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Prey selectivity of bacterivorous protists in different size fractions of reservoir water amended with nutrients

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Summary

An experiment designed to examine food preferences of heterotrophic nanoflagellates (HNF) grazing on bacterioplankton was performed in the freshwater Římov reservoir (Czech Republic). Water samples were size-fractionated to obtain < 5 μm filtrate containing bacteria and HNF. To manipulate resource availability, < 5 μm treatments were incubated in dialysis bags submerged in the barrels filled with the unfiltered reservoir water amended with either orthophosphate or glucose or combination of both. We employed rRNA-targeted probes to assess HNF prey preferences by analysing bacterial prey in HNF food vacuoles compared with available bacteria. *Actinobacteria* (the HGC69a probe) were avoided by HNF in all treatments. *Cytophaga-Flavobacterium-Bacteroidetes* bacteria (the CF319a probe) were positively selected mainly in treatments in which bacteria were heavily grazed, the < 5 μm treatments, but this trend was less pronounced towards the end of the study. The members of a small subcluster of *Betaproteobacteria* (the R-BT065 probe) were mostly positively selected. The nutrient amendments differentially affected bacterioplankton dynamics in almost all treatments, and together with the size fractionation, altered HNF overall bacterivory as well as prey selection. Analyses of bacterivores in unfiltered treatments allowed to detect the effect of different protists on shifts in HNF selectivity observed in <5 μm compared with unfiltered treatments.

Introduction

There are three main regulators of the dynamics of freshwater and marine bacterioplankton: resources, grazing and viral lysis (Wright, 1988; Sanders *et al*., 1992; Fuhrman, 1999). Impacts of protistan grazing, mainly that of heterotrophic nanoflagellates (HNF) and ciliates, on bacterioplankton dynamics has received the most attention (e.g. Sanders *et al*., 1989; Berninger *et al*., 1991; Šimek *et al*., 1995).

In the laboratory experiments, flagellate grazing on bacteria has been observed to be taxon-specific with regard to both feeding mechanisms and selection (Fenchel, 1986). Grazing selectivity on bacteria is directly influenced by many features such as size of bacterial prey, its motility, physiological status, and differences in the sensitivity and defence mechanisms of bacterial species to grazing (Chrzanowski and Šimek, 1990; González, 1996; Hahn and Höfle, 2001; Matz and Jürgens, 2005). Grazing by different species of bacterivorous protists results in distinct responses in the composition of bacterial community (Pernthaler *et al*., 2001a) as different bacterial genotypes are likely differently ingested, digested and utilized.

The effects of protozoan grazing on bacteria and the different strategies used by bacteria to escape predation (size alterations, forming of aggregates, etc.), have been well illustrated (see, for example, the review of Hahn and Höfle, 2001). In addition, various non-morphological traits such as motility, physicochemical surface characteristics and toxicity are known to affect bacterial vulnerability to protistan feeding (Jürgens and Matz, 2002).

Field studies of the effects of protistan grazing on bacterioplankton communities have been conducted mainly in freshwaters (Jürgens *et al*., 1999; Šimek *et al*., 1999; Gasol *et al*., 2002). Selective ingestion of different bacterial types is generally inferred from changes in bacterial community composition. So far, there is only limited direct evidence from analysing HNF food vacuole content that under typical bacterial prey densities present in a freshwater environment, protists differentially ingest certain groups of bacterial genotypes (Jezbera *et al*., 2005). The study presented there employed a size-fractionation approach (for details see Šimek *et al*., 2005) in combination with a transfer of samples between areas of the Římov reservoir that differed in nutrient concentrations. Fluorescence *in situ* hybridization (FISH) was used to compare relative proportions of different bacterial groups targeted by oligonucleotide probes in the natural bacterioplankton compared with those detected inside of food vacuoles of a plankton community of HNF.

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There have been a few attempts to employ FISH in the analyses of protistan food vacuoles contents but these have largely concerned laboratory populations (Gunderson and Goss, 1997; Pernthaler *et al.*, 1997; Eisenmann *et al.*, 1998; Diederichs *et al.*, 2003). Here we present results of a complex study focusing on the selection of bacterial prey by protists under natural conditions with different water fractions manipulated by addition of glucose, phosphorus or the combination of both. We sought to alter the resource available to bacteria, and in turn bacterial community composition, to permit examination of bacterivory in a variety of natural bacterioplankton communities.

The present study builds on our previous work (Jezbera *et al.*, 2005), in which the grazer community and selectivity of HNF were specifically analysed in < 5 μm treatments, as opposed to unfiltered treatments where differentially targeted grazing pressure was assumed for a more complex grazer community consisting of small *Spumella*-like HNF, choanoflagellates of the genus *Salpingoeca* and ciliates. Here we exploit the unique possibility of examining the impact of the different grazer communities on bacterial community composition with specific feedback on prey availability and thus also prey selectivity. We focused on several phylogenetically distinguishable groups of bacteria, mainly those that were found to be important in the previous studies in the Římov reservoir, i.e. bacteria targeted by the R-BT065 probe (subcluster of *Betaproteobacteria*), bacteria targeted by the HGC69a probe (*Actinobacteria*) and *Cytophaga/Flavobacterium* group (the CF319a probe).

