Altering the balance between bacterial production and protistan bacterivory triggers shifts in freshwater bacterial community composition

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Abstract

Bacterivorous protists are known to induce changes in bacterial community composition (BCC). We hypothesized that changes in BCC could be related quantitatively to a measure of grazing: the ratio of bacterial mortality to growth rate. To test this hypothesis, we analyzed time-course changes in BCC, protistan grazing rate, and bacterial production from 3 *in situ* studies conducted in a freshwater reservoir and three laboratory studies. In the field experiments, samples were manipulated to yield different levels of protistan bacterivory and incubated in dialysis bags. Laboratory investigations were continuous cultivation studies in which different bacterivorous protists were added to bacterial communities. BCC was assessed using 4–6 different rRNA-targeted oligonucleotide probes for community analysis. Change in BCC (Δ BCC) was estimated as the sum of changes in the proportions of the two phylogenetic groups that showed the largest shifts. Analysis of a set of 22 estimates of shifts in the ratio of grazing to production rate over periods of 48–72 h and Δ BCC showed that Δ BCC was positively and tightly correlated ($r^2 = 0.784$) with shifts in the ratio of grazing mortality to cell production. While the nature of a shift in BCC is unpredictable, the magnitude of the change can be related to changes in the balance between bacterial production and protistan grazing.

Abbreviations: BP – bacterial production; TGR – total protistan grazing rate; BCC – bacterial community composition

Introduction

In aquatic ecosystems, predation is considered to be a major factor affecting size structure, food web complexity, and community composition of different trophic levels (Brooks & Dodson 1965; Hairston & Hairston 1993). With regard to planktonic bacteria, grazing impacts of protists and metazooplankton are associated with a variety of effects. However, the relative importance of different mechanisms or factors controlling bacterioplankton dynamics and community structure are currently topics of debate in aquatic ecology. Recent studies have shown that bacterioplankton can be regulated by resources (bottom-up control), by grazing (top-down control), and/or by viral lysis

in both marine and freshwaters (e.g. Wright 1988; Sanders et al. 1992; Fuhrman 1999). Among grazers, heterotrophic nanoflagellates (HNF) and ciliates have been identified as the major consumers, regulating bacterial abundance and production in aquatic systems (e.g. Sanders et al. 1989; Berninger et al. 1991; Šimek et al. 2000). The identification is based in part on findings of tight relationships between HNF abundance and their bacterivory on the one hand, and bacterial abundance and production on the other (Pace 1988; Berninger et al. 1991; Dolan & Gallegos 1991; Sanders et al. 1992).

Many experimental studies have also shown that protistan predation induces distinct changes in bacterioplankton communities. They seem to be associ-

ated with selective protistan grazing on particular bacterial strains (e.g., Mitchel et al. 1988; Pernthaler et al. 2001), or more generally on large bacterial cells (e.g., Chrzanowski & Šimek 1990; Gonzalez et al. 1990). Thus, in natural systems, bacterial production can be affected by the selective removal of possibly larger, dividing and metabolically more active cells (Sherr et al. 1992). As a consequence, grazing could lead to the accumulation and persistence of a relatively large pool of dormant or less metabolically active bacteria (del Giorgio et al. 1996). Other phenomena typically associated with protistan grazing are changes in average physiological rates or cell morphology (e.g., Posch et al. 1999; Hahn & Höfle 2001). Not surprisingly then, recent studies of the impact of protistan grazing on natural or mixed freshwater communities (e.g., Pernthaler et al. 1997b; Šimek et al. 1997, 1999; Jürgens et al. 1999; Hahn et al. 1999; van Hannen et al. 1999; Langenheder & Jürgens 2001) have shown that protistan bacterivory is associated with changes in bacterial community composition (BBC).

While there is little doubt that protist-induced mortality causes changes in BCC, the underlying mechanisms are still unclear. For example, Pernthaler et al. (1997b) speculated that a chemical stimulus might have caused a rapid BCC shift in a continuous cultivation study on a mixed bacterial community. Hahn & Höfle (1998) suggested that the change in relative proportions of two bacterial strains, observed in their laboratory experiments, was a response to increased growth rates induced by flagellate grazing. Manipulating grazing mortality among natural communities during different seasons (Jürgens et al. 1999; Šimek et al. 1999, 2001) also induced distinct responses in the growth rate of several phylogenetic groups of bacteria.

