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Changes in bacterial community composition and microbial activities along the longitudinal axis of two canyon-shaped reservoirs with different inflow loading

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Abstract

Changes in microbial activities and bacterial community composition (BCC) were studied using fluorescence *in situ* hybridization (FISH) along the longitudinal axis of two canyon-shaped reservoirs with contrasting retention times and nutrient and organic matter loads. The reservoirs showed different specific longitudinal changes in plankton communities and BCC shifts that were related mainly to allochthonous input and longitudinal patterns of primary production and nutrient availability. The studied branch of the Orlík reservoir is entered by a large river and so had a short retention time (23 days) and a large allochthonous input. Primary production, bacterial abundance, bacterial production and protozoan bacterivory showed a consistent trend, decreasing gradually from the river inflow downstream. Overall, shifts in BCC were minor and statistically insignificant. The relatively large input of the river-borne allochthonous plankton communities and the short retention time of the reservoir probably limited the effect of the autochthonous in-reservoirs processes. In contrast, the Římov reservoir has a long retention time (∼100 days) and had a limited input of allochthonous organic matter to its lacustrine area. The autochthonous processes of primary and bacterial production were tightly linked and they showed peaks in the middle part of the studied stretch. This together with a trend of downstream decreasing nutrient concentrations and increasing protistan grazing pressure seem to support the development of specific BCC in the lacustrine part of the reservoir that significantly differed from that in river inflow. Apart from the marked differences between the limnological variables of reservoirs we found some commonalties in BCC – in both systems the phylogenetic groups of β-Proteobacteria and *Cythophaga/Flavobacterium* clearly dominated bacterioplankton assemblages.

Introduction

Compared to marine ecosystems and lakes of different trophic status and morphology (e.g., Pomeroy, 1974; Azam et al., 1983; Riemann & Sondergaard, 1986), much less attention has been paid to the role of pelagic microbial food webs in reservoirs and rivers.

There are several important differences between lake and reservoir ecosystems, these differences are most pronounced in canyon-shaped reservoirs, which are spatially highly heterogeneous because of their relatively short retention times and longitudinal heterogeneity (Thornton et al., 1990; Straškraba, 1998). The biogeochemical processes that occur in the upper parts of canyon-shaped reservoirs are not yet fully un-

derstood. For example, in the upper parts of highly eutrophic reservoirs, primary producers often have a very limited role as water is relatively turbid (Hejzlar & Vyhnálek, 1998; Comerma et al., 2001). Most matter transformation and nutrient cycling thus occur through enhanced activities of heterotrophic microbial communities (Armengol et al., 1999; Comerma et al., 2001; Šimek et al., 2001a).

A continuous supply of substrates by a river and high growth rate of microorganisms result in a clear gradient of nutrient and substrate concentration and microbial succession downstream (Hejzlar & Vyhnálek, 1998; Armengol et al., 1999; Kalff, 2002). In the upper, riverine part of canyon-shaped reservoirs the main source of carbon that fuels microbial processes is the allochthonous organic matter from the river inflow, while downstream, during transition to lacustrine areas, the autochthonously produced organic carbon by phytoplankton becomes more important.

Differences in water temperature and chemical composition between the river inflow and the lacustrine part of a reservoir determine how much of the inflow river water mass mixes with the reservoir epilimnion, which in turn affects plankton community development. For instance. in summer the lacustrine area is clearly separated from the riverine one by a short transient zone where inflowing water plunges to the deeper strata of the reservoir.

The pattern of longitudinal succession of phytoplankton and zooplankton communities in reservoirs have been known for some time (e.g., Urabe, 1989; Pinel-Alloul, 1995). Recently studies appeared that mapped longitudinal changes in microbial food web in relation to other biological and chemical variables (Comerma et al., 2001; Šimek et al., 2001a; Gasol et al., 2002). These studies were carried out in the highly eutrophic canyon-shaped Sau reservoir in NE Spain, with well developed longitudinal gradients in particulate organic and inorganic matter and nutrient concentration that probably drive the marked longitudinal succession of plankton food webs.

The upper part of the reservoir has a high input of allochthonous (easily decomposable) organic material, low oxygen saturation of water and almost no large metazooplankton (Armengol et al., 1999; Comerma et al., 2001). This implies that heterotrophic microbial processes dominated nutrient cycling and organic matter transformation. Detailed studies on protistan bacterivory conducted in the Sau reservoir showed that the communities of protistan grazers displayed a clear longitudinal pattern: bacterivory of heterotrophic nanoflagellates dominated the upper inflow part of the reservoir while ciliates (mainly due to small oligotrichs) were the most important bacterivores in the lacustrine area (Šimek et al., 1999a; Comerma et al., 2001).

It has been widely accepted that predators can change bacterial community composition (BCC) by feeding on larger, more active cells (e.g., delGiorgio et al., 1996) and/or specific groups of bacteria (Šimek et al., 1997; Jürgens et al., 1999; Pernthaler et al., 2001). The important role of protistan bacterivory in shaping longitudinal succession of BCC was confirmed also in experiments conducted in the Sau reservoir (Šimek et al., 1998; Gasol et al., 2002). The important impacts of likely microzooplankton grazing on shaping BCC was confirmed also in the study by Lindström (2000) in five lakes with different trophic status. BCC may also be affected by nutrient supply (e.g., Pace & Cole, 1994; Thingstad & Lignell, 1997; Simek et al., 2003, in press) or by host-specific viral lysis (e.g., Fuhrman, 1999). *In situ* hybridization with oligonucleotide rRNA-targeted probes and finger printing techniques – DGGE (Lindström, 2000) or T-RLFP – have brought information about BCC in freshwater systems ranging from oligotrophic (e.g., Alfreider et al., 1996; Pernthaler et al., 1997), through oligomesoeutrophic (Methé et al., 1998; Glöckner et al., 1999; Jrdillier et al., submitted), to eutrophic lakes (Šimek et al., 1999a,b, 2001a).

Canyon-shaped eutrophic reservoirs are the most likely to show dramatic changes in BCC along their longitudinal axes in the riverine to lacustrine transitional zones due to marked spatial changes in chemical and biological parameters, nutrient and substrate availability and, consequently, in the development of pelagic grazers (Šimek et al., 2001a; Gasol et al., 2002).

To our knowledge, however, there is no study aimed at a specific comparison of the longitudinal changes of microbial processes and BCC that compares canyon-shaped reservoirs with different nutrient and organic matter loads from river inflow. In the present study, we have analyzed the changes in BCC, bacterial biomass and production, protistan bacterivory and primary production along the longitudinal axes of two canyon-shaped reservoirs in the Czech Republic, of different trophic status and with quite distinct inflow water parameters. We analyzed planktonic communities in the meso-eutrophic Římov reservoir in five sampling campaigns in the season 1999, and in the eutrophic Orlík reservoir in four sampling campaigns

Table 1. The major background limnological data of the Orlík and Římov reservoirs. The data represent mean value of four and five longitudinal sampling campaigns in the Orlík and Římov reservoirs, respectively

	Orlík reservoir		Římov reservoir	
	River	Station 4*	River	Dam
Total inorganic N (mg l^{-1})	1.27	1.23	1.58	1.45
Total P (μg l^{-1})	220.5	74.8	72.9	26.1
Dissolved reactive P (μg l^{-1})	61.4	21.7	35.5	1.77
Dissolved organic C (mg l^{-1})	6.68	6.18	5.01	5.61
Chlorophyll *a* (μg l^{-1})	102	31.4	10.3	12.3
Primary production (μg l^{-1} h^{-1})	179	65.4	9.73	19.0

*Corresponds to the end of the Vltava branch of the Orlík reservoir.

in the season 2000. The following questions should be answered: (i) Is the bacterial community in both reservoirs dominated by the same microbial phyla? (ii) Can the different nutrient load cause a different longitudinal pattern of pelagic microbial communities? (iii) Does bacterial assemblage in both reservoirs differ between their riverine and lacustrine parts? (iv) Which other factors, besides nutrient and organic carbon loads, can significantly influence longitudinal pattern of pelagic microbial communities?

