resistant portion of the bacterial community. In studies elsewhere, it has been shown that bacteria within the size range of 2–5 μm are already less vulnerable to HNF grazing (Šimek et al. 1997; Jürgens et al. 1999). Whereas the relative proportions of all bacterial groups changed in the Rixdorf and Freudenholm experiments, only modest changes in the BCC were observed in Hasselburg (Table 2), which agrees with the lack of morphologically resistant bacteria (filaments and aggregates) in this experiment.

When HNFs increased, there was a clear shift toward smaller bacteria and also a shift toward higher structural complexity within the bacterial community in two of the experiments (Rixdorf and Freudenholm). The latter observation was mainly attributable to an increase of bacteria at-

tached to detrital particles, which proved to be ''microniches'' with a deviating taxonomic composition. Although our approach for classifying particle-attached bacteria was not ideal, the results strongly suggest that a different taxonomic composition prevails, compared with free-living bacteria. This is not surprising, since detrital particles constitute a special microhabitat, probably with higher substrate supply and lower grazing pressure (Caron 1987; Kirchman 1993). It has been shown already in marine (e.g., DeLong et al. 1993; Rath et al. 1998), estuarine (Crump et al. 1999), and limnetic (Weiss et al. 1996) habitats that particles (marine or lake ''snow'') harbor different bacterial communities.

Overall, we conclude that grazing by *Daphnia* or HNFs, respectively, leads to a selection of different phylogenetic groups, mainly because some groups have the ability to develop complex morphologies resistant to protozoan grazing. Changes in bacterial community composition due to protozoan grazing have also been observed in other chemostat and enclosure experiments, although clear controls without grazers were not always present in these studies. As methodological approaches, either FISH (Pernthaler et al. 1997; Šimek et al. 1997, 1999; Jürgens et al. 1999) or denaturing gradient gel electrophoresis (Van Hannen et al. 1999) has been used. The advantage of FISH is that taxonomic changes can be correlated directly to changes in the morphological composition of the bacterial community. The disadvantages are that (1) only the most drastic changes in the BCC can be detected when using group-specific probes and (2) not the whole bacterial assemblage is covered with this method.

The latter point includes the problem that not all DAPI-stained cells are detectable by FISH. Our hybridization efficiencies were between 46% and 86% and are therefore similar to values reported in other freshwater studies (e.g., Glöckner et al. 1996; Jürgens et al. 1999). The second problem is that not all of the cells that hybridize with the EUB probe can be assigned with one of the group-specific probes (in our experiments, 8%–47% remained unaffiliated). The reasons are that not all bacterial groups present in the plankton are covered by the available rRNA database or that no matching oligonucleotide probes had been designed yet (Amann et al. 1995)—also, the existing group-specific probes do not cover the whole phylogenetic branch they are designed for (e.g., Glöckner et al. 1999).

General considerations—Numerous studies have investigated the impact of grazing (''top-down'') versus nutrient supply (''bottom-up'') on bacterial abundance and production. On an ecosystem scale, special attention has been given to this question by Pace and Cole (1996), who investigated these impacts in whole-lake experiments and proved that bacteria are mainly influenced by nutrients (especially P). They suggested that grazing (mainly by metazooplankton) plays a role only when bacterial abundance exceeds a certain threshold concentration. However, the systems we investigated can be seen as an exception to this pattern because, first, inorganic nutrients were unlimited, and second, very high grazer densities were present (either *Daphnia* or HNFs). So we expected top-down effects to be much more important for the overall succession, although the composition of available dissolved organic carbon might have influenced the succession within the bacterioplankton.

Daphnia-dominated situations similar to those in our experiments occur temporarily in many temperate lakes and ponds, when predation pressure on large-sized zooplankton is low. *Daphnia* is considered to be a keystone species because of its strong grazing impact on several trophic levels, yielding control on protozoans, edible phytoplankton, and bacteria (Jürgens 1994). Bacteria and detritus contribute substantially to the carbon demand of *Daphnia* (Hessen et al. 1990). Therefore, we could expect that zooplankton would exhibit almost exclusively direct effects on bacterioplankton and rarely indirect effects mediated by phytoplankton (Pace 1993).

If we assume that the maximum bacterial biomass reached after the removal of zooplankton is an indicator of the carrying capacity of the system, we can conclude that grazing in general suppresses bacterial biomass to levels that are clearly below the maximum value. The experiments also showed that grazing by *Daphnia*—at least when present in high densities—can be a stronger regulating factor for bacterial communities than grazing by HNFs. In the latter situation, bacteria were able to partly compensate grazing losses because of the formation of grazing-resistant morphotypes (see above). In systems with more diverse zooplankton, we expect to find situations between the extreme cases of our experiments: high *Daphnia* grazing in DAPH(+) and high HNF grazing in DAPH(−).

Such compensation mechanisms occur at probably all trophic levels and contribute to the stability of ecosystems, coexistence of predator and prey organisms, and truncation of cascading predation effects (Pace et al. 1998). In an enclosure experiment in a mesotrophic lake (Jürgens et al. 1994), it was shown that the development of filamentous bacteria fully compensated biomass losses due to grazing by HNF. Compensation of predation losses by shifts in bacterial community structure might also be a reason why, in various studies, no effects at the bacterial level (with respect to abundance or biomass) were recorded after manipulation of higher trophic levels (Pace and Funke 1991; Mikola 1998). In contrast, when parameters of the bacterial community structure were included, significant effects of higher trophic levels became visible (Jürgens et al. 1994; Cochran-Stafira and von Ende 1998; Šimek et al. 1999). The consideration of the heterogeneity of planktonic bacteria, with respect to vulnerability toward grazers as well as with respect to taxonomy and physiological capability, would probably solve some of the contradictions that have been observed in studies that treated the bacterial community as a homogenous trophic level.

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