Short-term shifts in concentrations of HNF and the characteristics of bacterioplankton communities, in terms of cell volumes and division rates, have been noted (e.g., Psenner & Sommaruga 1992). Surprisingly, no attempts have been made to relate short-term changes of total protistan grazing rates (TGR) and bacterial production (BP) to changes in BCC. Here we attempt to relate changes in BCC to shifts in the ratio of bacterial cell production (BP) to cell loss to grazers (TGR). We assembled a large data set from three field and three laboratory studies. Field studies (Šimek et al., 1999, 2001) were conducted in a meso-eutrophic freshwater reservoir in which differential filtration was used to create different levels of protistan predation to which natural bacterioplankton exposed in dialysis bags were subjected. Data from field studies were pooled with the results from three continuous cultivation studies (Šimek et al. 1997; Posch et al. 1999; Kojecká & Šimek, in prep.). In the experiments, pure cultures of different bacterivorous protists were added to mixed bacterial assemblages that had previously been maintained under grazer-free conditions.

Materials and methods

We analyzed data from three published field investigations conducted in the meso-eutrophic Rímov Reservoir (South Bohemia, for details see Šimek et al. 1999, 2001), and from three continuous cultivation studies (see below) which combined provided 22 sets of data. Each data set comprised three rate estimates: bacterial production, grazing-induced mortality and change in the genetic composition of the bacterioplankton community.

Field studies

The reservoir studies were conducted during the clear water phase, 9–13 June 1997 (RES-I), the late phase of a summer phytoplankton peak, 12–18 September 1997 (RES-II, for details see Šimek et al. 1999), and again during the clear water phase, 28 May–1 June 1999 (RES-III; for details see Šimek et al. 2001). The studies RES-I and RES-III (clear water phase) were conducted during the period with low to moderate grazing impacts of protists on bacterioplankton and a high abundance of metazooplankton. In contrast, the study RES-II fall into the late summer period with the maximum of bacterial mortality induced by protistan grazing along with negligible grazing impacts of metazooplankton in the reservoir (cf. Šimek et al. 1990). The experimental design of the field studies and protocols are described in detail in Šimek et al. (1999) and Šimek et al. (2001).

Manipulation of grazing impact

Different size fractions of the reservoir plankton, produced via sequential filtration of water samples through 20-μm, 5.0-μm, and 0.8-μm pore size filters, were incubated in dialysis bags (diameter 75 mm, MWC 12 000–16 000 daltons, Poly Labo, Switzerland) at a depth of 0.5 m. Treatments with no additional concentration or dilution of samples were divided into two categories: (1) 'Bacterivore-free' incubations, i.e. prefiltration of reservoir water through 0.8 μm (the experiment RES-II and RES-III) or 1 μm

(RES-I) pore size filters (47 mm diameter, OSMONIC INC., Livermore, USA), which removed most bacterivorous protists. (2) Treatments that yielded 'increased bacterivory', i.e. via prefiltration through 5.0 μm (RES-I–RES-III) or 20 μm (RES-I and RES-II; see Table 1) pore size filters, which removed predators of heterotrophic nanoflagellates (HNF) and of small bacterivorous ciliates. In RES-II, an additional treatment, the batch cultures of mixed HNF populations from the reservoir were added to a bag containing <5 μm prefiltered water (assigned as <5.0 μm + HNF treatment in Table 1, for detail see Šimek et al. 1999). In addition, unfiltered samples (all bacterivores and protistan predators present) were incubated in dialysis bags (RES-I and RES-II), or the samples were taken directly from the surrounding reservoir water (RES-III). The later modification was adopted, since in the previous experiments (RES-I and RES-II) the transfer process of zooplankton into the dialysis bags yielded a certain deviation from the ambient zooplankton community, and thus also the top-down control of protistan populations was partially disrupted. The dialysis bags were incubated for 4 days in RES-I and RES-III or 6 days in RES-II and subsamples taken at time zero and then daily (RES-III) or at 2-day (RES-I), or 3-day intervals (RES-II). The five different treatments are referred to as: <20 μm, <5 μm, 'protistan free', 'unfiltered in bags' and 'reservoir' treatments.

