

# Effects of a *Microcystis aeruginosa* bloom and bacterivory on bacterial abundance and activity in a eutrophic reservoir

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**ABSTRACT:** We investigated the changes in bacterioplankton abundance, composition, and fractions of cells incorporating leucine associated with a *Microcystis aeruginosa* bloom and enhanced protozoan grazing in a eutrophic reservoir. Bacterial community composition and leucine incorporation were studied by means of fluorescence *in situ* hybridization and microautoradiography. To alter the levels of protozoan grazing and the interactions of bacteria with the *M. aeruginosa* bloom, a manipulation experiment using size-fractionation and dialysis bag techniques was conducted. Samples collected from the *Microcystis*-poor dam area and the *Microcystis*-rich middle part of the reservoir were incubated as <0.8 µm, <5 µm, and unfiltered treatments in dialysis bags at their collection sites and, in parallel, one of each of the treatments from one site was transferred to and incubated at the other site. The sample transfer from the dam to the middle part induced a decrease in abundances and doubling times of total bacteria, *Betaproteobacteria*, and of the R-BT065 cluster (a subgroup of *Betaproteobacteria*), whereas the transfer to the dam station revealed the opposite trends. In the presence of flagellates and the *M. aeruginosa* bloom, both abundance and fractions of cells incorporating leucine of *Betaproteobacteria* and of the R-BT065 cluster transferred to the middle station decreased dramatically, while members of the *Sphingobacteria*–*Flavobacteria* lineage became the dominant part of the community. While *Gammaproteobacteria* accounted for a minor proportion of the total community, their relative proportions and activity increased at the middle station, likely being stimulated by the increased resource availability.

**KEY WORDS:** Reservoir · *Microcystis aeruginosa* · Bacterial activity · Leucine incorporation · Protozoan grazing

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## INTRODUCTION

In eutrophic environments, massive cyanobacterial blooms frequently develop during summer, producing potentially toxic or noxious compounds and negatively affecting water quality (Chorus & Bartram 1999). Effects of intense cyanobacterial blooms on bacteria are related mainly to the production of various organic compounds, representing an important source of dissolved organic carbon (DOC) for bacteria (Baines & Pace 1991). The amount and nature of cyanobacterial

exudates specifically influence bacterial growth and activity (e.g. Kirkwood et al. 2006), causing significant shifts in bacterial community composition (Christoffersen et al. 2002, Eiler & Bertilsson 2004). In addition, production of allelopathic substances (Østensvik et al. 1998), competition for nutrients (e.g. Chorus & Bartram 1999), zooplankton grazing (Gobler et al. 2007), or viral lysis (van Hannen et al. 1999) also influence the cyanobacteria–bacteria interactions.

Various cyanobacteria, e.g. some strains of *Microcystis* spp., are common producers of several classes of

secondary metabolites, with microcystins (MCs) being the most frequently studied group of cyanotoxins (Codd 1995). The release of MCs depends on many factors, such as light and nutrient conditions, temperature, or physiological status (Park et al. 1998). Colonies of *Microcystis* spp. are often associated with heterotrophic bacteria (Worm & Søndergaard 1998), constituting a significant portion of total bacterial abundance and production (Brunberg 1999). In contrast, growth and activity of bacterial assemblages not previously subjected to the specific cyanobacterial effects, e.g. various cyanotoxins, may be suppressed when exposed to *Microcystis* blooms.

Bacteria are also subjected to various mortality factors such as protistan (e.g. Pace 1988) and zooplankton grazing (Jürgens et al. 1994) and viral lysis (Weinbauer & Höfle 1998). Predation by heterotrophic nanoflagellates (HNF) and ciliates controls bacterial abundance, biomass, and production (e.g. Hahn & Höfle 2001) and also induces significant changes in the bacterioplankton composition and cell-size structure, e.g. by selective ingestion of certain prey cells within the entire bacterial assemblage (e.g. Jezbera et al. 2006).

Capabilities of bacteria to incorporate various compounds substantially differ even at the group-specific level (e.g. Cottrell & Kirchman 2000, Vila et al. 2004). Both relative abundance and activity of bacteria may be notably modified by changes in substrate concentration, bacterivory, or structure of dissolved organic matter (Alonso & Pernthaler 2006, Horňák et al. 2006, Pérez & Sommaruga 2006).

The purpose of this study was to examine the potential interactions between the summer bloom of *Microcystis aeruginosa* and *in situ* abundances and activity of major bacterioplankton groups in the eutrophic Římov reservoir (Czech Republic). Bacterial community composition in this model system is both seasonally and spatially dominated by *Betaproteobacteria*, namely by its R-BT065 cluster (subcluster of *Rhodospirillum rubrum* sp. BAL47 lineage; Šimek et al. 2001, Zwart et al. 2002). The R-BT065 group represents the fastest-growing part of the community and is likely responsible for the majority of production and activity processes in the reservoir (Horňák et al. 2006, Šimek et al. 2006). We hypothesized that bacterioplankton community dynamics are likely differently affected by pronounced differences in the development of an *M. aeruginosa* bloom at 2 stations (5 km apart) of the same canyon-shaped reservoir. Aspects that are most likely to be affected by the bloom are related to shifts in the bacterial community and its capabilities to take up organic substrates. Correspondingly, we focused on: (1) the influence of different *M. aeruginosa* bloom intensities in the dam area and the middle part of the reservoir on bacterial community composition and

fractions of cells incorporating leucine, (2) the response of dominant groups of free-living bacterioplankton to different levels of protistan grazing and to the presence of *M. aeruginosa*, using the dialysis bag technique and sample transfers along the longitudinal axis of the reservoir.