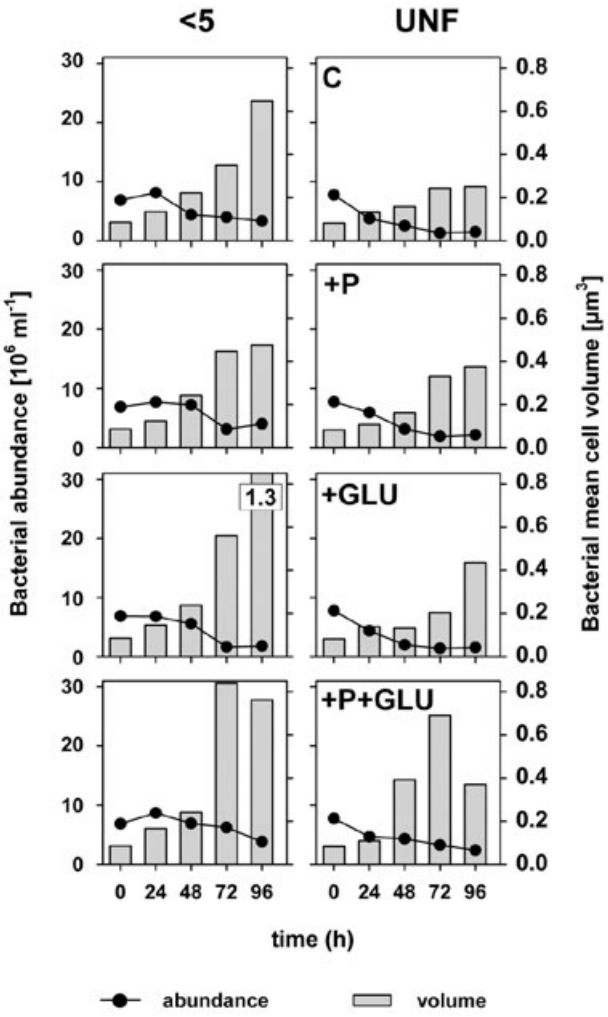

Fig. 1. Bacterial abundance and bacterial mean cell volume in < 5 μm and UNF treatments.

Results

Bacterial abundance and mean cell volume

Increase in bacterial abundance was most pronounced in +P (KH_2PO_4 added) and +P+GLU (KH_2PO_4 and glucose added) treatments (see Fig. 1, for the experimental set-up see cf. Fig. 5). In all < 5 treatments (bacteriovore-enhanced), a drop in bacterial abundance was recorded, as a consequence of heavy protistan grazing, and coincided with an increase in mean cell volume (MCV) that was most prominent in +GLU (glucose added) and +P+GLU incubations. In terms of bacterial concentrations, the same trends were observed in the UNF (unfiltered water) treatments, bacterial concentrations decreased in parallel with increasing protistan bacterivory (cf. Fig. 3), but less marked increases were observed in MCV.

Protozoan abundance and bacterivory, bacterial production

In all < 5 and UNF treatments, numbers of HNF increased substantially, starting from 3×10^3 ml^{-1}, achieving ~43×10^3 ml^{-1} in the +P variant (Fig. 2). The largest increase in cell numbers was observed at time 48 h. In UNF treatments ciliate densities also increased with time, the most markedly in the +P variant (Fig. 2).

Bacterial production (Fig. 3) showed variable trends in all treatments. It exhibited a strong increase during the first 48 h observed in the < 5 and UNF treatments, especially in the +P and +P+GLU treatments.

Heterotrophic nanoflagellates grazing in < 5 variants (Fig. 3) efficiently controlled bacterial biomass accumulation chiefly during the second half of the experiment, at times 48 and 96 h. In three cases (< 5C at 96 h, < 5+P at 48 h and 96 h) the total HNF grazing exceeded bacterial cell production, and this observation corresponded with the overall decreasing bacterial abundance in those treatments. In the UNF treatments, ciliate grazing formed rather a small portion of the aggregate grazing of both