Continuous culture experiments

The design of all three continuous culture systems (see Table 1) was adopted from Šimek et al. (1997), where the setup of the experiment CULT-I is described in detail. The second system, CULT-II, (Kojecká and Šimek, unpub. data) followed basically the setup as in Šimek et al. (1997), and the third system, CULT-III, is described in detail elsewhere (Posch et al. 1999). General features of all experiments were: (1) *Cryptomonas* sp. and the accompanying microflora from a batch culture were introduced to the first stages of the systems. Algae were grown phosphorus-limited on an inorganic medium at concentrations between 70 and 200 μg PO$_4$–P l^{-1}. Thus, organic carbon for the growth of the bacterial consortia was provided exclusively by primary production of *Cryptomonas* sp. (2) The outflow of the first, protist-free stage was fed into several parallel second stage vessels. After reaching a quasi steady state, these vessels were inoculated with pre-rinsed cultures (for details see below) of either a heterotrophic flagellate, Bodo saltans (CULT-I

and CULT-II), a mixotrophic flagellate, *Ochromonas* sp., or a bacterivorous ciliate, *Cyclidium glaucoma* (CULT-III, see Table 1).

To minimize the risk of contamination with allochthonous bacteria from the protistan stock cultures, all protists were precultured on non-axenic batch cultures of *Cryptomonas* sp. Prior to the protozoan inoculation to the algal culture, flagellates and ciliates maintained on wheat seed culture infusions were gently concentrated from 25 ml to about 5 ml by gravity filtration in an autoclaved filter holder on 2-μm or 5-μm pore-size filters, respectively. The protozoa were washed five times (gravity filtration) with 20 ml of autoclaved bacteria-free (0.2 μm pore-size filters) tap water. This procedure removed most bacteria originally present in the maintenance cultures (for details see Šimek et al. 1997). The protists contained in 2 ml of the last wash in the filtration funnel (no bacteria observed via epifluorescence microscopy) were aseptically inoculated into 50 ml of a 2-μm filtrate of the subsample from the first, protist-free stage of the continuous cultivation system containing bacteria only. Protists were allowed to grow on these bacteria adapted to the algal monoculture for 30 h, and then two additional runs of the above 'wash' and reinoculation procedure were conducted. After the last wash using this treatment, protists contained in 2 ml of the bacteria-free subsample were aseptically inoculated into the experimental vessel.

One of the second stage vessels served as unmanipulated control. Changes in bacterial community composition (BCC) were monitored 10–12 days after the protistan inoculation (for details of the respective experiments see Šimek et al. 1997; Posch et al. 1999; Pernthaler et al. 2001).

Sample analysis

Using identical protocols, the following parameters were determined in all 18 field treatments and the four laboratory continuous cultivation studies: (1) Bacterial abundances, (2) bacterial production (BP), (3) total protistan grazing rate (TGR), and (4) bacterial community composition (BCC).

Bacterial abundance and production
Bacterial abundance was determined in formalin fixed samples (2% final conc.), stained with DAPI (final conc. 0.2% wt/vol), and enumerated by epifluorescence microscopy (Posch et al. 1999; Šimek et al. 1999). Bacterial production was measured via