## MATERIALS AND METHODS

**Sampling and field experiment.** The experiment was carried out in the eutrophic canyon-shaped Římov reservoir (for details, see Šimek et al. 2006) during the summer phytoplankton maximum (18 to 22 August 2003) close to the dam of the reservoir (Stn Dam), and in the middle part of the reservoir (Stn Middle), where a heavy phytoplankton bloom, dominated by *Microcystis aeruginosa*, had developed. In brief, ~40 l of water were collected from each station from a depth of 0.5 m. To alter the levels of bacterivory, the water was size-fractionated using 5 and 0.8 µm polycarbonate filters (Poretics). The <5 µm treatment contained bacteria, HNF, and small algae, whereas zooplankton and ciliates were removed. The <0.8 µm treatment contained only bacteria, whereas all bacterivores were removed. Unfiltered water treatments containing bacteria, protists, zooplankton, and large colonies of *M. aeruginosa* were taken in parallel from each station. All size fractions (<0.8 µm, <5 µm, and unfiltered treatments) were incubated in dialysis bags (each containing ~2 l; diameter of dialysis tubes: 75 mm; molecular weight cutoff: 12 to 16 kDa; incubated 3 times in Milli-Q water at 70°C for 30 min and washed with Milli-Q water; Spectrapore, Poly Labo). Samples from Stns Dam and Middle were incubated at their site of collection and, in parallel, one of each of the treatments from one site was transferred to and incubated at the other site, yielding 4 treatments (Dam *in situ*: D; Dam transplanted to Middle: D→M; Middle *in situ*: M; and Middle transplanted to Dam: M→D). All treatments were incubated in the reservoir in duplicates for 4 d at a depth of 0.5 m. Samples for bacterial and HNF abundances and bacterial biomass and production were collected daily at 24 h intervals ( $t_0$ ,  $t_{24}$ ,  $t_{48}$ ,  $t_{72}$ , and  $t_{96}$ ). Samples for protozoan grazing and fluorescence *in situ* hybridization (FISH) were sampled at  $t_0$ ,  $t_{48}$ , and  $t_{96}$ . Samples for microautoradiography were taken at  $t_0$  and  $t_{96}$ .

**Phosphorus and chlorophyll a.** Concentrations of total phosphorus (TP) and dissolved reactive phosphorus (DRP) were determined spectrophotometrically (Kopáček & Hejzlar 1993). Prior to analysis, samples were filtered through a 200 µm mesh size filter. Samples for chlorophyll a (chl a) were filtered through Whatman GF/C filters, extracted in 90% acetone, and measured

spectrophotometrically (Lorenzen 1967). Live and Lugols-preserved samples were analyzed for phytoplankton species composition by using light microscopy.

#### **Bacterial abundance, biomass, and production.**

Samples of 1 to 2 ml for bacterial abundance were fixed with 0.2  $\mu\text{m}$  prefiltered formaldehyde (final concentration 2%), stained with DAPI (final concentration 1  $\mu\text{g ml}^{-1}$ ), and concentrated on 0.2  $\mu\text{m}$  black polycarbonate filters (Poretics). Bacteria were enumerated using epifluorescence microscopy (BX-60, Olympus). At least 500 DAPI-stained cells were counted per sample. To determine bacterial cell size, between 500 and 800 DAPI-stained cells were recorded per sample at 1000 $\times$  magnification with an analog monochrome camera (Cohu) and processed with the semiautomatic image analysis system LUCIA D (Laboratory Imaging). Bacterial biomass was calculated from the allometric relationship between bacterial volume and carbon cell content (Norland 1993). Bacterial production was measured by a thymidine incorporation method modified from Riemann & Søndergaard (1986) as described by Šimek et al. (2001). To convert the thymidine incorporation rate to bacterial cell production rate, empirical conversion factors (ECFs) were determined using data from the <0.8  $\mu\text{m}$  treatments. The cell production rate was calculated from the slope of the increase of the natural logarithm of bacterial abundance over time ( $t_0$  to  $t_{48}$ ). ECFs of  $2.48 \times 10^{18}$ ,  $4.55 \times 10^{18}$ ,  $4.39 \times 10^{18}$ , and  $3.97 \times 10^{18}$  cells  $\text{mol}^{-1}$  thymidine were established for treatments D, D $\rightarrow$ M, M, and M $\rightarrow$ D, respectively.

**FISH and microautoradiography.** Catalyzed reporter deposition (CARD)–FISH and microautoradiography (Teira et al. 2004) were used for bacterial genotypic identification and analysis of active leucine incorporation. Briefly, samples of 5 ml were incubated with L-[ $^3\text{H}$ ]-leucine, Leu+ (final concentration 20 nmol  $\text{l}^{-1}$ ; specific activity 6.4 TBq  $\text{mmol}^{-1}$ ; MP Biomedicals) for 2 h at *in situ* temperature in darkness, preserved in formaldehyde (final concentration 2%), and filtered through 0.2  $\mu\text{m}$  polycarbonate filters (Poretics; details in Horňák et al. 2006). Prefiltration through a 30  $\mu\text{m}$  filter was used to remove large phytoplankton aggregates from the unfiltered treatments. Filters with bacteria were covered with low melting point agarose, followed by incubations with lysozyme and achromopeptidase (Sekar et al. 2003). Bacteria were hybridized with the horseradish peroxidase-labeled probes EUB338 I–III (targeting most *Bacteria* [EUB]), BET42a (targeting *Betaproteobacteria* [BET]), GAM42a (targeting *Gammaproteobacteria* [GAM]), R-BT065 (targeting the R-BT065 subcluster [R-BT] of the *Rhodospirillum rubrum* sp. BAL47 lineage [also termed the beta I clade]), CF319a (targeting the *Sphingobacteria-Flavobacteria* lineage [SF] of *Bacteroidetes*), and HGC69a, targeting *Actinobacteria* (Sekar et al. 2003).

Subsequently, the filters were transferred onto the slides coated with the autoradiography emulsion (NTB, Kodak). After 24 to 48 h of exposure, the cells were stained with DAPI (final concentration 1  $\mu\text{g ml}^{-1}$ ). The relative abundances of hybridized cells were determined by epifluorescence microscopy (PROVIS AX-70, Olympus). Bacteria incorporating leucine were surrounded by black silver grains. At least 500 DAPI-stained cells were counted per sample.

**Protistan grazing and abundance.** Protistan grazing on bacterioplankton was estimated with short-time uptake experiments (details in Šimek et al. 2001) with fluorescently labeled bacteria (FLB; Sherr & Sherr 1993) prepared from the Římov reservoir water (details in Šimek & Straškrabová 1992). FLB uptake rates were enumerated in all <5  $\mu\text{m}$  and unfiltered treatments at  $t_0$ ,  $t_{48}$ , and  $t_{96}$ . FLB tracer accounted for 10 to 15% of natural bacterial abundance. Subsamples of 5 to 20 ml were stained with DAPI, filtered through 1  $\mu\text{m}$  black filters (Poretics), and enumerated via epifluorescence microscopy. At least 100 HNF and 50 ciliates (unfiltered treatment only) were inspected per sample. To determine the total grazing rate, average FLB uptake rates of HNF and ciliates were multiplied by their abundances.