Methods

Study sites and sampling scheme

We studied two canyon-shaped reservoirs with contrasting primary production, organic matter and phosphorus loads in the river inflow (see Table 1):

1. The meso-eutrophic Římov reservoir (South Bohemia, 470 m a.s.l., area 2.06 km^2, volume 34.5×10^6 m^3, maximum depth 43 m, mean depth 16.5 m, mean retention time 100 days, dimictic) is 13.5 km long, canyon-type impoundment located in the middle stretch of the Malše River. Water samples were collected at six sampling points along the longitudinal axis of the reservoir at the river inflow (R), then at four points downstream (stations 1–4, the precise position of which depended on the type of water stratification and the position of the plunge point) and at the dam of the reservoir (D). During five sampling campaigns in 1999 (8 April, 5 May, 25 May, 10 August, and 14 September) samples were taken from a boat with a 2-l Friedinger sampler (seven samples from each station were mixed in a 20-l plastic container) from epilimnetic layer (0.5-m depth) water samples were collected from all sampling stations.

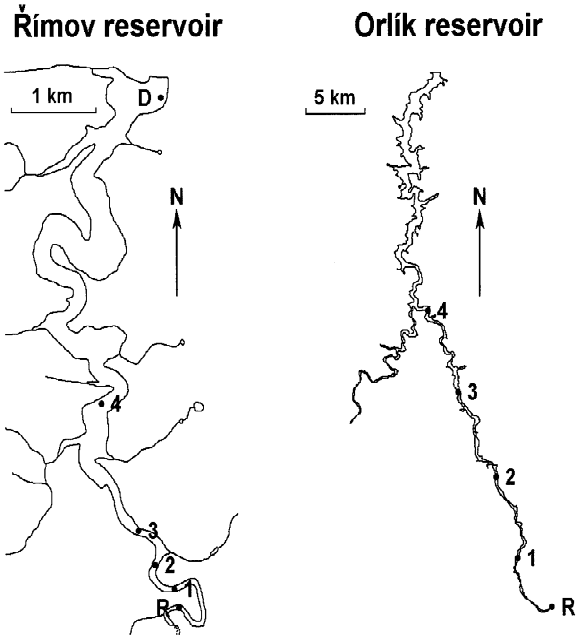

Figure 1. The rough map of the Orlík and Římov reservoirs showing the positions of the sampling stations.

2. The eutrophic Orlík reservoir consists of two large V-oriented branches (the Vltava and Otava Rivers) that merge in the middle of the reservoir. For this study, only the upper Vltava River branch of the reservoir was studied (see Fig. 1, located in South Bohemia) with the following major parameters: 354 m a.s.l., area 27.3 km^2, volume 715×10^6 m^3, maximum depth 74 m, mean depth 26.2 m, mean retention time 23 days, dimictic. This 30-km long,

deep-valley branch of the reservoir is located in the middle stretch of the river Vltava. The same sampling device and a similar sampling strategy as described above for the Římov reservoir was used. However, water samples were collected at only five sampling points along the longitudinal axes of the reservoir: the Vltava River inflow and four fixed points situated roughly equidistantly (~7.5 km) downstream (stations 1–4). Station 4 was situated just above the confluence of both reservoir branches. Samples were collected four times in 2000 (3 May, 27 June, 8 August, 26 September) from the epilimnetic layer.

Bacterial abundance and production

Duplicated subsamples were fixed with formaldehyde (2% final concentration), stained with DAPI (final concn 0.2% wt/vol), and counted by epifluorescence microscopy (Olympus BX 60). Bacterial production was measured from the method of thymidine incorporation, modified from Riemann & Søndergaard (1986). Duplicate 5-ml subsamples were incubated for 30 min at *in situ* temperature with 10 nmol l^{-1} of [*methyl*-^{3}H]thymidine (Amersham), then preserved with neutral buffered formaldehyde (2% final concentration, v/v), filtered through 0.2-μm membrane filters (Poretics) and extracted 10 times by 1 ml of ice-cold 5% TCA. Replicate blanks prefixed by 2% formaldehyde were processed in parallel. We used a mean empirical conversion factor (2.15×10^{18} cells mol^{-1} thymidine) to calculate bacterial cell production rate from thymidine incorporation rate. This conversion factor was previously determined for the Římov reservoir using data from three different experiments where bacterivore-free treatments were incubated in dialysis bags directly in the reservoir (for details see Šimek et al., 1999b, 2001b). The cell production rate was calculated from the slope of the increase of ln bacterial abundance over time (0, 24, 48 h). A theoretical conversion factor of 2×10^{18} cells mol^{-1} thymidine incorporated was used for the data from the Orlík reservoir.

Fluorescence in situ *hybridization of bacteria with group specific oligonucleotide RNA-targeted probes*

An analysis of bacterial community structure was carried out by fluorescence *in situ* hybridization (FISH) with oligonucleotide probes on membrane filters (Amann et al., 1995; Alfreider et al., 1996). Reservoir samples of water were prefixed with alkaline Lugol's solution followed by formaldehyde (2% w/v, final concentration) for at least 1 h, and decolorized by addition of several drops of a 3% sodium thiosulphate solution to prevent cell disruption of fragile planktonic algae. Bacterial cells from 10–20-ml subsamples were concentrated on white 0.2-μm pore-size filters (Poretics Corp., 47-mm diameter), rinsed with distilled water and stored frozen at −20 °C until further processing. FISH of filter sections with six oligonucleotide probes targeted to the kingdom *Bacteria* (EUB338), the α-, β-, γ-subclasses of Proteobacteria (ALF, BET42a, GAM42a), to the Cytophaga/Flavobacterium group (CF319a), and to a small subcluster of the β-Proteobacteria (R-BT065, for the specific targets of the sequence and the nucleotide sequence accession number see Šimek et al., 2001b) was carried out as described in Alfreider et al. (1996). The probes were fluorescently labelled with the indocarbocyanine dye Cy3 (Interactiva, Ulm, Germany). After hybridization, the filter sections were stained with 4′,6-diamino-2-phenylindole (DAPI), and the percentage of hybridized bacterial cells counted by epifluorescence microscopy was determined. At least 500 DAPI-stained cells per sample were inspected. The mounting medium Citifluor (Citifluor Ltd., Kent, UK) was amended with about 20% of VectaShield (Vector Lab., Burlingame, CA, U.S.A.). This modification resulted in significantly reduced fading of the probe signal (Pernthaler, pers. comm.).

Protozoan grazing and abundance

To measure protozoan grazing upon bacterioplankton, we used fluorescently labeled bacterioplankton concentrated from the reservoir (FLB, for more details see Šimek et al., 1999b). For grazing experiments, 250 ml of samples were dispensed into acid soaked and rinsed 0.5-l flasks and incubated at *in situ* temperature. Flagellate and ciliate uptake rates were determined in the same treatment where FLB added constituted 5–15% of bacterial natural abundances. Forty-ml subsamples for protozoan counting and tracer ingestion determinations were taken at 0, 5, 10, 15, 20 and 30 min after tracer addition and fixed by adding 0.5% of alkaline Lugol's solution, immediately followed by 2% borate-buffered formaldehyde (final concentration) and several drops of 3% sodium thiosulphate to clear the Lugol's colour (Sherr & Sherr, 1993). We determined ciliate grazing rate in time series from 5 to 15 min subsamples and flagellate grazing rate in

subsamples from 10 to 30 min, respectively (for details see Šimek et al., 1999b). Five-ml (flagellates) or 20–30-ml (ciliates) subsamples were stained with DAPI, filtered through 1-μm black Poretics filters, and inspected via epifluorescence microscopy. Non-pigmented, heterotrophic nanoflagellates (HNF) and phytoflagellates were always differentiated. At least 50 ciliates and 100 flagellates were inspected for FLB ingestion in each sample. To estimate total protozoan grazing, we multiplied the average uptake rate of ciliates and flagellates by their *in situ* abundances.