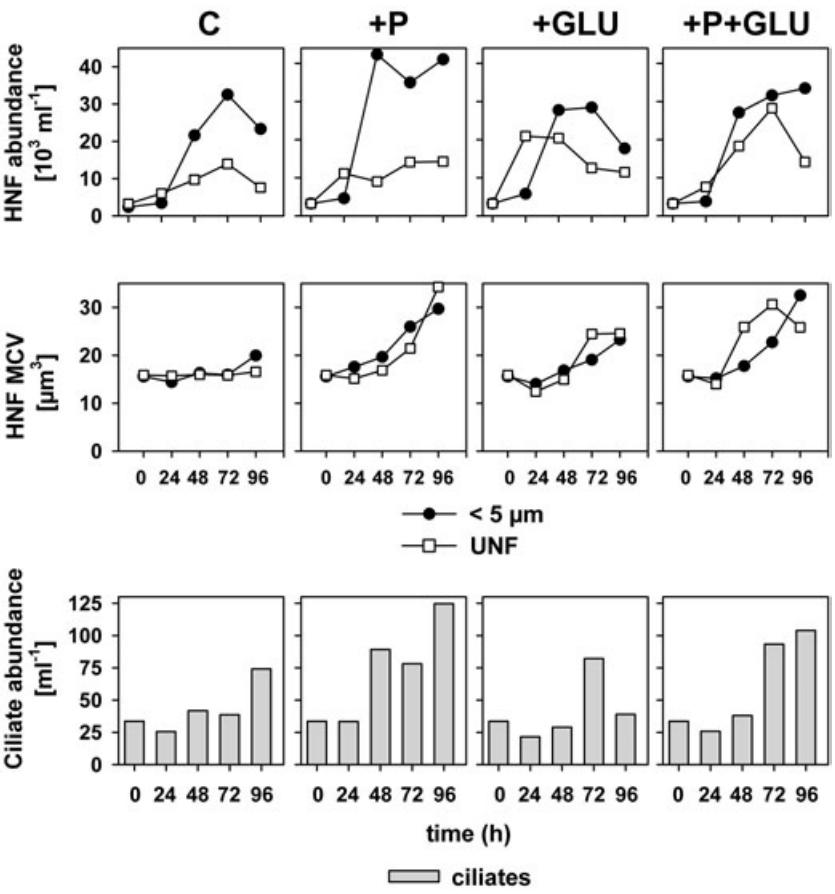

Fig. 2. Abundance of HNF, their MCV (top, two rows) in < 5 µm and UNF treatments, ciliate abundance (bottom) in UNF treatments.

HNF and ciliates, representing always < 25%. A large number of choanoflagellates of the genus *Salpingoeca*, found attached to diatoms in the UNF treatments, contributed significantly (Fig. 3, right) to the total HNF grazing, especially in the +P treatment, where usually more than one half of the total HNF bacterivory could be attributed to the grazing of these choanoflagellates (for details see Šimek *et al.*, 2004). The addition of glucose, contrary to +P treatment, stimulated growth of small, free-swimming *Spumella*-like HNF, so that the relative contribution of *Salpingoeca* sp. to the total HNF grazing was much lower and comparable to the Control treatment.

Mean cell volumes of HNF (cf. Fig. 2) during the experiment were rather stable in the incubations without nutrient additions, the control treatments (< 5C and C-UNF), compared with all the nutrient-amended ones, where an increase in the HNF MCV was detected. This was most evident in the unfiltered treatments, especially in the +P-UNF treatment. The +GLU-UNF and +P+GLU-UNF treatments showed a decline in the MCV of HNF at the end of the study, probably as a result of a shift in the species composition towards smaller *Spumella*-like chrysomonads. An exponential increase in MCV of HNF along with an increasing proportion of bacterivorous *Salpingoeca* sp. attached to colonial diatoms, *Asterionella formosa* and *Fragilaria crotonensis* occurred in the +P addition alone or in combination (+P+GLU treatments, for details see Šimek *et al.*, 2004).

Proportions of probe-detected bacterial prey available compared with those ingested by HNF

Table 1 shows mean values (pooled over treatments) of relative proportions of bacteria targeted by FISH probes expressed as percentage of total DAPI counts. Proportions of bacteria detected by BET42a probe ranged usually from 30% to 45% within the both size fractions, and increased towards the end of the experiment. The proportions of the R-BT065-positive bacteria (subgroup of *Betaproteobacteria*, 9–19% of total) increased at time 48 h and decreased at time 96 h in both treatments. The GAM42a probe-detected cells accounted for 2.2–4.6% of the community and showed no clear temporal trends. The proportions of the *Cytophaga-Flavobacterium-Bacteroidetes* group (the CF319a probe, ~8–20% of a total bacteria) displayed markedly different patterns: they increased in < 5 treatments while an opposite trend was observed in UNF treatments (Table 1). Proportions of the *Actinobacteria* group (HGC69a) dramatically dropped from 33%–36% at time 0 h towards the end of the exper-