Table 1. A summary of the data used to calculate parameters plotted in Figure 1

Experiment	/ Treatment	Parameter				Interval	Shifts in two probes						Reference
		BP_0	BP_1	TGR_0	TGR_1	(h)	Code	Shift (%)		Code	Shift (%)		
		10^6 bact ml^{-1} day^{-1}		10^6 bact ml^{-1} day^{-1}				t_0	t_1		t_0	t_1	
RES-I – 97	/ <1 μm	1.32	23.30	0.32	0	0–48	ALF1b	10.6	10.7	CF319a	12.0	16.3	Šimek et al. (1999)
	/ <5 μm	1.65	1.40	0.13	4.36	48–96	ALF1b	10.6	18.7	CF319a	13.7	29.9	
	/ <20 μm	1.68	1.77	0.29	3.14	48–96	ALF1b	10.5	25.9	CF319a	14.9	22.3	
	/ unfiltered in bag	1.88	1.96	0.11	3.14	0–48	ALF1b	11.4	18.4	CF319a	17.6	20.0	
RES-II – 97	/ <0.8 μm	1.13	4.45	1.64	0	0–72	BET42a	14.4	28.3	CF319a	13.2	10.9	Šimek et al. (1999)
	/ <5 μm	0.77	1.38	0.44	6.14	0–72	ALF1b	7.8	22.3	BET42a	18.1	11.9	
	/ <5 μm + HNF	0.77	2.56	2.40	4.27	0–72	ALF1b	9.2	16.3	BET42a	17.3	13.0	
	/ <20 μm	0.81	1.15	0.67	3.64	0–72	ALF1b	11.5	13.7	BET42a	13.9	10.8	
	/ unfiltered in bag	1.32	1.41	0.87	2.66	0–72	ALF1b	9.1	8.5	CF319a	13.4	10.9	
RES-III – 99	/ <0.8 μm, A	2.37	12.16	1.25	0	12–72	R-BT065	14.5	35.3	CF319a	5.1	8.0	Šimek et al. (2001)
	/ <0.8 μm, B	2.51	12.50	1.88	0	12–72	R-BT065	15.6	31.0	CF319a	5.0	7.3	
	/ <0.8 μm, C	1.48	13.06	1.47	0	12–72	R-BT065	16.9	34.1	CF319a	5.1	9.6	
	/ <5 μm, A	2.15	3.87	0.66	7.05	12–72	BET42a	29.8	40.9	CF319a	11.8	19.3	
	/ <5 μm, B	4.11	2.23	0.46	7.76	12–72	BET42a	25.7	45.1	CF319a	10.8	15.5	
	/ <5 μm, C	3.43	3.06	0.56	9.79	12–72	BET42a	26.1	43.7	CF319a	11.9	17.5	
	/ reservoir, A	2.90	3.75	0.99	2.04	12–72	BET42a	25.7	25.5	CF319a	5.2	9.7	
	/ reservoir, B	3.12	3.33	0.96	1.60	12–72	BET42a	21.2	24.5	CF319a	7.3	6.4	
	/ reservoir, C	3.10	2.77	1.02	1.78	12–72	BET42a	23.1	24.1	CF319a	7.9	5.6	
CULT – I	/ + *Bodo*	5.52	4.57	0.24	24.00	24–72	ALF1b	19.4	45.5	BET42a	53.0	21.6	Šimek et al. (1997)
CULT – II	/ + *Bodo*	5.20	1.30	3.60	6.88	48–96	ALF1b	29.0	57.6	BET3-447	51.1	18.9	Kojecká & Šimek, unpub. data
CULT – III	/ + *Ochromonas*	4.33	1.14	1.60	7.84	24–72	BET3-447	38.0	14.0	Ac1-847	0.1	43.1	Pernthaler et al. (2001)
	/ + *Cyclidium*	7.56	5.94	7.74	13.90	24–72	BET3-447	52.1	30.0	CF1-853	5.0	12.0	

[a]RES - *in situ* dialysis bag experiments conducted in the Římov Reservoir in 1997 and 1999; indexes A, B, C are assigned to the respective triplicate within the same treatments (RES-III – 99). CULT – continuous cultivation studies conducted in 1995, 1996 and 1998, in which cultivation vessels were inoculated by different bacterivorous protists (*Bodo saltans, Ochromonas* sp., *Cyclidium* glaucoma). For details of the experimental designs see the corresponding references. BP_0 and BP_1 – bacterial production, TGR_0 and TGR_1 – total protistan grazing rate at the beginning (t_0) and of the end of the incubation interval (t_1).

thymidine incorporation method following the protocol of Riemann and Søndergaard (1986). Duplicate 5-ml subsamples were incubated for 30 min at *in situ* temperature with 5–10 nmol l^{-1} of [methyl-^{3}H] thymidine (Amersham), preserved with neutral buffered formalin (2% final conc.), filtered through 0.2-μm membrane Poretics filters (25 mm diameter, OSMONIC INC., Livermore, USA) and extracted with ice-cold 5% TCA (Posch et al. 1999; Šimek et al. 1999).

An empirical conversion factor (ECF) between thymidine incorporation rate and bacterial cell production rate was determined for all the *in situ* reservoir experiments using data from protist-free, < 1- or 0.8-μm, treatments exposed directly in the reservoir. The bacterial production rate was calculated (based on regression relationship) from the slope of the increase of ln bacterial abundance over time (0, 24, 48 h). We determined ECFs of 2.0 × 10^{18} cells mol^{-1} (RES-I

and RES-II) and 2.3 × 10^{18} cells mol^{-1} thymidine (RES-III) for further calculations. For the CULT-II experiment, we also established an ECF, 0.93 × 10^{18} cells mol^{-1} thymidine, while for CULT-I and CULT-III experiments the theoretical conversion factor of 2 × 10^{18} cells mol^{-1} thymidine was applied (e.g. Bell et al. 1983).