Primary production

Primary production was measured with the ^{14}C-method. Water samples were incubated *in situ* (two light and two dark bottles at each depth) for ~4 h. Each bottle (volume of about 120 ml) received 0.1–0.2 MBq of carrier-free [^{14}C]bicarbonate (final concentration <10 μg C l^{-1}). The assimilated ^{14}C was fractionated using a combination of filtration and acidification methods (for details see Straškrabová et al., 1999) into the following fractions: (i) >2 μm, algae, (ii) 0.2–2 μm, bacteria, (iii) <0.2 μm, dissolved organic carbon. The gross primary production was calculated as A+B+C, the net primary production was assumed to be equal to A. To obtain carbon fluxes (μg C l^{-1} h^{-1}), the rates of ^{14}C incorporation into each fraction (in % h^{-1} of the added inorganic ^{14}C) were multiplied by the DIC concentration calculated from alkalinity and pH.

Other methods

Chl *a* concentrations in prescreened water (100 μm) were determined after filtration through Whatman GF/C filters. Filters with retained seston were ground, extracted in 90% acetone, and measured spectrophotometrically after the method of Lorenzen (1967). Total phosphorus (TP) was determined colorimetrically after mineralization with nitric and perchloric acids (Borovec & Hejzlar, 2001). Dissolved reactive phosphorus (DRP) concentrations were determined according to Murphy & Riley (1962). Dissolved organic carbon (DOC) was determined in filtered samples (0.4 μm, glass-fiber filters MN-5, Macherey Nagel, Germany) with LiquiTOC analyzer (Foss-Heraeus, Germany). NH_4-N, NO_3-N, NO_2-N were analyzed using ion chromatography (Thermo Separation Products, U.S.A.). Samples for ion chromatography were frozen (-20 °C) and analyzed between 12 h and 1 month after sampling.

Results

Background limnology of the reservoirs

Table 1 shows the major limnological data of the river inflow and the downstream parts of the reservoirs averaged over the whole study period. The major differences between the reservoirs were: (i) Orlík had a significantly larger river-borne allochthonous input of primary producers and a generally higher trophic status, indicated by a markedly higher concentration of total phosphorus (TP) and dissolved reactive phosphorus (DRP), chlorophyll-*a* and primary production (Table 1). (ii) The latter variables had their maxima in the river inflow, but decreased only moderately downstream. There was thus little evidence that primary production was limited by DRP, even at station 4 in the Orlík reservoir. (iii) In contrast, near the Římov dam DRP concentration was about one order of magnitude lower than that in the river inflow. Despite this, primary production and chlorophyll-*a* concentration were higher near the dam, indicating the dominant role of autochthonous phytoplankton production in the lacustrine area of the Římov reservoir. Interestingly, neither total inorganic nitrogen nor dissolved organic carbon showed marked differences between the reservoirs and a clear longitudinal gradient within a reservoir.

Changes in microbial dynamics and community composition in the reservoirs

In the Orlík reservoir, the four longitudinal samplings yielded variable patterns. Primary production and the proportion of bacterial production consumed by protists usually peaked at the river inflow and decreased downstream (Fig. 2). For both parameters there was tendency to a secondary increase towards the station 4. Bacterial abundance and production changed downstream from the river inflow in a variable manner, generally, there was a decrease (Fig. 2) from the river inflow towards the station 4.

The data from 3 May (the late stage of a spring phytoplankton peak and the beginning of the clear water stage) deviated from the other sampling dates. The maximum of bacterial abundance and production occurred in the middle of the stretch (downstream from station 2), while primary production showed the typical trend decreasing downstream (Fig. 2) and protists consumed a lower proportion of bacterial production compared to summer months.

In the Orlík reservoir, that has a very short mean retention time (23 days, calculated only for the Vltava branch), the changes in bacterial community composition (BCC) showed no clear longitudinal pattern (Fig. 3). Typically, our FISH detection limit with the universal EUB338 probe ranged between ∼35% and>60% of total DAPI-stained bacteria and mostly the BET42a and CF319a lineages dominated the community. It is perhaps worth noting the higher proportion of GAM42a (June, August) and ALF968 (September) lineages in the whole transects.

In the Římov reservoir, we analyzed samples from five longitudinal sampling campaigns conducted between April and September 1999. They yielded very different patterns (Figs 4 and 5) from those observed in the Orlík reservoir (cf. Figs 2 and 3). Bacterial production, abundance and primary production in Římov were mostly lowest at the river inflow and highest between stations 1 and 2 (see 5 May, 10 August and 26 September). The largest proportion of bacterial production removed by protists was consistently found at the downstream lacustrine parts of the reservoir (stations 3, 4 and 'D') throughout the whole season. In contrast to this general pattern, marked differences were found for the longitudinal transect conducted during the spring mixing period (8 April). We observed a downstream increase in bacterial abundance and primary production that peaked at the dam area (Fig. 4), while maximum bacterial production was at station 2 (Fig. 4). Data from 25 May also did not closely follow the previously described general pattern: bacterial abundance, production and primary production showed additional peaks at the dam area that spatially coincided with the maximum value of chlorophyll-*a* concentration (data not shown).

In Římov, that had a longer mean retention time (∼100 days), changes in BCC mostly displayed a clear longitudinal pattern (Fig. 5). With a few exceptions (see, e.g., the CF319a lineage in the May transect), members of BET42a and CF319a lineages showed the highest proportions at the river inflow, usually followed by a dramatic drop towards the station 1, and then they gradually decreased towards the reservoir dam. The proportion of bacteria targeted by the probe EUB338 ranged between 56 and 83% of total DAPI-stained bacterial cells (Fig. 5), peaking either in the inflow (station R – May, August and September) or in the upper part of the reservoir (station 2 or 3 – April and May transects). Surprisingly, almost no trend was observed for the small cluster of β-Proteobacteria targeted by probe R-BT065. The proportions of bacteria hybridizing with ALF968 and GAM42a probes were consistently low (<2%, not shown), and they thus could not be used for any statistical treatment.

Significant differences in bacterial community composition between the reservoirs

Differences in the contributions of various phylogenetic groups of bacteria to total bacterial counts were tested: (i) between the reservoirs for the whole data sets regardless of the longitudinal position of sampling points, (ii) between the riverine and lacustrine parts of the two reservoirs (Table 2), and (iii) within each reservoir, i.e., the riverine versus the lacustrine section (Table 3). The differences in the contributions of the EUB338, BET42a and CF319a lineages to total DAPI-stained bacteria were significant in the comparison of reservoirs across the whole data set (see Mann–Whitney test, Table 2). Moreover, the BET42a and CF319a lineages were consistently higher and differed significantly in relative proportions between the inflow to the Římov reservoir and the Vltava river inflow to Orlík (Table 2). Finally, when data were tested within a reservoir, no significant differences between the river inflow and the station 4 of Orlík were found for either of the phylogenetic lineages tested, while in the Rimov reeservoir the contributions of all but GAM42a lineages were significantly different in the river inflow compared to the dam area.

Discussion

Differences in the major limnological parameters between the reservoirs

The major features of the longitudinal changes in limnological parameters can be summed up as follows:

1. The moderately P-limited Římov reservoir showed longitudinal changes in bacterial production, which were tightly linked to the changes in predominantly autochthonously produced primary production (Fig. 6), peaking usually in the first lacustrine stations 1 and 2 (Fig. 4). This was likely related to the growth potential and community composition of typical river-borne algae (diatoms), growing mostly surface-bound to the shallow river bottom, moreover at lower river temperature and under light-limited conditions, caused by a shelter produced by a tall forest growing on both river banks.