**Doubling times.** In the grazer-free <0.8  $\mu\text{m}$  treatments, increases in absolute numbers of total bacteria as well as of different identified bacterial groups (BET, R-BT, SF, GAM) were used to calculate the bulk- as well as the group-specific net growth rates (details in Šimek et al. 2006). Growth rates were calculated during the fastest phase of bacterial exponential growth ( $t_0$  to  $t_{48}$  or  $t_{48}$  to  $t_{96}$ ) and expressed as doubling times.

**Viral abundance.** At  $t_{96}$ , samples of 1 ml for abundances of virus-like particles (VLPs) were fixed with glutaraldehyde (final concentration 0.5%), frozen in liquid nitrogen, and stored at  $-80^\circ\text{C}$ . After thawing, samples were diluted 10 $\times$  in TE-buffer, stained with SybrGreen I (final concentration 5  $\mu\text{M}$ , Molecular Probes), heated at  $80^\circ\text{C}$  for 10 min in the dark, and analyzed by flow cytometry (Brussaard 2004).

**Zooplankton composition.** At  $t_0$ , zooplankton was concentrated from  $\sim 5$  l collected at Stns Dam and Middle, preserved with formaldehyde (final concentration 4%), and quantified by direct microscopic counting of several subsamples (McCauley 1984). At  $t_{96}$ , the same procedure was applied to analyze the zooplankton composition in the remaining water (600 to 800 ml) in the unfiltered dialysis bag treatments.

**Statistical analysis.** Data on relative abundances (as % of DAPI-stained cells) and fractions of cells incorporating leucine (as % of hybridized cells) of bacterial groups were normalized by arcsine transformation. A 2-way ANOVA, using replicate data ( $n = 2$ ) for every sample, was applied to test the effects of size-fraction-

ation (<0.8  $\mu\text{m}$ , <5  $\mu\text{m}$ , unfiltered) and longitudinal transfer (D versus D→M, and M versus M→D) on the relative abundances and fractions of cells incorporating leucine. We compared (1) treatments D and M at  $t_{96}$  versus D and M at  $t_0$ , and (2) treatments D→M versus D, and M→D versus M at  $t_{96}$ , respectively. Significant ANOVAs were followed by Bonferroni tests applied to pair-wise comparisons of differences in relative abundances and fractions of cells incorporating leucine of bacterial groups. All statistics were performed using GraphPad Prism (GraphPad Software).

## RESULTS

### Temperature, phosphorus, and chl *a*

Water temperature at a depth of 0.5 m varied from 19.5 to 24.5°C at both stations. Concentrations of TP and DRP in the reservoir water samples were markedly higher at Stn Middle compared to Stn Dam over the duration of the experiment (Table 1). TP and DRP concentrations inside the dialysis bags were also higher at Stn Middle at  $t_{96}$  with increasing concentrations from <0.8  $\mu\text{m}$  to unfiltered M and M→D treatments (Table 1).

Table 1. Concentrations of total phosphorus (TP) and dissolved reactive phosphorus (DRP) in natural samples at Stns Dam and Middle at  $t_0$  and  $t_{96}$ , and in <0.8  $\mu\text{m}$ , <5  $\mu\text{m}$ , and unfiltered D, D→M, M, and M→D dialysis bag treatments at  $t_{96}$

	TP ( $\mu\text{M l}^{-1}$ )	DRP ( $\text{nM l}^{-1}$ )
<b>Natural samples</b>		
Start – $t_0$		
Dam	0.34	54.9
Middle	1.25	132.4
End – $t_{96}$		
Dam	0.97	77.5
Middle	2.87	371.3
<b>Treatments</b>		
D – $t_{96}$		
<0.8 $\mu\text{m}$	0.62	12.9
<5 $\mu\text{m}$	0.63	45.2
Unfiltered	0.69	32.3
D→M – $t_{96}$		
<0.8 $\mu\text{m}$	1.13	38.7
<5 $\mu\text{m}$	0.63	45.2
Unfiltered	0.79	25.8
M – $t_{96}$		
<0.8 $\mu\text{m}$	0.73	42
<5 $\mu\text{m}$	1.19	54.9
Unfiltered	2.79	119.5
M→D – $t_{96}$		
<0.8 $\mu\text{m}$	0.78	42
<5 $\mu\text{m}$	1.21	54.9
Unfiltered	2.67	100.1

Chl *a* concentrations at Stn Dam varied between 16 and 25  $\mu\text{g l}^{-1}$ , whereas elevated chl *a* concentrations in the range of 52 to 65  $\mu\text{g l}^{-1}$ , corresponding to the bloom event, were found over the course of the experimental period at Stn Middle. Large colonies of *Microcystis aeruginosa* accounted for >50% of the total phytoplankton biomass at Stn Dam and 80 to 90% at Stn Middle at  $t_{96}$ .

### Bacterial abundance and biomass

The Bacterioplankton assemblage in the grazer-free (<0.8  $\mu\text{m}$ ) treatment attained higher abundances at Stn Dam compared to Stn Middle (Fig. 1). Transferring the samples from Stn Dam to Stn Middle (D→M treatment) yielded a less pronounced increase in total bacterial numbers, while the opposite transfer (M→D treatment) induced a clear increase in bacterial numbers (Fig. 1). After  $t_{24}$  or  $t_{48}$ , bacterial abundances in the grazer-enhanced (<5  $\mu\text{m}$ ) and unfiltered treatments were markedly lower compared to respective <0.8  $\mu\text{m}$  treatments (Fig. 1), due to rapid development and grazing activity of HNF (Fig. 2) and partially also ciliates (in unfiltered treatment only, data not shown).

Changes in bacterial biomass closely followed the changes in total bacterial numbers (Fig. 1). Compared to the initial samples, bacterial biomass markedly increased in all grazer-free (<0.8  $\mu\text{m}$ ) treatments, showing maxima in D and M→D treatments (Fig. 1). Bacterial biomass was substantially lower in corresponding <5  $\mu\text{m}$  and unfiltered treatments, especially during  $t_{48}$  to  $t_{96}$  (Fig. 1).

### Bacterial production, HNF abundance, and grazing

Bacterial production (BP) declined during the first day of incubation, but then continuously increased in all treatments (Fig. 2). BP typically peaked at  $t_{96}$ , particularly in the M and M→D <5  $\mu\text{m}$  and unfiltered treatments. The highest numbers of HNF were observed at  $t_{72}$ , followed by a marked decline at  $t_{96}$  (Fig. 2). Total grazing rates accounted for 4 to 88% of BP, but in D and D→M <5  $\mu\text{m}$ , and D and M unfiltered treatments temporarily exceeded BP rates at  $t_{48}$  (Fig. 2).