Bacterial community composition
BCC was analyzed by fluorescence *in situ* hybridization with rRNA-targeted oligonucleotide probes (FISH) on membrane filters (Alfreider et al. 1996). Bacterial cells from 10 to 20 ml subsamples were concentrated on white 0.2-μm-pore-size filters (Poretics Corp.; 47-mm diameter), then fixed with 4% paraformaldehyde in phosphate buffered saline (PBS; pH 7.2) and stored at −20 ° C (Alfreider et al. 1996), and counted as in Pernthaler et al. (1997b). Four different oligonucleotide probes (Interactiva, Ulm, Germany) targeted to larger taxonomic groups were used

Table 2. Probe sequences used in dialysis bag and continuous cultivation studies. For details of hybridization conditions and the sequence GenBank accession numbers see references

Probe	rRNA target, position	Sequence (5′ - 3′)	Specificity	Reference
ALF1b	16S, 19–35	CGT TCG YTC TGA GCC AG	alpha subclass of Proteobacteria, several members of delta Proteobacteria, most spirochetes	Manz et al. (1992)
BET42a	23S, 1027–1043	GCC TTC CCA CTT CGT TT	beta subclass of Proteobacteria,	Manz et al. (1992)
R-BT065	16S, 65–83	GTT GCC CCC TCT ACC GTT	specific cluster within beta Proteobacteria	Šimek et al. (2001)
BET3-447	16S, 447–464	AGC GCA GAC CGT TTC TTC	DGGE sequence BET3	Pernthaler et al. (2001)
CF319a	16S, 319–336	TGG TCC GTG TCT CAG TAC	*Cytophaga/Flavobacterium* cluster of CFB phylum[a]	Manz et al. (1996)
CF1-853	16S, 853–873	TAA TGC TTT CGC TCA GAC AC	*Flavobacterium ferrugineum* *Flexibacter elegans* *F. sancti* *Cytophaga arvensicola*	Pernthaler et al. (2001)
Ac1-847	16S, 847-875	TTA GCT GCG TCG CAC AAA C	DGGE sequence Ac1	Pernthaler et al. (2001)

[a]CFB: *Cytophaga–Flavobacterium–Bacteroides*.

to count the relative abundances of cells affiliating with the kingdom Bacteria (Eubacteria, EUB338), the alpha-, and beta-subclasses of the class Proteobacteria (ALF1b and BET42a), and with the Cytophaga-Flavobacterium (CF319a) lineage of the Cytophaga-Flavobacterium-Bacteroides phylum (Amann et al. 1995). In addition, bacterial diversity was examined by amplification via polymerase chain reaction (PCR) in several cases, by denaturing gradient gel electrophoresis (DGGE) (Muyzer et al. 1998), and by cloning and sequencing of 16S rDNA genes from the different treatments or experimental cultivation vessels. Specific oligonucleotide probes (R-BT065, CF1-853, Ac1-847, BET3-447) were designed from this sequence information and used for further analysis of BCC (Šimek et al. 2001; Pernthaler et al. 2001). Probe sequences and details on the application of different probes in the experiments are given in Tables 1 and 2.

Protozoan grazing

Protozoan grazing on bacteria was estimated using fluorescently labeled bacteria (FLB; Sherr and Sherr 1993; Šimek et al. 1999, 2000). The uptake experiments for the FLB were performed in subsamples from the bags or vessels containing protists and in samples of unfiltered reservoir water. HNF and ciliate FLB uptake rates were determined in short-term direct uptake experiments with FLB accounting for 5–15% of natural bacterial concentration as determined after adding the FLB to natural samples. Ciliate uptake rates as FLB cell^{-1} were estimated at times 5, 10 and 15 min, and flagellate uptake rates were estimated at times 10, 20 and 30 min. Uptake rates were calculated by linear regression of average number of tracers per protozoan cell versus time. Subsamples (10–25 ml) for the determination of protozoan abundance and tracer ingestion were preserved and evaluated microscopically (Sherr and Sherr 1993; Šimek et al. 1999). At least 40 ciliates and 50 flagellates were counted for the FLB ingestion in each sample to derive the average ingestion rates. To estimate total protozoan grazing, we multiplied average uptake rates of ciliates and flagellates by their *in situ* abundances.