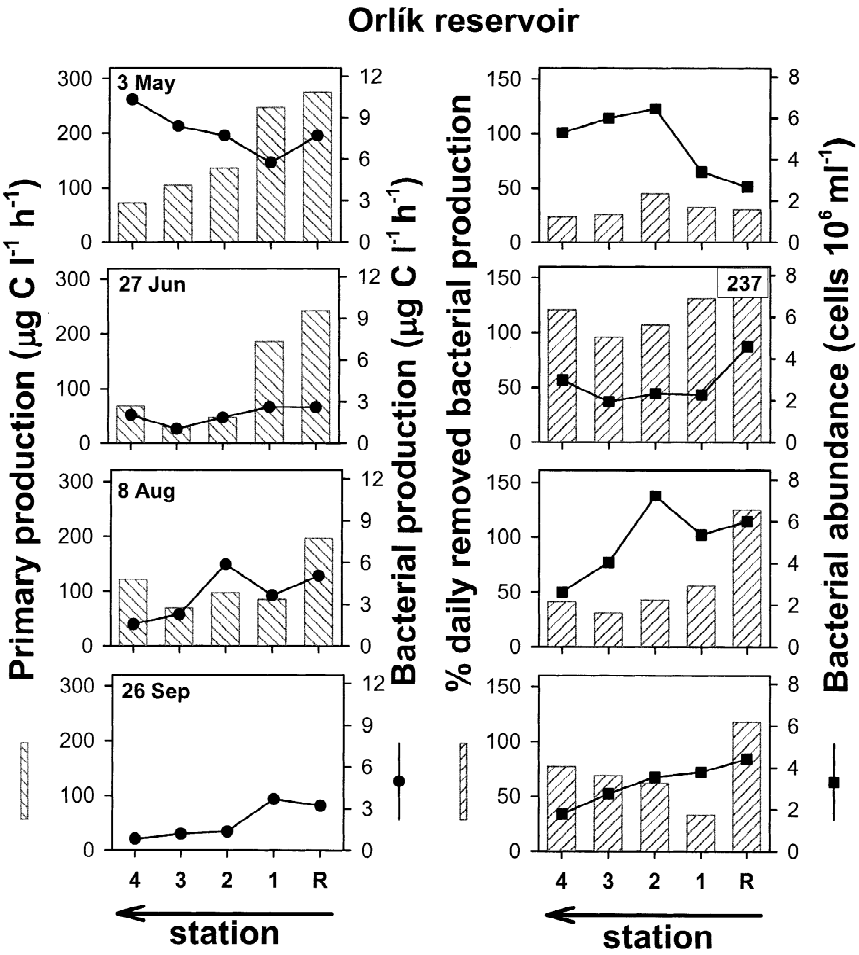

Figure 2. The Orlík reservoir – longitudinal changes in primary production (not determined on 26 September) and bacterial production (left panels), in the proportion (%) of bacterial production daily removed by protists and bacterial abundance (right panels).

Table 2. The comparison between the Orlík and Římov reservoirs – statistical analyses of differences in proportions (%) in total DAPI-stained bacteria of the cells targeted by the probe EUB338, BET42a, CF319a, the Mann–Whitney test of means of two replicate counts. Probability values are shown for the tests of all data from all sampling sites in both reservoirs (a) and for sampling points Orlík-River versus Římov-River and Orlík-Station 4 versus Římov-Dam (b). $^{*}P<0.05$, $^{**}P<0.01$

(a)	Probe	Orlík (n=20) versus Římov (n=25)	
Surface	EUB338	P=0.0317*	
	BET42a	P=0.0317*	
	CF319a	P=0.0317*	
(b)		Orlík-River (n=4) versus Římov-River (n=5)	Orlík-Station 4 (n=4) versus Římov-Dam (n=5)
Surface	BET42a	P=0.0159*	P=0.556
	CF319a	P=0.0159*	P=0.905

Table 3. A comparison of the inflow part and downstream part of both reservoirs, i.e., River versus Station 4 in the Orlík and the River versus Dam stations in the Římov. Statistical differences in the proportions (% of total DAPI-stained bacteria) of the cells targeted the respective oligonucleotide probes in the surface layer. Tested were means of replicate counts, the Paired *t*-test. Nd – not determined data. $^{*}P<0.05$, $^{**}P<0.01$

	Probe	Orlík (n=4) River versus Station 4	Římov (n=5) River versus Dam
Surface	EUB338	P=0.266	P=0.039*
	BET42a	P=0.417	P=0.0036**
	CF319a	P=0.233	P=0.0016**
	R-BT065	Nd	P=0.076
	GAM42a	P=0.899	Nd

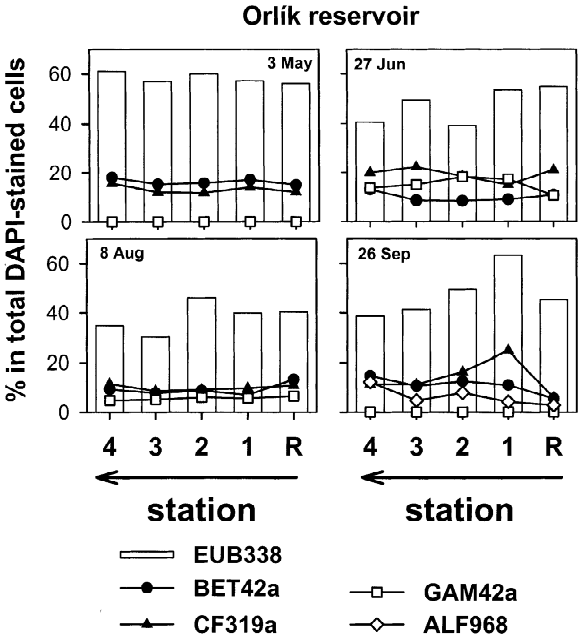

Figure 3. The Orlík reservoir – longitudinal changes in the proportions (%) of the bacteria targeted by the probe EUB338, BET42a, GAM42a, ALF968 (only on 26 September above the detection limit) and CF319a.

2. Orlík (Vltava river branch) had five-times shorter retention time than the Římov and no substantial differences in water temperature between the river inflow and the main body of the reservoir. The short retention time with no additional nutrient input at the lower parts yielded gradual decreases of the studied parameters (in particular primary production) from the maximum in the river inflow downstream towards the station 4. Overall, it indicated the dominant role of allochthonous plankton communities brought by the river mainly from a smaller upstream reservoir loaded by nutrients from densely populated upstream parts of watershed.

In the Sau reservoir used for comparison in Figure 6 (with the retention time 90–100 days, comparable to Rimov), the high allochthonous organic matter load supported the dominance of heterotrophic processes and low water transparency in its inflow parts (cf. Comerma et al., 2001, Šimek et al., 2001a), yielding thus an apparent uncoupling between PP and BP processes in the upper half of the reservoir.

Factors shaping the dynamics of microbial communities

Overall, our study and the available literature data (Armengol et al., 1999; Šimek et al., 1999a, 2001a), clearly indicate that reservoir morphology, retention time, and quality of river water, especially the grazing-induced bacterial mortality can significantly contribute to the establishment of the typical longitudinal patterns of bacterial parameters in the studied reservoirs.

There have been detailed studies of bacterivory of heterotrophic nanoflagellates (HNF) and ciliates (e.g., Šimek et al., 1995, the Římov reservoir) in the Římov and Orlík reservoirs (Šimek et al., 1999a; Jezbera et al., 2003), as well as in the similarly sized canyon-shaped Sau reservoir (Šimek et al., 1999a, 2001a; Comerma et al., 2001). The results of these studies can be summarized as: (i) along with the increasing trophic status of the reservoirs there is an increasing role of ciliate bacterivory compared to HNF bacterivory, and (ii) there is an overall gradient in the role of total protistan bacterivory accounting, on average,