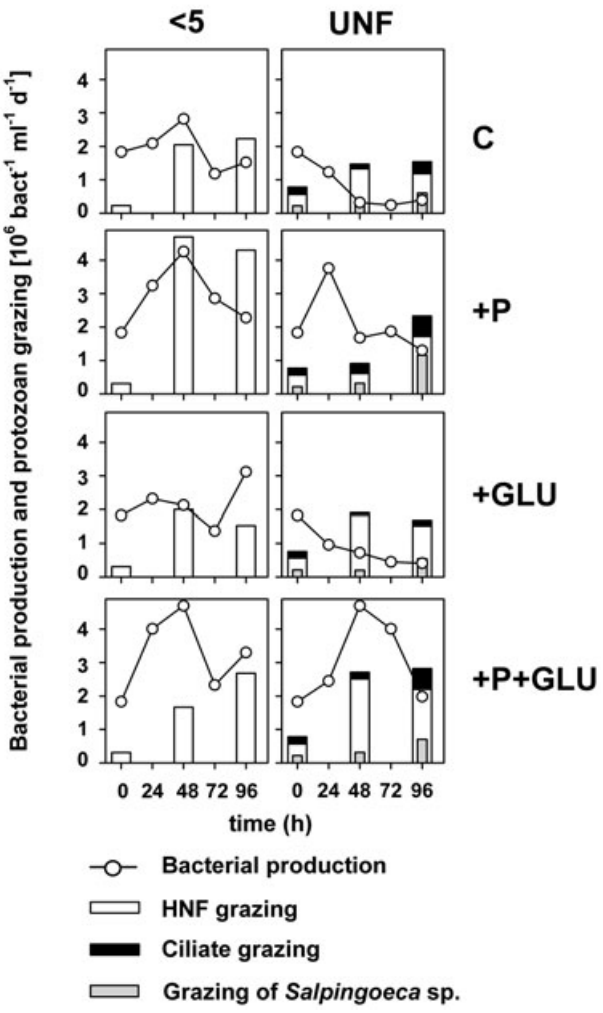

Fig. 3. Bacterial production for < 5 μm and UNF treatments and protozoan grazing subdivided into the (1) HNF grazing (then divided into the grazing of *Salpingoeca* sp., and other HNF) and (2) ciliate grazing.

iment (10%), although they were consistently negatively selected for by protists in all treatments (see below).

Selectivity indexes (Fig. 4) were calculated as the ratio between the relative abundance of a given probe-detected bacterial group inside food vacuoles divided by its relative abundance in the ambient bacterioplankton. Both proportions were normalized to the percentage of total cells targeted by the universal EUB338 probe. Due to the low initial HNF numbers (see time 0 h in Fig. 2), only values from 48, 72 and 96 h series are presented here, when sufficient HNF numbers enabled the appropriate evaluation of cell vacuole contents.

Overall, bacteria targeted by the BET42a probe (*Betaproteobacteria*) were discriminated against by HNF as indicated by the selectivity index being almost always < 1. The largest differences between the < 5 and UNF treatments were detected among the resource-enriched treatments – +P, +GLU and +P+GLU. Consistently, this bacterial subgroup was more negatively selected by HNF in all < 5 nutrient-amended treatments.

Members of the *Rhodoferax* BAL 47 cluster, targeted by the R-BT065 probe (subgroup of *Betaproteobacteria*), were subjected to both positive and negative selection. Preference for these bacteria generally increased in the 5 μm treatments with less marked changes in the unfiltered communities. An opposite trend was recorded for the GAM42a bacterial cells.

The GAM42a probe (*Gammaproteobacteria*) detected bacteria (accounting for < 10% of total *Eubacteria*) were more positively selected for by HNF by the end of the experiment but mostly among populations of HNF in the unfiltered treatments (see, for example, Control, +P and +P+GLU treatments).

Bacteria targeted by the CF319a probe were increasingly avoided during the time-course of the study in all the treatments. The largest difference between UNF and < 5 treatments was observed in the Control and +P treatments with initially strong positive selection of the CF319a-positive cells in the unfiltered treatments (Fig. 4). Interestingly, this trend changed to mostly negative selection towards the end of the study. To elucidate this remarkable switch in the prey selectivity, we sized also the cell volumes of CF319a- and of R-BT065-targeted bacteria (Table 2).

Mean cell volume of the members of the CF319a probe-targeted group remained relatively stable during the study, except for the increase observed in the < 5+P treatment. However, bacteria targeted by the R-BT065 probe increased markedly their MCV at time 96 h, mainly in the < 5+GLU and +P+GLU-UNF variants (see Table 2). Bacteria belonging to other tested groups did not show any traceable trend in size (data not shown). The difference

Table 1. Percentage of bacteria targeted by different FISH probes, expressed as percentage ± SD of total DAPI bacterial counts for times 0, 48 and 96 h found, pooled for each probe-detected group across all < 5 μm and UNF treatments.