Data analysis

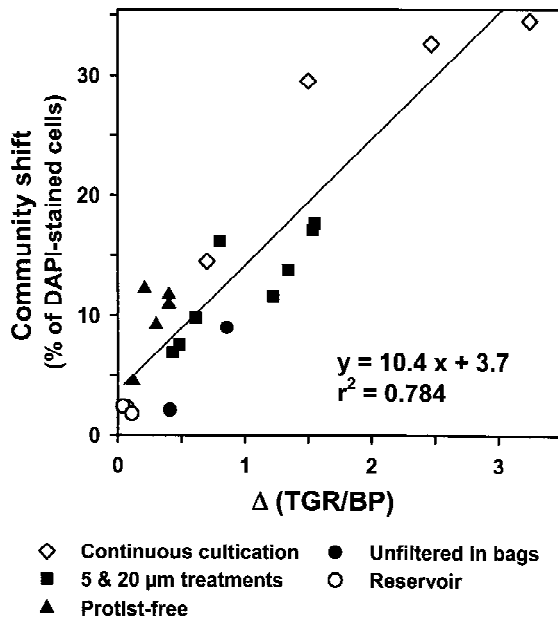Short-term changes in BCC, as revealed by changing proportions of different phylogenetic groups of bac-

Figure 1. Linear regression of the sum of the absolute changes in the proportions of two phylogenetic groups of bacteria and the magnitude of changes in the ratio of total protistan grazing rate (TGR) to bacterial production (BP). The plot of the parameters (normalized per day) summarizes data from: (i) Continuous cultivation systems, and (ii) Field experiments which employed different size-fractionation treatments: 'protists-free' treatments with samples screened through 1- or 0.8-μm filters; '5 and 20 μm' – the treatments with samples screened through 5- or 20-μm filters; 'Unfiltered in bags' – unfiltered samples exposed in dialysis bags; and 'Reservoir' – samples taken directly from surrounding reservoir water (for details see the text, Table 1 and references therein).

teria, were related to the changes in the ratio of total protistan grazing rate (TGR) to bacterial production (BP). Results from previous studies (e.g. Pernthaler et al. 1997b; Hahn et al. 1999; Šimek et al. 2001) showed the most significant changes in TGR, BP and BCC approximately 48–72 h after the experimental manipulation of samples. Therefore, in all treatments we analyzed differences in the latter parameters over incubation intervals of 48-72 h (for details see Table 1). We calculated changes in the ratio of grazing to production (TGR/BP) as follows:

$$\Delta(TGR/BP) = |TGR_1/BP_1 - TGR_0/BP_0|/$$
$$(t_1 - t_0) \quad [d^{-1}] \qquad (1)$$

where TGR_0, and BP_0 represent total protistan grazing rate, and bacterial production, respectively, at time zero (t_0) and TGR_1 and BP_1 grazing rate and bacterial production at time t_1 (48–72 h, for details see Table 1). In Equation (1), we used the absolute values of the parameters because experimental manipulations resul-

ted in an either positive value of ratio changes over time, i.e. treatments of 'increased bacterivory', or in a negative value, i.e., 'bacterivore-free' treatments, in which grazing pressure was removed. The parameter Δ (TGR/BP) was normalized to per day rates and plotted as the independent variable in Figure 1.

Usually 1–2 group-specific rRNA-probes indicated a quantifiable change in BCC within 2–3 days after the experimental manipulation (see e.g. Pernthaler et at. 1997b; Šimek et al. 1999), which was reflected in an increase or decrease in the relative proportions (%) of these phylogenetic groups in the total number of DAPI-stained bacteria (=100%). As an estimate for the magnitude of shifts in BCC, we used the absolute values of the sum of the change in the proportions of those two phylogenetic groups (see Equation 2) that showed the largest shifts in their relative proportions during a period of 48–72 h. This parameter, characterizing the magnitude of the shift in bacterial community composition (Δ BCC), was calculated using the following equation:

$$\Delta BCC = (|ProbeA_1 - ProbeA_0| +$$
$$|ProbeB_1 - ProbeB_0|)/(t_1 - t_0) \quad [\%d^{-1}] \quad (2)$$

where ProbeA and ProbeB are relative proportions (as % of total DAPI-stained bacteria) of two probes at time zero (t_0) and times 2 to 3 days (t_1). Δ BCC was normalized per day, plotted as the dependent variable (Figure 1) against the parameter characterizing ration between grazing and bacterial production, Δ (TGR/BA), and the relationship evaluated using correlation analysis.