### Bacterioplankton composition

Bacterial community composition, as analyzed by CARD-FISH targeting broad phylogenetic groups, differed only slightly between the stations at  $t_0$  (Figs. 3, 4). Using the mixture of EUB338 I–III probes,

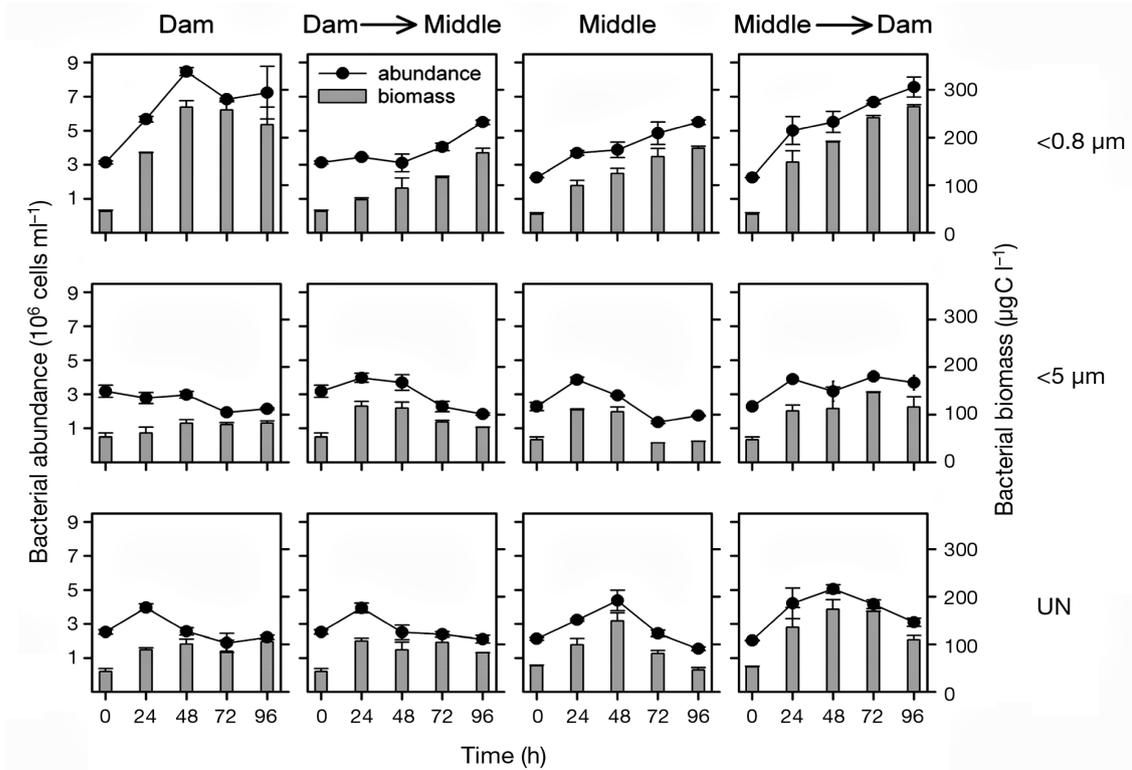


Fig. 1. Changes in bacterial abundance and biomass in <0.8, <5 μm, and unfiltered (UN) Dam, Dam→Middle, Middle, and Middle→Dam treatments (n = 2) over the course of the experiment. Error bars = range

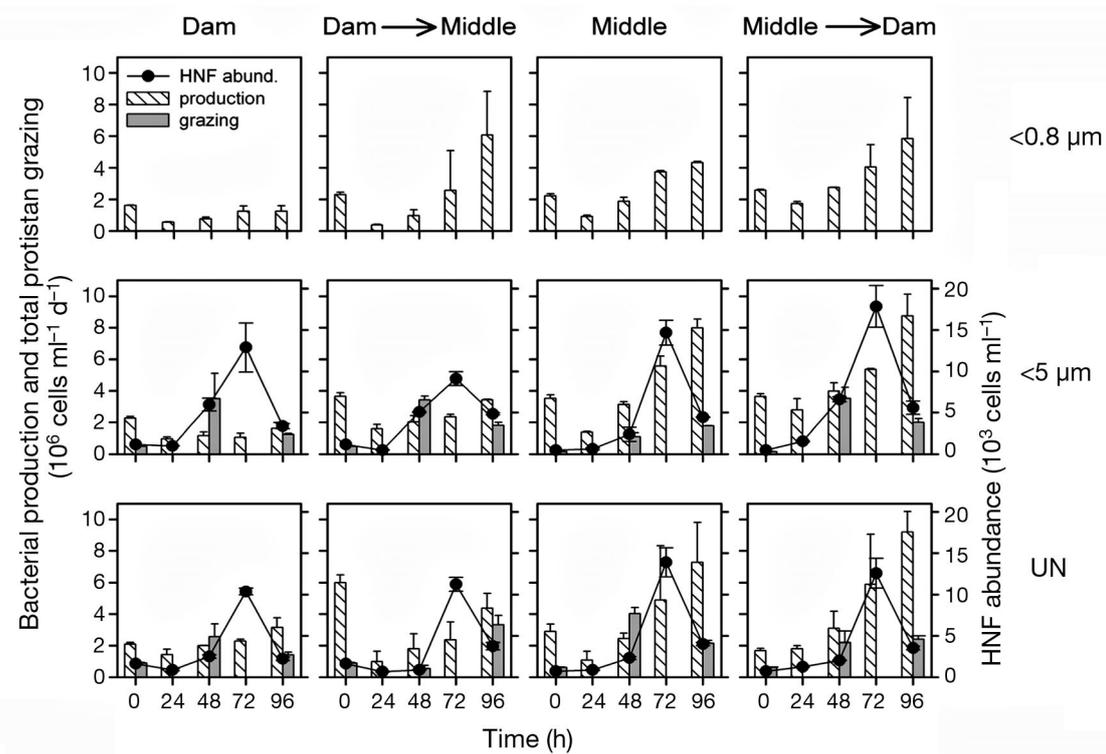


Fig. 2. Bacterial production in <0.8, <5 μm, and unfiltered (UN) Dam, Dam→Middle, Middle, and Middle→Dam treatments (n = 2), and total protistan grazing and abundance of heterotrophic nanoflagellates (HNF) in <5 μm and UN treatments over the course of the experiment. Error bars = range