	BET42a		R-BT065		GAM42a		CF319a		HGC69a	
Time (h)	< 5 μm	UNF	< 5 μm	UNF	< 5 μm	UNF	< 5 μm	UNF	< 5 μm	UNF
0	35 ± 2	30 ± 3	12 ± 2	9 ± 2	5 ± 0.2	3 ± 0.3	11 ± 1	11 ± 1	33 ± 3	36 ± 5
48	40 ± 9	37 ± 5	18 ± 8	19 ± 9	4 ± 0.4	5 ± 0.6	10 ± 2	6 ± 2	16 ± 3	17 ± 5
96	43 ± 5	45 ± 6	11 ± 6	12 ± 6	3 ± 1.0	2 ± 0.4	20 ± 6	8 ± 3	12 ± 3	9 ± 4

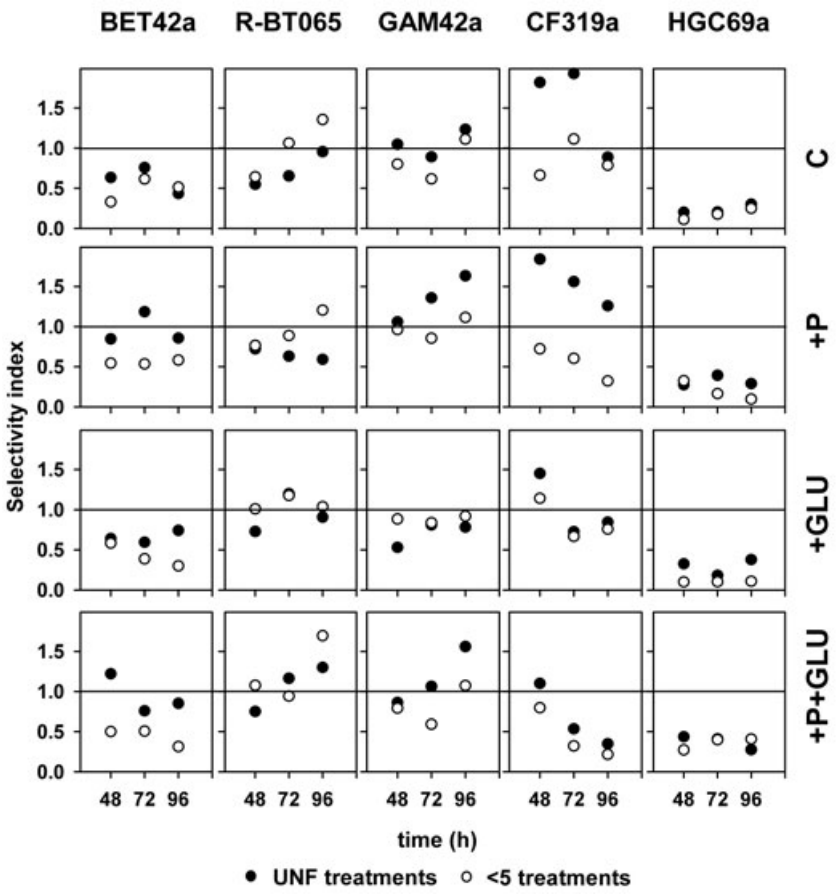

Fig. 4. Selectivity indexes for bacterial subgroups targeted by different oligonucleotide probes.

between UNF and < 5 treatments might be attributed to the presence of daphnids (10 ind l^{-1}) and the medium-sized diatom-attached choanoflagellates (*Salpingoeca* sp.) abundant in UNF treatments. These choanoflagellates accounted for a large portion of the aggregated HNF bacterivory (Fig. 3) as they showed very high cell-specific grazing rates (cf. Šimek *et al*., 2004) compared with small HNF numerically dominating in UNF treatments. Bacteria targeted by the HGC69a probe (*Actinobacteria* group) were consistently negatively selected for by HNF in both < 5 and UNF treatments, with the selectivity index ranging from 0.1 to 0.5.

Comparing nutrient treatments, overall, it appeared that the addition of only glucose alone did not have any marked effects on HNF grazing selectivity in terms of triggering temporal changes. This was in contrast to the +P or +P+GLU additions together, where temporal shifts in positive or negative selectivities were detected (Fig. 4).

Discussion

Recently, Jezbera and colleagues (2005) have presented the first study that dealt with employment of oligonucleotide probes (FISH method) for studying the food preferences of protists, at *in situ* conditions, in a freshwater reservoir. Here we have attempted to improve the resolution level of the analysis of food vacuole contents of protists, e.g. all FISH probes were applied using the catalysed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) protocol (Sekar *et al*., 2003) facilitating identification of ingested prey items. Natural communities were size-fractionated and subjected to nutrient enrichment to induce shifts in bacterial community composition to allow assessment of the response of the HNF community to shifts in prey composition and concentration. Indeed, three different combinations of the resource amendments (cf. Fig. 5) evoked also measurable changes in the uptake of different genotypes of bacteria by protists (cf. Fig. 4). Moreover, compared with the previous study (Jezbera *et al*., 2005) a detailed analysis of protistan bacterivores in UNF treatments was available (Šimek *et al*., 2004) allowing detection of the possible effect of different bacterivores on the bacterial community composition.