Results

Table 1 gives data from all six presently available studies in which information on BP, BCC and direct measurements of protistan TGR over short time intervals were made simultaneously. It should be recalled that these parameters were determined by identical methods so that our data set was not affected by the potential biases introduced by the conversion of different methodological approaches. Potential errors inherent in our determinations of BCC, BP and TGR have been discussed elsewhere (Pernthaler et al. 1997b; Šimek et al. 1999, 2000).

Our data (Figure 1) clearly supported the hypothesis that changes in the relationship between TGR and BP are directly related to the magnitude of shifts induced in BCC. The parameter estimating

community composition shift, Δ BCC, was highly significantly correlated ($r^2 = 0.784$, $P < 0.0001$) to the parameter characterizing the stability of production and loss processes, Δ (TGR/BP).

We could detect between 50 and 90% of the total DAPI-stained bacterial cells in the different treatments by FISH with probe EUB338 (covering all the phylogenetic clusters targeted by group-specific probes used, see Table 2). This range is similar to those reported from other natural systems (e.g., Pernthaler et al. 1997a; Glöckner et al. 1999; Langenheder & Jürgens 2001). Nevertheless, the changes in relative proportions of different phylogenetic groups of bacteria could potentially be biased by a systematic increase in the proportions of cells visualized with probe EUB338 in heavily grazed treatments in RES-I and RES-II (for details see Šimek et al. 1999). Such an increase would not indicate a real BCC shift but rather an enhanced physiological state and a higher number of ribosomes per cell in heavily grazed bacterial community. To test for the potential effect of this factor in our data, we normalized the parameter Δ BCC to the proportions of EUB338-positive bacteria (rather than % DAPI) in different treatments at the respective time points. Relating community data for Δ BCC to % EUB338 still yielded a highly significant correlation with the Δ (TGR/BP) and the correlation could explain a very similar amount of variability ($r^2 = 0.785$, $P < 0.0001$). This suggests a rather minor effect of the changes in proportions of EUB338 on the overall significance and predictive power of the regressions (Figure 1).

Discussion

Presently, it is not possible to determine accurately diversity in natural bacterioplankton communities, that is, to determine the relative contributions of all members at the species level. Rather, most studies today resemble ours, focusing on large groupings of bacteria or a small number of individual species. Hence, it is remarkable that a robust relationship was found employing a very simple and crude metric of community change. This parameter, Δ BCC, was calculated as the sum of absolute changes (thus weighting equally both increments and decreases of relative proportions) of those two phylogenetic groups of bacteria that exhibited the largest changes during the study interval (for details see Table 1). Potentially, summing such changes might overestimate the significance of BCC

shifts as in simple replacement of one phylogenetic group by another. This was, however, not the case in our samples, since the increments and decreases in the relative proportions of the two evaluated groups of bacteria within any single treatment never balanced each other.

On the other hand, the actual magnitude of community shifts may have been underestimated by our approach, as only the two probes showing the largest changes were utilized for the estimation of community shifts. Our strategy represents a compromise between the limits of the methodology (i.e., detection limit of the counting procedure in case of rare community members) and the need for quantification of BCC changes. It is presently impossible to estimate microbial community shifts in natural systems by classical diversity indices, because specific phylogenetic stains for smaller taxonomic entities (e.g., genera) of many common freshwater bacteria are yet to be developed. Therefore, a varying number of probes with different specificity were available for the analysis of samples from different experiments, and this might have strongly biased our estimation of community changes. In spite of the potential reduction of the true community dynamics, we observed a tight relationship between the studied parameters (Figure 1).