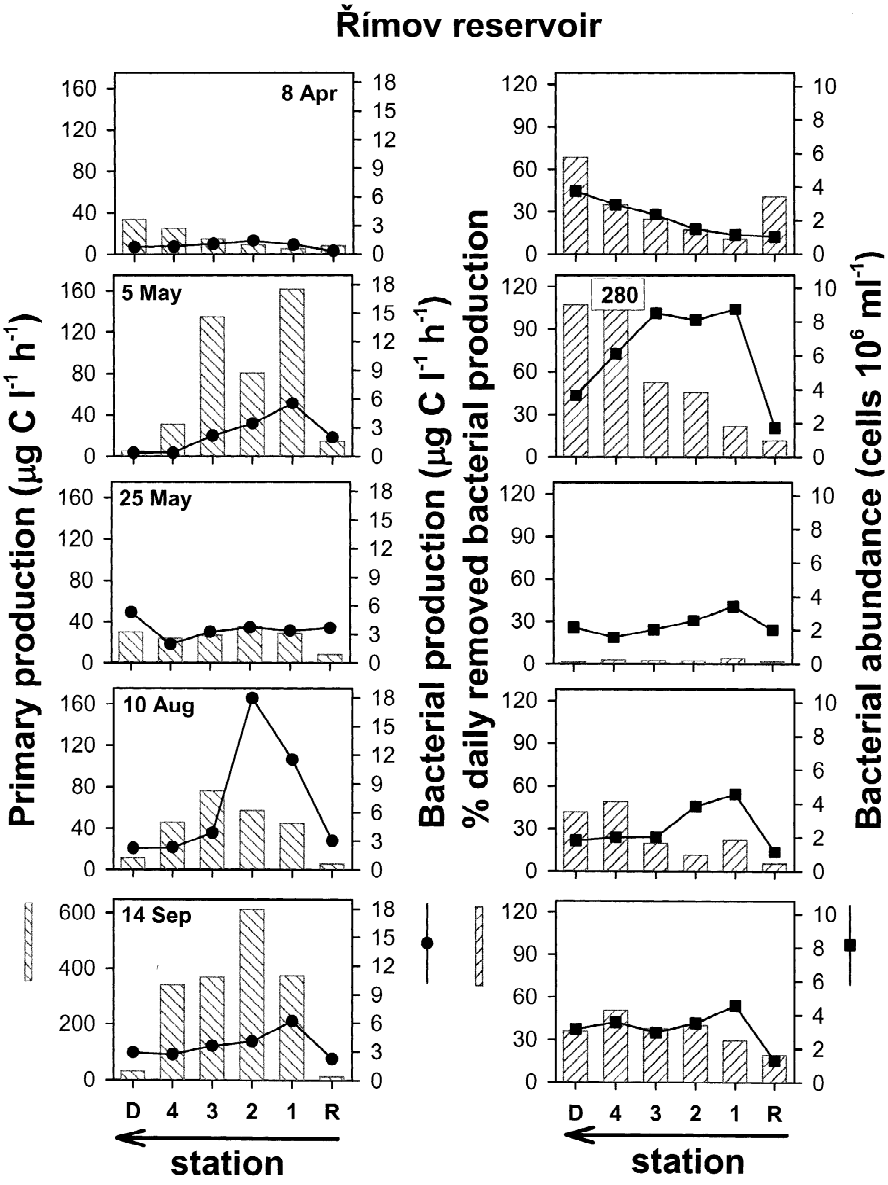

Figure 4. The Římov reservoir – longitudinal changes in primary production and bacterial production (left panels), in the proportion (%) of bacterial production daily removed by protists and bacterial abundance (right panels). Note different scale for primary production 14 September.

for 35, 70 and 70–90% of bacterial production in the Římov, Orlík, and Sau reservoirs, respectively. However, all these reservoirs showed specific longitudinal trends and seasonal variability of protistan dynamics and bacterivory (see, e.g., Figs 2 and 4 in this study; Jezbera et al., 2003).

Thus, in the Římov reservoir, bacterial production (BP) peaked, except for 8 April (Fig. 4), in the middle or upper parts of the reservoir, while the maximum of protistan bacterivory was shifted closer to the reservoir dam. A rather limited development of protistan populations in the upstream parts of the reservoir (for details see Jezbera et al., this issue) spatially coincided with enhanced abundances of zooplankton, namely of daphnids, but also of copepods and rotifers (Sed'a, pers. comm.). Strong grazing impacts of zooplankton on protozoan populations have been frequently reported for lakes (for review, see Jürgens, 1994), but they were also specifically documented for the Římov reservoir during its clear water phase (Šimek et al.,

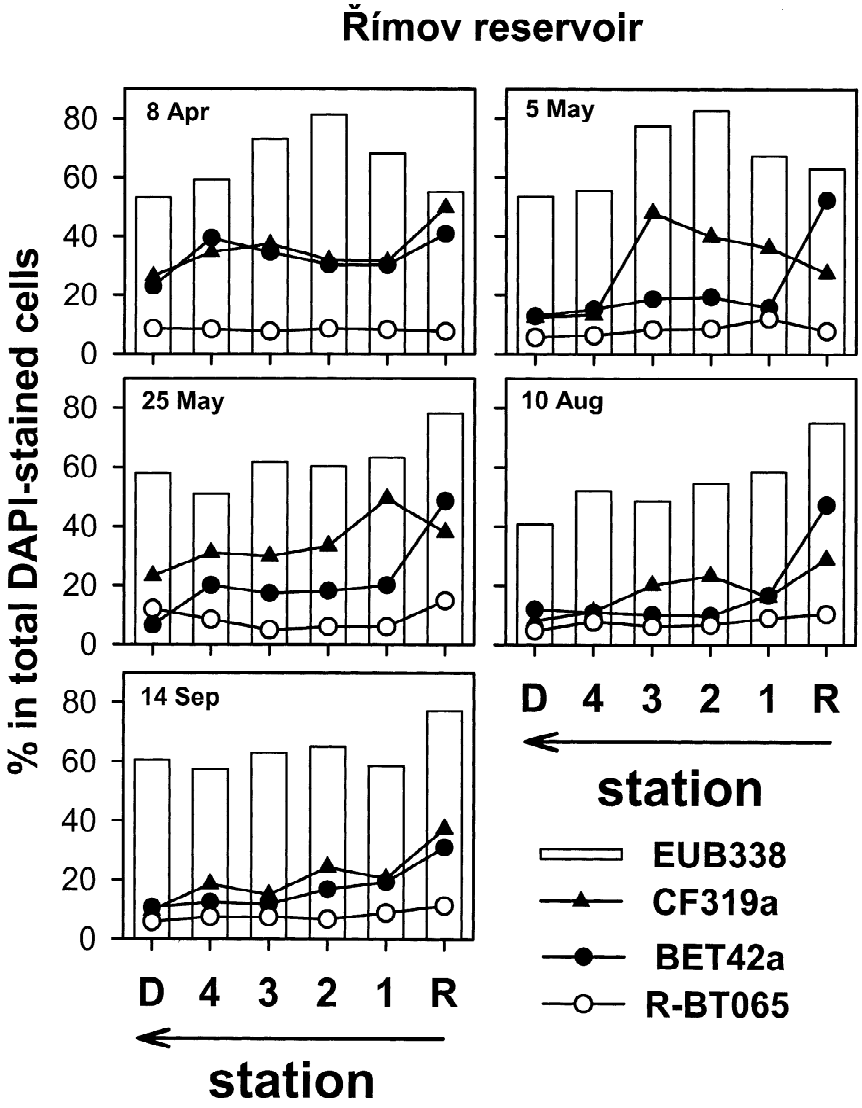

Figure 5. The Římov reservoir – longitudinal changes in the proportions (%) of the bacteria targeted by the probe EUB338, BET42a, R-BT065 and CF319a.

1990). In our present study we did not conduct any direct measurements of zooplankton bacterivory or its grazing on protistan populations.

In the Orlík reservoir, with a few exceptions, the values of BP and protistan bacterivory showed a roughly similar downstream decreasing trend. It indicated the major role of a large inoculum of bacterivorous protists (both HNF and ciliates, Jezbera et al., 2003) in the control of allochthonous BP both likely originating from the upstream placed reservoir. The short reservoir retention time and well-pronounced longitudinal trophic gradient of Orlík (resulting from the high allochthonous input) yielded a distribution of zooplankton abundance with the maximum in the inflow part (Sed'a, pers. comm.) followed by a downstream decrease resembling the typical patterns found for microbial and phytoplankton parameters (cf. Figs 2 and 6).