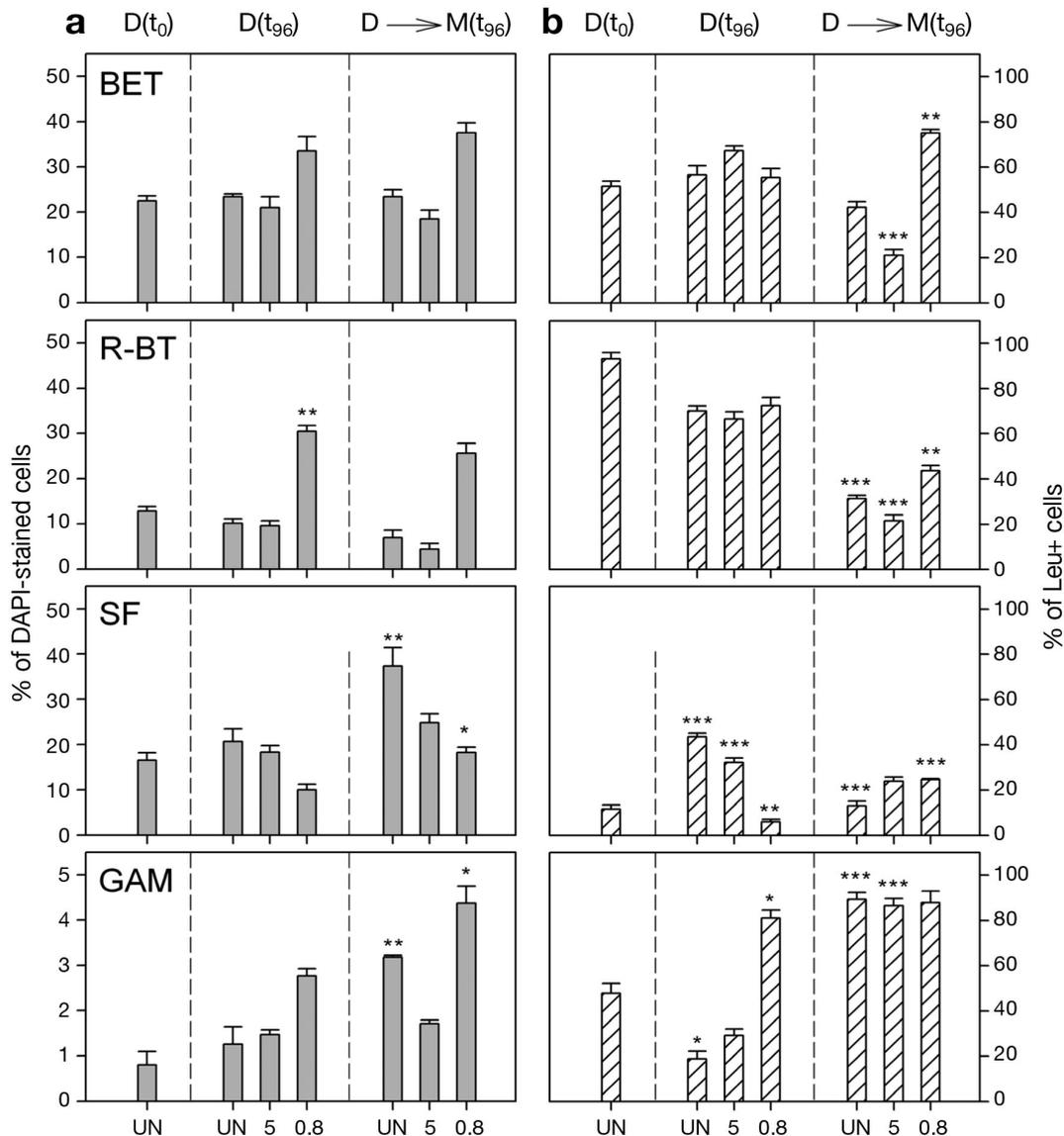


Fig. 3. Relative abundances (a) of DAPI-stained cells (% total bacterial abundances) and (b) of cells incorporating leucine (Leu+ cells as % of hybridized cells) of *Betaproteobacteria* (BET), R-BT065 bacteria (R-BT), *Sphingobacteria-Flavobacteria* (SF), and *Gammaproteobacteria* (GAM) at Stn Dam (D) at t<sub>0</sub> in unfiltered (UN) samples, and at Stn Dam and samples transferred from Stn Dam to Stn Middle (D→M) at t<sub>96</sub> in UN, <5 μm (5), and <0.8 μm (0.8) treatments. n = 2, error bars = range. Asterisks above bars either indicate (1) that treatments D(t<sub>96</sub>) were different from D(t<sub>0</sub>) or (2) that treatments D→M(t<sub>96</sub>) were different from the respective treatment type (UN, 5, 0.8) in the D(t<sub>96</sub>) sample. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

we detected 65 to 78% of total DAPI-stained bacteria in all treatments (data not shown). In all treatments, *Actinobacteria* comprised only ~1% of total DAPI-counts (data not shown). At t<sub>96</sub>, the bacterial assemblage was dominated at both stations by BET, accounting for 18 to 38% of DAPI-stained bacteria (Figs. 3, 4). BET dominated particularly in the <0.8 μm treatments (34 to 38% at t<sub>96</sub>), while it consistently accounted for lower proportions in the unfiltered and <5 μm treatments (18 to 32%). R-BT comprised 5 to 30% of DAPI-stained cells (Figs. 3, 4) and followed the pattern of

BET. R-BT bacteria represented the vast majority of BET (57 to 94%), except for the unfiltered and <5 μm M and D→M treatments at t<sub>96</sub> (24 to 40%). SF made up 9 to 38% of the total bacterial community and became the most abundant group in all unfiltered and <5 μm treatments at t<sub>96</sub> (25 to 38%) except for the D treatment (Figs. 3, 4). SF represented considerably lower proportions (9 to 18%) in all <0.8 μm treatments at t<sub>96</sub>. In all treatments, GAM accounted for 1 to 4% (Figs. 3, 4), with the highest percentages (3 to 4%) found in all <0.8 μm treatments at t<sub>96</sub>.