Three groups, i.e. the bacteria targeted with the R-BT065, HGC69a and CF319a probes showed interesting and consistent trends in our treatments. The members of the R-BT065 cluster (affiliated with the *Rhodoferax* sp. BAL47 cluster of *Betaproteobacteria*) abundant in many

Table 2. Bacterial mean cell volume (in μm^3) for the cells targeted by the CF319a and R-BT065 probes at times 0, 48 and 96 h.

	< 5C		< 5+P		< 5+GLU		< 5+P+GLU		+P-UNF		+P+GLU-UNF	
Time (h)	CF 319a	R-BT065	CF 319a	R-BT065	CF 319a	R-BT065	CF 319a	R-BT065	CF 319a	R-BT065	CF 319a	R-BT065
0	0.066	0.079	0.066	0.079	0.066	0.079	0.066	0.079	0.066	0.079	0.066	0.079
48	0.064	0.071	0.082	0.042	0.081	0.086	0.058	0.044	0.087	0.062	0.092	0.080
96	0.075	0.073	0.099	0.076	0.087	0.135	0.057	0.056	0.055	0.091	0.069	0.147

European lakes (Zwart *et al*., 2002; 2003) are thought to represent opportunistic bacterial strategists with large growth and uptake capabilities (Šimek *et al*., 2005; K. Hornák *et al*., submitted). Until recently, only limited information has been available about the ecology of this subcluster.

The major features of influence of top-down and bottom-up manipulations on the dynamics of the R-BT065-positive bacteria have been reported by Šimek and colleagues (2005). In their study, between 40% and 95% of BET42a-targeted bacteria belonged into the R-BT065 cluster and these phylotypes rapidly responded to the changing levels of protistan grazing pressure. In the grazer-free treatments (< 0.8 μm), R-BT065-positive bacteria showed the highest net growth rates (Šimek *et al*., 2005) while in the bacterivore-enhanced treatments these phylotypes decreased in relative importance, but remarkably increased in single-cell uptake activity (K. Hornák *et al*., submitted).

The data shown here suggest that the R-BT065 phylotypes were grazed with a slightly increasing tendency in almost all variants (cf. Fig. 4), except for +GLU treatment. This trend was most evident for +P+GLU variant. However, a marked difference was detected between the UNF and < 5 treatments. This difference is perhaps because the < 5 treatments resulted in an overall dominance of small *Spumella*-like chrysomonads in HNF bacterivory (cf. Šimek *et al*., 2004). It was characterized by increases in positive selectivity indexes among some < 5 treatments at the end of the experiment (see time 96 h in Fig. 4). It seems that the HNF selectivity exerted on R-BT065-positive bacteria was probably treatment-specific (cf. Jezbera *et al*., 2005). Nutrient-induced shifts in bacterial community composition (i.e. shifts in the composition of non-R-BT065 cells) could re-target the grazing pressure of the bacterivores.

Marked differences between the UNF and < 5 treatments (especially in case of the P-enriched treatments) in the selection indexes may be attributed to the presence of the distinct bacterivore communities in the UNF treatments. In the latter treatments, one has to take into account the combined effect and variable contributions to the total protistan bacterivory (cf. Fig. 3) of the grazers with very different feeding modes: the free-swimming *Spumella*-like chrysomonads that are considered to be typical encounter feeders (for review see Boenigk and Arndt, 2002) in contrast to the filter-feeding *Salpingoeca* sp. (accounting on average for ~40% of total protistan bacterivory) and small oligotrichous ciliates (mainly *Halteria grandinella*, cf. Šimek *et al*., 2000) that also represent fine filter-feeding bacterivores. This complex assemblage of protists surely had a different impact on the bacterial community composition in UNF treatments compared with only HNF bacterivory in the < 5 treatments.

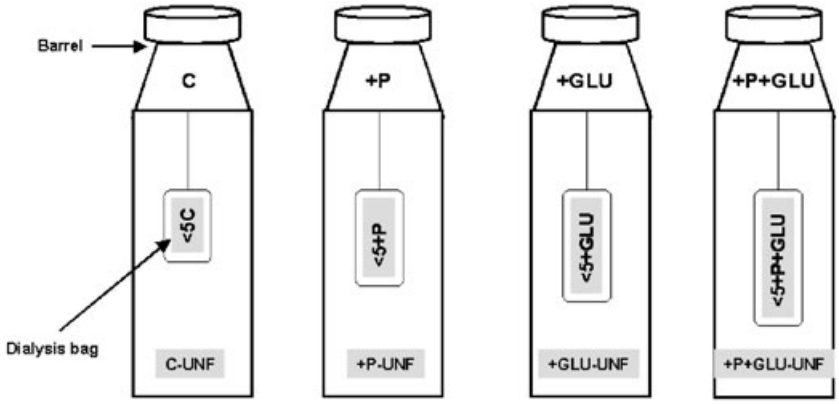

Fig. 5. The design of the experiment, 9–13 September 2002. Barrels were filled with unfiltered reservoir water (UNF), amended with nutrients – phosphorus (+P) or glucose (+GLU) or phosphorus and glucose (+P+GLU) and dialysis bags with different water fractions were incubated inside of them (2 per each barrel).