The data from 25 May 1999 (Římov) and 3 May 2000 (Orlík) displayed a certain deviation from the general trends described above (see also Fig. 6). The timing of these campaigns fell into the late stage of the spring phytoplankton bloom and early clear water phase in both reservoirs when the highest BP values

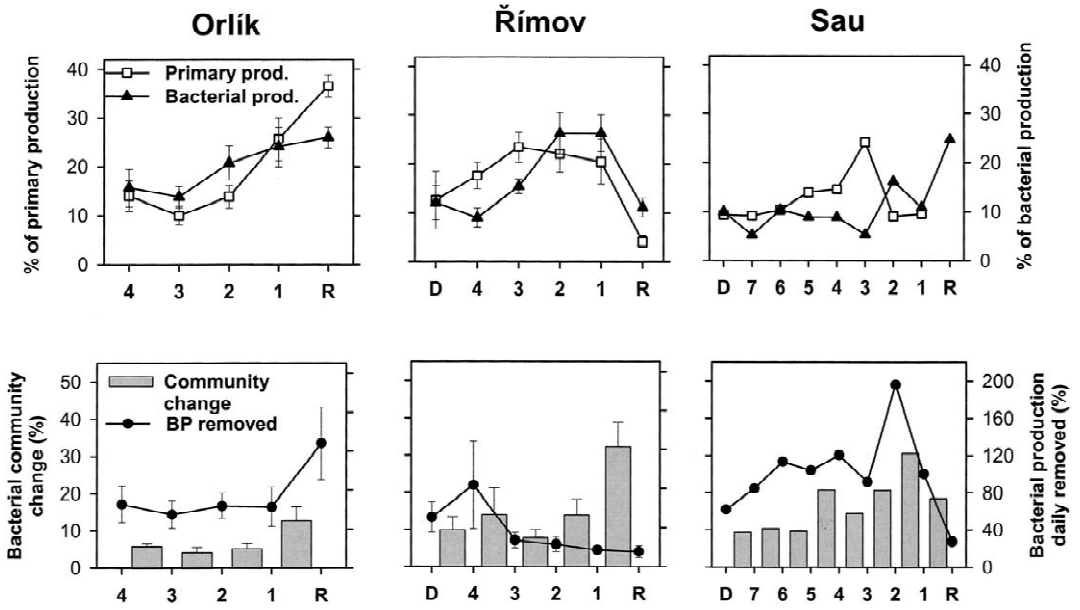

Figure 6. The mean values of primary and bacterial production showed as percent of the summary value along whole longitudinal profile (upper panels) and percentage of daily removed bacterial production for the each sampling point in the Orlík (n=4) and Římov (n=5) reservoirs compared to the Sau reservoir, where only two sampling campaigns with appropriate available data sets were averaged (i.e., April and July 1997, for details see Šimek et al., 2001a). The parameter of the community change was calculated as a difference in the sum of absolute changes in the proportions of four phylogenetic subgroups of the bacteria between two consecutive sampling points (lower panel). Error bars shows standard errors of the mean.

were commonly measured in their downstream areas, spatially coinciding with the maximum of phytoplankton biomass (data not shown). While the common trend of the increase in BP was also accompanied by a rather low proportion of BP removed by protistan bacterivory in the downstream stations of both reservoirs, no such a common trend of PP could be detected. For instance, a secondary peak in PP was followed by the peak of BP in the dam area of Římov (Fig. 4), while in Orlík it was obvious that these processes were uncoupled because of the rather inverse trends of PP and BP (see Fig. 2). Thus, this downstream located peak in BP in Orlík did not reflect changes in PP, but it was likely a result of enhanced organic carbon and nutrient supplies released from decaying algal cells brought from the upstream parts of the reservoir.

The literature data from the Sau reservoir (cf. also Fig. 6), showed highly specific trends (for details see Comerma et al., 2001; Šimek et al., 2001a; Gasol et al., 2002). The most distinct changes in production rates and in other plankton variables, were found around the plunge point, i.e., where river water sinks below reservoir's epilimnion (stations 1 and 2 in Fig. 6). A significant portion of the river water, rich in organic matter and allochthonous bacterial turbidity, was injected into the reservoir epilimnion (Armengol et al., 1999). This caused a highly specific longitudinal succession of microbes (see Šimek et al., 2001a) and marked changes in activities and composition of bacteria and protozoa. In the lacustrine part of the Sau reservoir, the large river-borne bacteria originally adapted to high nutrient supply (Šimek et al., 2001a) decreased their abundances, activities and production (see also Fig. 6), partially as a response to conspicuous peak in HNF numbers and bacterivory. Protistan bacterivory together with changing substrate nature and availability are considered as the most likely factors inducing a spatially distinct shift in BCC (Šimek et al., 2001a). Moreover, the intensive grazing on bacteria and enhanced sedimentation of river-carried particles lead to an increased water transparency (Comerma et al., 2001), relieving primary production from severe light limitation. These specific features of the Sau reservoir system can explain why the PP and BP processes were found to be largely uncoupled, yielding even the contrasting inverse trend in the upper parts of the reservoir (Fig. 6).

Changes in bacterial community composition in the reservoirs

Available literature indicates that more pronounced gradients in microbial variables are likely to be expected when the river water variables, in particular nutrient and organic matter loads, differ from those in the lacustrine part of the reservoir (Thornton et al., 1990, Šimek et al., 2001a; Gasol et al., 2002). Our study is the first one that compared longitudinal changes in bacterial parameters in two canyon-shaped reservoirs with different retention times and organic matter loads. Our working hypothesis was that under moderate nutrient and organic matter loads, the longer retention time of a reservoir will differently support mainly autochthonous processes of PP which can contribute to the development of BCC in the river inflow and the lacustrine parts of the reservoir. Generally, our data from the Římov reservoir showed quite distinct patterns, in case of BCC significantly different, from those found in Orlík reservoir.

To examine changes in BCC in the two different reservoirs, we used fluorescence *in situ* hybridization (FISH) with oligonucleotide rRNA-targeted probes. Our data showed a similar range of proportion in DAPI stained bacteria (40–80%) detected with the probe for the domain Bacteria (EUB338, Figs 3 and 5) as reported from different oligo- to eutrophic lakes and reservoirs (e.g., Hicks et al., 1992; Alfreider et al., 1996; Weiss et al., 1996; Pernthaler et al., 1998; Šimek et al., 2001a). However, our FISH detection limit with the probe EUB338 was consistently higher (Table 2) in the Římov than in Orlík. It is worth noting that a similar low FISH detection limit as in the more flow-through Orlík reservoir was also reported in a study monitoring changes of BCC along a river (Leff, 2000).

There are three possible reasons why the probe EUB338 targets a varying proportion of total DAPI-stained bacterial cells: (i) The cells may have too few ribosomes to produce a FISH-detectable signal, e.g., in case of inactive or dormant cells (Poulsen et al., 1993; Manz et al., 1999). (ii) The probe EUB338 may not reach the target site due to the very limited cell wall penetrability or a higher order structure of the ribosome (Frischer et al., 1996). Such a limited FISH-detectability has been recently reported for environmentally important groups of *Bacteria* belonging to *Planctomycetales* and *Verrumicrobia* (Daims et al., 1999). Finally, (iii) cells from domains other than the domain Bacteria may be present. These cells are not targeted by this bacterial probe (e.g., Archaea).

In the Římov reservoir, there was usually a marked drop in FISH detection limit between the station River and 1 (Fig. 5). This is likely to be expected at a transient zone between environments with very distinct properties, e.g., a river–reservoir mixing zone (Šimek et al., 2001a) or water masses with different salinity (Bouvier & delGiorgio, 2002). In such zones, bacteria are exposed to adverse conditions such as temperature and substrate shocks (e.g. Straškrabová, 1983), yielding potentially accelerated death rate of bacteria.

Longitudinal changes in the phylogenetic BCC in both reservoirs showed that the dominant groups were consistently β-Proteobacteria and *Cythophaga/Flavobacterium* (CF), thus supporting findings of other studies from fresh waters (e.g., Alfreider et al., 1996; Methé et al., 1998; Glöckner et al., 1999). Significant proportions of these two groups in the Římov reservoir (10–50% of total bacterial abundance) have been also previously documented in fractionation experiments (Šimek et al., 1999b, 2001b).

In the Orlík reservoir, other phylogenetic groups significantly contributed to BCC only temporarily, i.e., during the sampling campaigns conducted on 27 June and 26 September 2000, with enhanced proportions of γ- and α-Proteobacteria. However, on 27 June, γ-Proteobacteria became so abundant throughout the whole longitudinal profile (Fig. 3) that they accounted for a similar proportion as, e.g., otherwise dominating beta-Proteobacteria group. It is possible, that the γ-Proteobacteria, which have in the Římov reservoir usually much larger cell volume (4–5 μm) than cells affiliated to other groups (Mašín, unpubl. data), profited from the protistan grazing pressure (see Fig. 2) by heterotrophic flagellates targeted usually to <3 μm bacteria (see, e.g., Šimek et al., 1997; Hahn & Höfle, 2001; Pernthaler et al., 2001). In general, the data from the Orlík reservoir indicated relative longitudinal stability of BCC because no significant differences in BCC between the inflow and lacustrine parts were detected (Table 3).