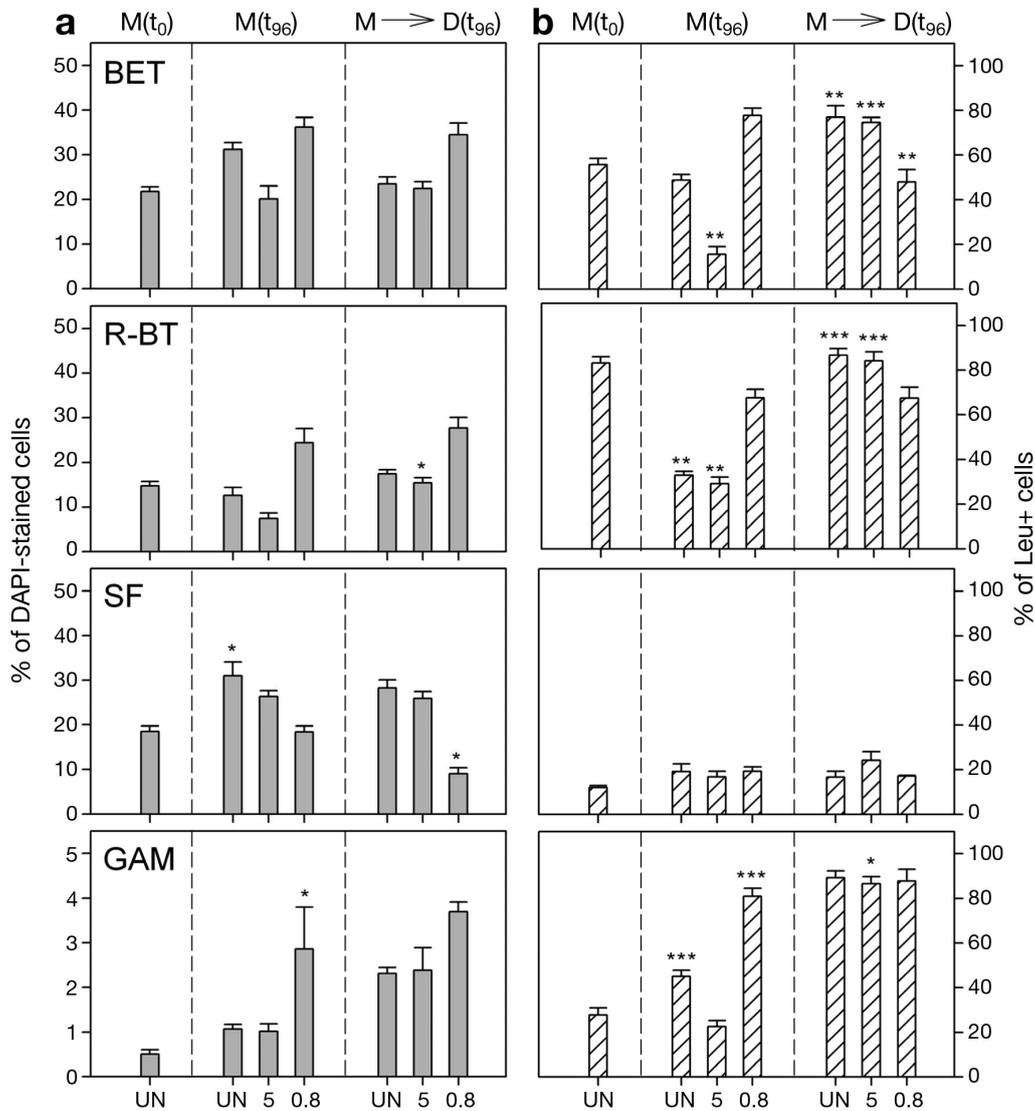


Fig. 4. Relative abundances (a) of DAPI-stained cells (% total bacterial abundances) and (b) of cells incorporating leucine (Leu+ cells as % of hybridized cells) of Betaproteobacteria (BET), R-BT065 bacteria (R-BT), Sphingobacteria–Flavobacteria (SF), and Gammaproteobacteria (GAM) at Stn Middle at t<sub>0</sub> in unfiltered (UN) samples, and at Stn Middle and samples transferred from Stn Middle to Stn Dam at t<sub>96</sub> in UN, <5 μm (5), and <0.8 μm (0.8) treatments. n = 2, error bars = range. Asterisks above bars either indicate (1) that treatments M(t<sub>96</sub>) were different from M(t<sub>0</sub>) or (2) that treatments M → D(t<sub>96</sub>) were different from the respective treatment type (UN, 5, 0.8) in the M(t<sub>96</sub>) sample. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

### Leucine incorporation

Between 52 and 56% of total BET incorporated <sup>3</sup>H-leucine (Leu+) at t<sub>0</sub> (Figs. 3, 4). Fractions of Leu+ BET at t<sub>96</sub> were significantly lower in the unfiltered and <5 μm D → M treatments (21 to 42%) compared to the unfiltered and <5 μm D treatments (57 to 67%, Fig. 3). In contrast, the opposite was true for the M → D (75 to 77%) and M treatments (15 to 38%, Fig. 4). Fractions of Leu+ R-BT bacteria followed the trends of BET and usually exhibited higher proportions of Leu+ cells than BET (Figs. 3, 4). The SF group typically showed the

lowest fractions of Leu+ cells (6 to 44%), with no clear trend in different treatments (Figs. 3, 4). In contrast to BET and R-BT bacteria, fractions of Leu+ GAM increased in the unfiltered and <5 μm D → M treatments (87 to 89%, Fig. 3) at t<sub>96</sub> and remained very high in all <0.8 μm treatments (81 to 91%, Figs. 3, 4).