It can be well exemplified on the very distinct time-courses in the relative contributions of the CF319a group to total bacterial community in the compared size treatments (data not shown). Thus, it is probable that the community composition of the HNF communities varies with presence or absence of other non-HNF bacterivores. The consideration here, for the first time, of the impact of the entire grazer community on the selectivity of HNF demonstrates the not-unexpected complexity of the bacterivorous community.

The members of the *Actinobacteria* group (bacteria targeted by the HGC69a probe) were consistently avoided by HNF (selectivity index fairly below 0.5, see Fig. 4), thus confirming our previous field study of Jezbera and colleagues (2005). Some of the actinobacterial strains exhibit a small size (Hahn *et al*., 2003); however, this might not be a rule. Bacteria belonging to *Actinobacteria* found in our experiment were almost exclusively of small cell size (data not shown) as holds true for the previous experiment (see Jezbera *et al*., 2005). *Actinobacteria*, representing Gram-positive bacteria with a high genomic G+C content, display a great variety of validly described species and isolates, despite the fact that their role in the water remains rather poorly understood (Hahn *et al*., 2003; Warnecke *et al*., 2004). Their detectability by FISH may be influenced by their small size (Hahn *et al*., 2003) resulting in low ribosome content. Therefore, to increase the microscopic resolution of the small Gram-positive cells, in this study we consistently applied the CARD-FISH protocol.

There is an ongoing discussion on what protects *Actinobacteria* from being grazed upon by predators. Definitely a small cell size, with MCV often being below $< 0.05\ \mu m^3$, suboptimal for most of the predators, mostly C-shaped morphology (Hahn *et al*., 2003; Jezbera *et al*., 2005), and specific cell wall properties to some extent related to the thick Gram-positive wall may account for a large part of the apparent grazing resistance of these phylotypes. Interestingly, there are only a few studies, performed under the laboratory conditions, which have actually reported these bacteria as grazing-resistant: Pernthaler and colleagues (2001a) reported *Actinobacteria* as a major group in a chemostat experiment clearly profiting from the presence of abundant bacterivorous flagellate, *Ochromonas* sp., that eliminated most of the bacterial competitors of different phylogenetic lineages (e.g. *Betaproteobacteria*). Hahn and colleagues (2003) observed grazing avoidance when only a single *Actinobacterium* strain was available as prey for *Ochromonas* sp.

Another group, the CF319a probe-detected bacteria, was subjected to an intriguing pattern of selectivity by protists. There were variable and frequently contrasting shifts in morphology and/or size of these bacteria between the < 5 and UNF treatments (Tables 1 and 2). We recorded in our study a constant decrease in the selectivity index of these cells with time in all nutrient-amended treatments (Fig. 4). Moreover, a marked divergence between the two size treatments was detected in the phosphorus-enriched treatments, where these phylotypes were clearly ingested much less in < 5 μm than in UNF treatments, although this pattern is, but less obviously, reflected also in other treatments. The difference is also apparent for the proportions of these bacteria available in the ambient environment as prey for HNF (see Table 1). In the course of the study, their fractions in < 5 μm treatments increased while they decreased in the UNF treatments. Last but not least, a small increase of CF319a-positive bacteria MCV was recorded in the < 5+P variant as opposed to relatively stable MCV of these cells in other nutrient-amended treatments (see cf. Table 2). Thus, the differences in the selectivity can be related to the treatment-specific compositional shift within the CF319a cluster that is very likely (unfortunately, not yet proved with more specific probes) induced by the presence of different grazer community in the size treatments.

Overall, based on the detailed analyses of HNF prey selectivity together with the specific investigations of protistan bacterivory and community composition, our data indicated the strong effect of distinct bacterivores on the bacterial community dynamics, consequently having

marked feedbacks on the prey availability resulting in different selectivity within the studied bacterial communities.

Experimental procedures

Study site and experimental design of size fractionation

The experiment was conducted between 9 and 13 September 2002 in the Římov reservoir (South Bohemia, 470 m above sea level, area 2.06 km^2, volume 34.5×10^6 m^3, mean retention time 100 days, dimictic, meso-eutrophic). It was run in duplicated four 50 l polyethylene barrels (acid soaked and MQ water rinsed) filled with unfiltered reservoir water collected from and then incubated in a depth of 0.5 m in the dam area. All barrels were attached to buoys and incubated for 4 days approximately 200 m from the reservoir dam. The water used for the experiment was phosphorus (1.9 $\mu g\ l^{-1}$ DRP and 20 $\mu g\ l^{-1}$ TP) but not nitrogen (1.5 $mg\ l^{-1}$) limited. Thus, by changing phosphorus and organic carbon availability, we intended to stimulate differently bacterial growth in the experimental containers, by adding KH_2PO_4 (assigned in the figures as +P treatment, final concentration 62 μl^{-1}) or glucose (the +GLU treatment, final concentration 2.5 mg C l^{-1}) or by combination of both (the +P+GLU treatment). For a detailed view of the experimental design see Fig. 5.