In contrast, there was a significant phylogenetic BCC shift between the river inflow and the station 1 of the Římov reservoir, i.e., the area where the river sinks beneath the epilimnion, representing a transient mixing zone between the typical river and lacustrine water masses. This phylogenetic shift can be roughly characterized as a change from the dominance of the β-Proteobacteria in the river towards the CF group, which dominated the lacustrine parts of the reservoir (see, e.g., 25 May, Fig. 5). Along with these most marked longitudinal changes in BCC, we also ob-

served a gradual decrease of the FISH detection limit based on the proportions of EUB338-positive bacteria towards the dam of Římov. This phenomenon was perhaps connected with a marked decrease in nutrient availability (Table 1). Limiting nutrients, mainly phosphorus availability, are known to regulate efficiently bacterial dynamics, in terms of abundance, production and biomass of bacteria (e.g., Schweitzer & Simon, 1995; Chrzanowski et al., 1995; Šimek et al., 2003, in press). There was also growing protozoan predation pressure towards the reservoir dam (cf. Fig. 6), which could specifically contribute to the lower proportion of FISH detectable bacterial cells, since particularly HNF are known to prefer larger, dividing and actively growing bacterial cells (e.g., Sherr et al., 1992).

A specifically designed probe for bacterioplankton in the Římov reservoir (R-BT065, for details see Šimek et al., 2001b) targets an abundant freshwater cluster of β-Proteobacteria (accounting usually for 5–30% of total bacteria) with a cosmopolitan appearance (Glöckner et al., 2000). Interestingly, this bacterial lineage showed a marked distinction between the two systems – it was totally absent in the allochthonously loaded Orlík reservoir while it composed consistently 8–10% of BCC with almost no spatial or seasonal variability in the Římov reservoir (see Fig. 5). From the previous study conducted in Římov (for detail see Šimek et al., 2001b), it is known that this group never form flocs or filaments. It seems that this group is permanently under grazing pressure in the reservoir. This idea is consistent with the finding that HNF are capable of selecting prey of the certain size or genotype (Šimek et al., 1997, 1999b; Hahn & Höfle, 1998).

Conclusion

This study documented that two canyon-shaped reservoirs with different nutrient load were dominated with the same phylogenetic groups of bacteria almost whole season although at least the β-Proteobacteria group was composed from different phylogenetic subclasters. The shorter retention time and higher nutrient load of the Orlík reservoir resulted in stabile phylogenetic composition of the bacterioplankton along the whole longitudinal profile. On the other hand, in lacustrine part of the Římov reservoir with long retention time BCC developed differently from that in the inflow part of the reservoir, and the lacustrine BCC and bacterial production were obviously more coupled to primary production and influenced by predation along longitudinal profile of the reservoir. Although large spatial and seasonal variability is inherent in our data sets from various transects, thus strongly limiting the applicability of usual statistical analysis, the studied canyon-shaped reservoirs showed specific longitudinal dynamic relatable to the markedly different major limnological variables of the systems.

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References

Alfreider, A., J. Pernthaler, R. I. Amann, B. Sattler, F. O. Glöckner, A. Ville & R. Psenner, 1996. Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake using *in situ* hybridization. Appl. environ. Microbiol. 62: 2138–2144.

Amann, R.I., 1995. Fluorescently labelled, rRNA-targeted oligonucleotide probes in the study of microbial ecology. Microb. Ecol. 4: 543–554.

Armengol, J., J. C. Garcia, M. Comerma, M. Romero, J. Dolz, M. Roura, B. P. Han, A. Vidal & K. Šimek, 1999. Longitudinal processes in canyon type reservoirs: The case of Sau (N.E. Spain). In Tundisi, J. G. & M. Straškraba (eds), Theoretical Reservoir Ecology and its Applications. Brazilian Academy of Sciences and Backhuys Publishers: 313–345.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyerreil & F. Thingstad, 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10: 257–263.

Borovec, J. & J. Hejzlar, 2001. Phosphorus fractions and phosphorus sorption characteristics of freshwater sediments an their relationship to sediment composition. Arch. Hydrobiol. 151: 687–703.

Bouvier, T. C. & P. A. delGiorgio, 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperare estuaries. Limnol. Oceanogr. 47: 453–470.

Chrzanowski, T. H., R. W. Sterner & J. J. Elser, 1995. Nutrient enrichment and nutrient regeneration stimulate bacterioplankton growth. Microb. Ecol. 29: 221–230.

Comerma, M., C. J. García, J. Armengol, M. Romero & K. Šimek, 2001. Planktonic food web structure along the Sau Reservoir (Spain) in summer 1997. Int. Rev. Hydrobiol. 86: 195–209.

Daims, H., A. Brühl, R. Amann, K. H. Schleifer & M. Wagner, 1999. The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: development and evaluation of a more comprehensive probe set. Syst. appl. Microbiol. 22: 434–444.

delGiorgio, P. A., J. M. Gasol., D. Vaque, P. Mura, S. Agusti & C. M. Duarte, 1996. Bacterioplankton community structure: Protists control net production and the proportion of active bacteria in a coastal marine community. Limnol. Oceanogr. 41: 1169–1179.

Frischer, M. E., P. J. Floriani & S. A. Nierzwicki-Bauer, 1996. Differential sensitivity of 16S rRNA targeted oligonucleotide probes used for fluorescence in situ hybridization is a result of ribosomal higher order structure. Can. J. Microbiol. 42: 1061–1071.

Fuhrman, J. A., 1999. Marine viruses and their biogeochemical and ecological effects. Nature 399: 541–548.

Gasol, J. M., M. Comerma, J. C. Garcia, J. Armengol, E. O. Casamayor, P. Kojecká & K. Šimek, 2002. A transplant experiment to identify the factors controlling bacterial abundance, activity, production, and community composition in a eutrophic canyon-shaped reservoir. Limnol. Oceanogr. 47: 62–77.

Glöckner, F. O., B. M. Fuchs & R. Amann, 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence *in situ* hybridization. Appl. environ. Microbiol. 65: 3721–3726.

Glöckner, F. O., E. Zaichikov, N. Belkova, L. Denissova, J. Pernthaler, A. Pernthaler & R. Amann, 2000. Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phyllogenetic clusters including an abundant group of actinobacteria. Appl. environ. Microbiol. 66: 5053–5065.

Hahn, M. W. & M. G. Höfle, 1998. Grazing pressure by a bacterivorous flagellate reverses the relative abundance of *Comamonas acidovorans* PX 54 and *Vibrio* strain CB5 in chemostat cocultures. Appl. environ. Microbiol. 64: 1910–1918.

Hahn, M. W. & M. G. Höfle, 2001. Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microb. Ecol. 35: 113–121.

Hejzlar, J. & V. Vyhnálek, 1998. Longitudinal heterogeneity of phosphorus and phytoplankton concentrations in deep-valley reservoirs. Int. Rev. Hydrobiol. 83 (Special issue): 139–146.

Hicks, R. E., R. I. Amann & D. A. Stahl, 1992. Dual staining of natural bacterioplankton with 4′,6-diamidino-2-phenylindole and fluorescent oligonucleotide probes targeting kingdom – level 16S rRNA sequences. Appl. environ. Microbiol. 58: 2158–2163.

Jezbera, J., J. Nedoma & K. Šimek, 2003. Longitudinal changes in protistan bacterivory and bacterial production in two canyon-shaped reservoirs of different trophic status. Hydrobiologia 504: 115–130.

Jürgens, K., 1994. Impact of *Daphnia* on planktonic microbial food webs. A review. Mar. Microb. Food Webs 8: 295–324.

Jürgens, K., J. Pernthaler, S. Schalla & R. Amann, 1999. Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. Appl. environ. Microbiol. 65: 1241–1250.