### Doubling times

Transferring the treatments upstream (D → M) yielded longer doubling times of all bacterial groups

Table 2. Net doubling times of total bacterioplankton, *Betaproteobacteria* (BET), the R-BT065 cluster (R-BT), *Sphingobacteria-Flavobacteria* (SF), and *Gamma-proteobacteria* (GAM) in the <0.8  $\mu\text{m}$  D, D→M, M, and M→D treatments. n = 2;  $\pm$  range is shown

	D	Net doubling time (h)		
		D→M	M	M→D
Bacterioplankton	33.4 $\pm$ 0.02 <sup>a</sup>	64 $\pm$ 21 <sup>b</sup>	65.4 $\pm$ 15.3 <sup>a</sup>	37.8 $\pm$ 4.5 <sup>a</sup>
BET	31.7 $\pm$ 0.7 <sup>a</sup>	34.5 $\pm$ 4.8 <sup>b</sup>	34.8 $\pm$ 3.8 <sup>a</sup>	32.9 $\pm$ 14.2 <sup>a</sup>
R-BT	14.1 $\pm$ 1.7 <sup>a</sup>	35.1 $\pm$ 11.8 <sup>b</sup>	22.1 $\pm$ 5.8 <sup>a</sup>	23.5 $\pm$ 7.1 <sup>a</sup>
SF	45.5 $\pm$ 1.8 <sup>a</sup>	69.5 $\pm$ 25.9 <sup>b</sup>	61.8 $\pm$ 14.5 <sup>b</sup>	91.8 $\pm$ 2.5 <sup>a</sup>
GAM	17.4 $\pm$ 0.01 <sup>a</sup>	30.8 $\pm$ 5.8 <sup>b</sup>	27.3 $\pm$ 2.8 <sup>a</sup>	16.3 $\pm$ 0.4 <sup>a</sup>

<sup>a</sup>t<sub>0-48</sub>, <sup>b</sup>t<sub>48-96</sub>

detected (Table 2). In addition, maximum doubling times in the <0.8  $\mu\text{m}$  D treatment yielded an interval of t<sub>0</sub> to t<sub>48</sub>, while in the <0.8  $\mu\text{m}$  D→M treatment doubling times were calculated for t<sub>48</sub> to t<sub>96</sub>. In contrast, the opposite transfer (M→D) accelerated doubling times of BET, GAM, and total bacterioplankton (Table 2), but doubling time of SF was much longer. Members of GAM and R-BT typically exhibited the shortest doubling times, and the R-BT group usually grew faster than BET (Table 2).

## DISCUSSION

During the course of the experiment, the 2 sites differed mainly in the concentrations of TP, DRP (Table 1), and chl *a*, all of which were markedly higher at Stn Middle. Concentrations of TP in the unfiltered water samples at t<sub>96</sub> at both stations were fairly similar to those measured inside the dialysis bags of the unfiltered treatments. These findings suggest that dialysis membranes were penetrable to P over the experimental period and that P supported growth of enclosed communities. In contrast, concentrations of DRP were obviously lower inside the dialysis membranes compared to surrounding environments at t<sub>96</sub>, particularly at Stn Middle (Table 1). Thus, the equilibrium of DRP concentrations between the enclosed assemblages and ambient environments might have been influenced by the reduced membrane permeability and altered DRP transport rates into the dialysis tubes, or by increased microbial DRP uptake kinetics inside the enclosures (cf. Posch et al. 2007), compared to supply processes driven by passive diffusion. However, temporal changes in bacterial abundance after removing the grazers in the <0.8  $\mu\text{m}$  treatments incubated at Stn Dam indicated no obvious effect of decreased P availability on bacterial growth.

Transplanting the <0.8  $\mu\text{m}$  treatment to Stn Middle yielded significantly decreased bacterial cell numbers

(Fig. 1) compared to increased BP rates (Fig. 2). It seems that total cell counts did not reflect the changes in BP as measured by the thymidine incorporation method, which is potentially due to the presence of rapidly growing bacteria with multiple genome copies or cells with larger genome size.

As in the eutrophic Římov reservoir, R-BT have also been found in the alpine Gossenköllesee (Pérez & Sommaruga 2006) and in the oligomesotrophic Piburgersee (Salcher et al. 2006). Commonly, R-BT formed a

substantial proportion of total BET. R-BT typically overgrew bacteria of other groups in the absence of predators but became mostly suppressed under enhanced grazing pressure (e.g. Šimek et al. 2005, Salcher et al. 2006). R-BT bacteria also exhibited the highest fractions of Leu+ cells of all bacterial groups examined during the clearwater phase (Horňák et al. 2006). Our data on bacterial composition and fractions of Leu+ cells of individual groups at t<sub>0</sub> are thus consistent with previous studies.

The fraction of Leu+ R-BT bacteria has been typically found to be stimulated by enhanced protistan grazing (Horňák et al. 2006) or by enrichment with algal-derived DOM (Pérez & Sommaruga 2006). Instead of changing the cell size and morphology toward grazing-resistant morphotypes, R-BT bacteria override the impacts of predation pressure by their ability to multiply fast (cf. Table 2) and seem to be well adapted to utilization of labile DOM of algal origin. However, in our study, both abundance and fractions of Leu+ R-BT cells markedly decreased in the presence of grazers and when transferred to Stn Middle (Fig. 3), while both parameters markedly increased after the transfer to Stn Dam (Fig. 4). It is likely that simultaneous effects of enhanced protozoan grazing and the massive cyanobacterial bloom suppressed the development of R-BT bacteria at Stn Middle.

Fractions of Leu+ SF cells were frequently ~20% (Figs. 3, 4). Members of the SF group seem to be more efficient in the uptake of high molecular weight compounds (Cottrell & Kirchman 2000). Leucine, commonly used as a general indicator for growth and metabolic activity measurements, thus may not be the most appropriate type of substrate for SF bacteria. Percentages of SF increased in all treatments at t<sub>96</sub> when transferred to Stn Middle (Fig. 3). It seems that under temporarily increased phytoplankton biomass related to the *M. aeruginosa* bloom, the SF group may dominate the bacterial community, although it typically accounts for 10 to 15% in the Římov reservoir. The

fraction of Leu+ GAM was clearly favored by the upstream transfer (Fig. 3), likely profiting from the increased availability of resources. Although GAM exhibited the opposite trend in the fraction of Leu+ cells than the 2 most prominent groups (BET and SF), GAM accounted for only 1 to 4% of total abundance, and thus it could hardly change the overall negative response of the entire bacterial community subjected to the *Microcystis* bloom.

Both transfers of the treatments along the longitudinal axis of the reservoir also had distinctly different impacts on doubling times of bulk bacterioplankton and of individual bacterial groups (Table 2). In the treatments incubated at Stn Dam, doubling times of total bacterioplankton and of the probe-defined subgroups fell fairly well into the range of group-specific doubling times observed in 8 manipulation experiments conducted in the Římov reservoir (Šimek et al. 2006). After transferring the treatments to the *Microcystis*-rich Stn Middle, however, doubling times of all bacterial groups were substantially prolonged (Table 2). In addition, pronounced increases in total bacterial numbers occurred from  $t_{48}$  to  $t_{96}$ , thus reflecting the suppressing effect of the upstream transfer. In contrast, in the treatments transferred to Stn Dam, doubling times of GAM and total bacteria were shorter, and members of the BET and R-BT groups showed comparable doubling times to those incubated *in situ*, except for the SF group (Table 2). These findings support the evidence of the possible negative effect of cyanobacteria on bacterial development and point to the possible recovery of the bacterial assemblage after transferring to Stn Dam, which was less affected by *Microcystis*.