In each barrel, dialysis bags filled with water subjected to two different treatments were incubated: unfiltered water (assigned as UNF, filled directly from the barrel), and bacterivore-enhanced treatment (< 5 μm filtrate) with fast-growing bacterivorous HNF. The latter treatment (assigned as < 5 treatments) was first gravity filtered and then used to fill 2.5 l (deionized water rinsed and boiled) dialysis bags (approximately 70 cm long, 75 mm diameter, and molecular weight cut-off 12–16 kDa, Poly Labo, Switzerland) and then immediately submerged into each barrel. For the details of filtration procedures, see Šimek and colleagues (2003). Such size-fractionated samples thus yielded different levels of bacterivory, and moreover, different bacterivore communities, so that we could induce marked changes in microbial communities caused by the different grazing pressure. Overall, we obtained eight different treatments, assigned throughout the figures and text as < 5C (Control), < 5+P, < 5+GLU, < 5+P+GLU, C-UNF (Control), +P-UNF, +GLU-UNF, +P+GLU-UNF.

Bacterial and protozoan dynamics, protistan grazing on bacteria

For details of most of these methods see the paper of Šimek and colleagues (2004), which described a part of the same experiment, but very specifically focused on the dynamics and bacterivory of diatom-attached choanoflagellates of the genus *Salpingoeca* present only in the unfiltered treatments.

Briefly, for bacterial abundance, subsamples were fixed with DAPI (Porter and Feig, 1980) and enumerated employing the Olympus AX70 Provis epifluorescence microscope. For details of cell sizing (MCV), see Šimek and colleagues (2003). Bacterial production was measured using ^{3}H-thymidine incorporation with a modified method of Riemann and Sondergaard (1986). The treatment-specific empirical conversion factors were established on the basis of bacterial cell number increase in the 0.8 μm filtered treatments incubated in dialysis bags (Herndl *et al.*, 1993; Šimek *et al.*, 1999), using a modified derivative method of Kirchman and Ducklow (1993).

Protozoan grazing on bacterioplankton was measured at times 0, 48 and 96 h using fluorescently labelled bacteria according to the protocol of Sherr and Sherr (1993) and detailed in Šimek and colleagues (1999). The fluorescently labelled bacteria tracer was added to constitute roughly 10–15% of natural bacterioplankton abundance and subsamples were taken at incubation times of 5, 15 and 30 min. For HNF enumeration and tracer ingestion determinations, 50 ml of subsample was taken and fixed with the lugol-formaldehyd-thiosulfate decolorization technique (Sherr and Sherr, 1993). Ciliate grazing was analysed simultaneously with HNF uptake in all unfiltered treatments and important groups of bacterivorous ciliates were distinguished according to their ability to ingest bacteria. Due to the problematic recognition of ciliates in the epifluorescence microscope, additional criteria such as size, organization of bacterial prey in food vacuoles, number and shape of nuclei were applied as detailed in Šimek and colleagues (2000).

CARD-FISH with rRNA-targeted oligonucleotide probes

Analysis of bacterioplankton in water samples as well as in the food vacuoles was performed using group-specific oligonucleotide probes (Amann *et al.*, 1995; Pernthaler *et al.*, 2001b). We applied CARD-FISH (Pernthaler *et al.*, 2002; Sekar *et al.*, 2003) protocol employing following probes: EUB338, BET42a, GAM42a, CF319a, R-BT065 (this probe targets a subcluster of the *Rhodoferax* sp. BAL47 cluster of *Betaproteobacteria*, Zwart *et al.*, 2002), and HGC69a probe (*Actinobacteria*) purchased from ThermoHybaid (Interactiva Division, Ulm, Germany). At least 100 HNF were inspected for bacteria hybridized directly in the vacuoles per each probe and each time point and treatment (see Jezbera *et al.*, 2005). On average, three to four bacteria hybridized with the universal EUB338 probe were found inside the food vacuole of each protist. In the following text, the probe-targeted bacterial groups are assigned as EUB338, BET42a, GAM42a, CF319a, R-BT065 and HGC69a.

Selectivity indexes were calculated by dividing the proportions of probe-targeted subgroups of ingested bacteria by the proportions available in bacterioplankton, both expressed as the percentage of EUB338-positive cells. Values of 1 represent random feeding, and values < 1 and > 1 indicate negative and positive selection respectively.

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