Kalff, J., 2002. Limnology: Inland water ecosystems. Prentice-Hall Inc., Upper Saddle River, NJ, 592 pp.

Leff, L. G., 2000. Longitudinal changes in microbial assemblages of the Ogeechee River. Freshwat. Biol. 43: 605–616.

Linsdtröm, E. S., 2000. Bacterioplankton community composition in five lakes differing in trophic status and humic content. Microb. Ecol. 40: 104–113.

Lorenzen, C. J., 1967. Determination of chlorophyll and phaeopigments. Spectrophotometric equations. Limnol. Oceanogr. 12: 343–346.

Manz, W., K. Wendt-Ponthoff, T. R. Neu, U. Szewzyk & J. R. Lawrence, 1999. Phylogenetic composition, spatial structure, and dynamics of lotic bacterial biofilms investigated by fluorescent *in situ* hybridization and confocal laser scanning microscopy. Microb. Ecol. 37: 225–237.

Methé, B. A., W. D. Hiorns & J. P. Zehr, 1998. Contrasts between marine and freshwater bacterial community composition: Analyses of communities in Lake George and six other Adirondack lakes. Limnol. Oceanogr. 43: 368–374.

Murphy, I. & J. P. Riley, 1962. A modified single-solution method for determination of phosphate in natural waters. Anal. Chim. Acta 27: 31–36.

Pace, M. L. & J. J. Cole, 1994. Comparative and experimental approaches to top-down and bottom-up regulation of bacteria. Microb. Ecol. 28: 181–193.

Pernthaler, J., A. Alfreider, T. Posch, S. Andreatta & R. Psenner, 1997. *In situ* classification and image cytometry of pelagic bacteria from a high mountain lake (Gossenkollesee, Austria). Appl. environ. Microbiol. 63: 4778–4783.

Pernthaler, J., F. O. Glöckner, S. Unterholzner, A. Alfreider, R. Psenner & R. Amann, 1998. Seasonal community and population dynamics of pelagic Bacteria and Archaea in a high mountain lake. Appl. environ. Microbiol. 64: 4299–4306.

Pernthaler, J., T. Posch, K. Šimek, J. Vrba, A. Pernthaler, F. O. Glöckner, U. Nubel, R. Psenner & R. Amann, 2001. Predator-specific enrichment of actinobacteria from a cosmopolitan freshwater clade in mixed continuous culture. Appl. environ. Microbiol. 67: 2145–2155.

Pinel-Alloul, B., 1995. Spatial heterogeneity as a multiscale characteristic of a zooplankton community. Hydrobiologia 300/301: 17–42.

Pomeroy, L. R., 1974. The ocean's food web, a changing paradigm. Bioscience 24: 499–504.

Poulsen, L. K., G. Ballard & D. A. Stahl, 1993. Use of rRNA fluorescence *in situ* hybridization for measuring the activity of single cells in young and established biofilms. Appl. environ. Microbiol. 59: 1354–1360.

Riemann, B. & M. Sondergaard, 1986. Regulation of bacterial secondary production in two eutrophic lakes and in experimental enclosures. J. Plankton Res. 8: 519–536.

Roberts, E. C., J. Laybourn-Parry, D. M. McKnight & G. Novarino, 2000. Stratification and dynamics of microbial loop communities in Lake Fryxell, Antarctica. Freshwat. Biol. 44: 649–661.

Schindler, D. W., 1977. Evolution of phosphorus limitation in lakes. Science 195: 260–262.

Schauer, M., R. Massana & C. Pedrós-Alió, 2000. Spatial differences in bacterioplankton composition along the Catalan coast (NW Mediterranean) assessed by molecular finger printing. FEMS Microb. Ecol. 33: 51–59.

Schweitzer, B. & M. Simon, 1995. Growth limitation of planktonic bakteria in a large mesotrophic lake. Microb. Ecol. 30: 89–104.

Shapiro, J., 1988. Introductory Lecture at the International Symposium 'Phosphorus in Freshwater Ecosystems, Uppsala, Sweden', October 1985. Hydrobiologia 179: 9–17.

Sherr, B. F., E. B. Sherr & J. McDaniel, 1992. Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblage. Appl. environ. Microbiol. 58: 2381–2385.

Sherr, E. B. & B. F. Sherr, 1993. Protistan grazing rates via uptake of fluorescently labeled prey. In Kemp, P. F., B. F. Sherr, E. B. Sherr & J. J. Cole (eds), Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, FL: 695–701.

Šimek, K., M. Macek, J. Sed'a & V. Vyhnálek, 1990. Possible food chain relationships between bacterioplankton, protozoans, and cladocerans in a Reservoir. Int. Rev. Hydrobiol. 75: 583–596.

Šimek, K., J. Bobková, M. Macek, J. Nedoma & R. Psenner, 1995. Ciliate grazing on picoplankton in a eutrophic reservoir during the summer phytoplankton maximum: a study at the species and community level. Limnol. Oceanogr. 40: 1077–1090.

Šimek, K., J. Vrba, J. Pernthaler, T. Posch, P. Hartman, J. Nedoma & R. Psenner, 1997. Morphological and genotypic shifts in an experimental bacterial community influenced by protists of contrasting feeding modes. Appl. environ. Microbiol. 63: 587–595.

Šimek, K., J.Armengol, M. Comerma, J. C. Garcia, T. H. Chrzanowski, M. Macek, J. Nedoma & V. Straškrabová, 1998. Characteristics of protistan control of bacterial production in three reservoirs of different trophy. Int. Rev. Hydrobiol. 83: 485–494.

Šimek, K., J. Armengol, M. Comerma, J. C. Garcia, T. H. Chrzanowski, P. Kojecká, M. Macek, J. Nedoma & V. Straškrabová,1999a. Impacts of protistan grazing on bacterial dynamics and composition in reservoirs of different trophy. In Tundisi, J. G. & M. Straškraba (eds), Theoretical Reservoir Ecology and its Applications. Brazilian Academy of Sciences & Backhuys Publishers: 267–282.

Šimek, K., P. Kojecká, J. Nedoma, P. Hartman, J. Vrba & J. R. Dolan, 1999b. Shifts in bacterial community composition associated with different microzooplankton size fractions in a eutrophic reservoir. Limnol. Oceanogr. 44: 1634–1644.

Šimek, K., J. Armengol, M. Comerma, J. C. Garcia, P. Kojecká, J. Nedoma & J. Hejzlar, 2001a. Changes in the epilimnetic bacterial community composition, production, and protist-induced mortality along the longitudinal axis of a highly eutrophic reservoir. Microb. Ecol. 42: 359–371.

Šimek, K., J. Pernthaler, M. G. Weinbauer, K. Horňák, J. R. Dolan, J. Nedoma, M. Mašín & R. Amann, 2001b. Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesotrophic reservoir. Appl. environ. Microbiol. 67: 2723–2733.

Straškraba, M., 1998. Limnological differences between deep valley reservoirs and deep lakes. Int. Rev. Hydrobiol. (Spec Issue) 83: 1–12.

Straškrabová, V., 1983. The effect of substrate shock on populations of starving aquatic bacteria. J. Appl. Bacteriol. 54: 217–224.

Straškrabová, V., C. Callieri, P. Carrillo, L. Cruz-Pizarro, J. Fott, P. Hartman, M. Macek, J. P. Medina-Sánchez, J. Nedoma & K. Šimek, 1999. Investigation on pelagic food webs in mountain lakes – aims and methods. J. Limnol. 58: 77–87.

Thingstad, T. F. & R. Lignell, 1997. Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. Aquat. Microb. Ecol. 13: 19–27.

Thornton, K. W., B. L. Kimmel & F. F. Payne, 1990. Reservoir Limnology: Ecological Perspectives. Wiley and Sons, New York: 246 pp.

Urabe, J., 1989. Relative importance of temporal and spatial heterogeneity in zooplankton community of an artificial reservoir. Hydrobiologia 184: 1–6.

Weiss, P., B. Schweitzer, R. Amann & M. Simon, 1996. Identification *in situ* and dynamics of bacteria on limnetic organic aggregates. Appl. environ. Microbiol. 62: 1998–2005.