In this study, we focused almost exclusively on abundance and fractions of Leu+ cells of free-living bacterioplankton. However, in the unfiltered treatments at  $t_0$ ,

5.6 and 20.8% of DAPI-stained bacteria were associated with phytoplankton in Stns Dam and Middle, respectively. The presence of these aggregates likely underestimated bacterial abundances, either because of difficult microscopic enumeration or because of complete elimination of the large particles in the  $<0.8$  and  $<5$   $\mu\text{m}$  treatments. Additionally, prefiltration through a 30  $\mu\text{m}$  filter used in all unfiltered treatments before the samples for CARD-FISH and microautoradiography techniques were processed might partially exclude these algal-associated bacteria from the analyses. Nevertheless, the vast majority of total bacteria (78 to 94%) were free-living cells, easily accessible to analyses.

We did not quantify the impact of viruses in our study, although this factor may significantly contribute to the specific removal of bacteria (e.g. Weinbauer & Höfle 1998). At  $t_{96}$ , abundances of VLPs ranged from 1 to  $3.5 \times 10^8 \text{ ml}^{-1}$  in all treatments at both stations. Interestingly, ratios between abundance of VLPs and bacterial abundance were stable in all  $<0.8$   $\mu\text{m}$  treatments (18 to 23), while they were significantly higher in  $<5$   $\mu\text{m}$  and unfiltered treatments (53 to 188), indicating increased viral activity in the presence of grazers (Weinbauer et al. 2003). Although VLP abundances were similar at both stations, the presence of cyanobacteria-related viruses was expected at Stn Middle.

The most important groups of zooplankton likely preying on protists in the unfiltered treatments were filter-feeding cladocerans (*Daphnia galeata*, *Diaphanosoma brachyurum*), copepods, and various rotifers (Table 3). Although our experimental setup using the dialysis bag enclosures might discriminate against certain zooplankton species, we did not observe any marked differences in zooplankton composition between the enclosures and initial reservoir sam-

Table 3. Zooplankton abundances (ind.  $\text{l}^{-1}$ ) at Stns Dam and Middle at the beginning of the experiment in reservoir water and at the end of the experiment in different unfiltered treatments (UN). Only those zooplankton species or groups with an abundance of  $>1$  ind.  $\text{l}^{-1}$  are shown

	Stn Dam $t_0$	UN treatment $t_{96}$		Stn Middle $t_0$	UN treatment $t_{96}$	
		D	D→M		M	M→D
<i>Daphnia galeata</i>	2	6	5	15	11	14
<i>Diaphanosoma brachyurum</i>	41	34	52	61	75	39
<i>Eudiaptomus gracilis</i> , adults	1	3	1	4	1	1
<i>Eudiaptomus gracilis</i> , copepodites	10	3	2	8	4	3
Adults of cyclops <sup>a</sup>	3	6	3	4	11	13
Copepodites of cyclops	17	18	13	10	8	5
Total nauplii	59	7	3	60	25	19
Rotifers <sup>b</sup>	152	141	170	196	241	186

<sup>a</sup>Adults of *Cyclops vicinus*, *Mesocyclops leukarti*, *Thermocyclops* sp., and *Acanthocyclops* sp.  
<sup>b</sup>*Keratella cochlearis*, *K. quadrata*, *Kellicotia longispina*, and *Polyarthra* sp.

ples over the experimental period, suggesting comparable levels of zooplankton grazing in these treatments.

Potential cyanobacterial effects on bacteria may also be related to the release of toxic MCs and other compounds (Casamatta & Wickstrom 2000). Dissolved MCs have been commonly found in many reservoirs of the Czech Republic, mostly in the range of 2 to 20  $\mu\text{g l}^{-1}$ , and *Microcystis* spp. were recognized as the most important taxa producing MCs (e.g. Bláha & Maršálek 2003). In the Římov reservoir, 3 to 5  $\mu\text{g}$  of dissolved MCs  $\text{l}^{-1}$  were found at Stn Dam just after the experiment (Znachor et al. 2006). Free MCs may, due to their small size (~1 kDa), easily penetrate through dialysis membranes and directly affect the enclosed bacterioplankton community (e.g. the fractions of metabolically active cells; K. Horňák unpubl. data) subjected to these supposedly toxic compounds.

Some members of the *Alphaproteobacteria* (ALF) belonging to the microcystin-degrading bacteria have often been found in the mucilage of *Microcystis* spp. (e.g. Maruyama et al. 2003). These bacteria may thus profit in the presence of a *Microcystis* bloom. We did not determine the proportions of ALF in our study, since they typically account for a negligible proportion in the total community in the Římov reservoir, likely being below the detection limit of CARD-FISH. Moreover, BET and SF groups covered the vast majority of total free-living bacteria determined with the EUB338 I–III probe mixture (EUB). Although the fractions of EUB cells (65 to 78% of total DAPI-stained bacteria) were in the range of values repeatedly reported for the Římov reservoir (e.g. Šimek et al. 2006), we could have underestimated the real abundances of hybridized cells likely due to the presence of the tiny detrital particles, unspecifically stained by DAPI, occurring particularly at Stn Middle.

In summary, the combination of environmental conditions related to the presence of the *Microcystis aeruginosa* bloom at Stn Middle negatively affected total bacterial cell numbers and biomass as well as doubling times of bulk bacterioplankton and of individual genotypic groups, while the treatments incubated at the *Microcystis*-poor Stn Dam revealed the opposite trends. In comparison to a previous study conducted in the Římov reservoir during the clearwater phase (Horňák et al. 2006), both the abundance and fractions of Leu+ *Betaproteobacteria* and of its R-BT065 cluster, typically accounting for the majority of total bacteria, were strongly suppressed by the presence of grazers and the *Microcystis* bloom. In contrast, members of the *Sphingobacteria-Flavobacteria* lineage numerically dominated the bacterial community under these specific conditions. The fraction of Leu+ *Gammaproteobacteria* was stimulated by the upstream

transfer to the P-rich Stn Middle, but it comprised only very small proportions of the total community. Our study thus suggested group-specific responses of bacteria to the temporary cyanobacterial bloom event.

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