Our treatments represented a large range of manipulation-induced changes in predator community resulting in a mismatch between BP *versus* protistan TGR. Δ BCC was linearly related to Δ (TGR/BP) and the 'bacterivore enhanced' treatments were associated with greatest shifts in BCC. Overall, different types of treatments formed distinguishable clusters of points (Figure 1). The magnitude of the correlation coefficient at least partially reflects the extreme values that were derived from the three laboratory continuous cultivation systems where four different treatments inoculated by protists were analyzed (Figure 1, Table 1). The shifts of BCC induced during these experiments might be rather unrealistically high for natural plankton systems. After excluding data from CULT-I, CULT-II and CULT-III, the regression, Δ (TGR/BP) versus Δ BCC based only on 18 *in situ* treatments, showed the same trend, and slightly weaker correlation ($r^2 = 0.599$, $P < 0.001$).

In the protist-free treatments, changes in BCC were related to the reduction of protistan grazing pressure as compared to pre-incubation levels. Correspondingly, in four of five protist-free treatments we found moderate (Figure 1), but significant values of Δ BCC, which were proportional to the change in

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TGR when bacterioplankton subjected to heavy predation in the reservoir was transferred to the protist-free bag. In contrast, almost no change in BCC was observed in the protist-free treatment in RES-I, where bacterioplankton subjected to negligible protistan bacterivory were transferred to the protist-free bag (for details see Šimek et al. 1999). In fact, this point closely relates to the triplicate 'Reservoir' treatments (Figure 1) that represented a stable background against which changes in the manipulated communities could be evaluated. This was not the case for the unfiltered treatments exposed in dialysis bags (RES-I and II, Table 1, Figure 1), which clearly deviated from their assigned role as the experimental control treatments. Again, this discrepancy can be readily accounted for by considering the enhanced protistan removal of bacteria in these treatments. Compared to the ambient reservoir water, the top-down control of the protistan community by metazooplankton was apparently disrupted by the transfer process into the dialysis bags (for details see Šimek et al. 1999).

Overall, tight predator–prey relationships have been reported between populations of bacterivorous protists and bacterial abundance and production over a large scale of trophic states in various marine and freshwaters (Pace 1988; Berninger et al. 1991; Dolan & Gallegos 1991; Sanders et al. 1992). Then logically, invariant time-courses or proportional changes in the parameters of BP and TGR within a certain system should thus support a relative stability of BCC. A general scheme illustrating the interplay between those parameters and their impact on changes in BCC is given in Figure 2. Imbalances between production and consumption correspond with changes in BCC while matches are associated with stable community composition. For example, the invariant time courses of BP and TGR observed in the "Reservoir" treatments (Šimek et al. 2001, see Figure 1 in this study) represent a quasi-steady state characterized by a minimum value of Δ (TGR/BP). Such a situation can likely persist until a marked change occurs in environmental factors that disturbs the balance between the growth and removal rates of the dominant members of the bacterioplankton community. Intuitively, one expects shifts in grazing pressure or resource availability (top-down or bottom-up) to correspond with changes in community composition. We attempted to quantify the imbalance and identify the temporal scale on which shifts in BCC occur.

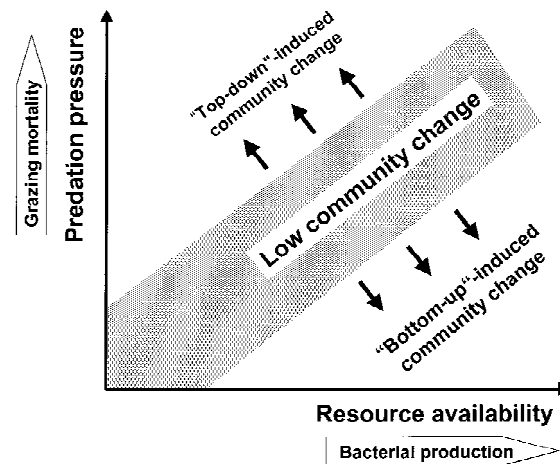One may ask what natural phenomena may result in a sudden change in the ratio of production

Figure 2. A simple model, based on the idea that under steady state conditions of growth and grazing, the bacterial community composition will also be in steady state, while change in the ratio between growth and grazing will result in shifts in bacterial community composition with larger changes in the proportion of these parameters (either top-down or bottom-up induced system imbalance) yielding more significant shifts in composition. A shadowed area represents a moderate range of the ratio at which the relative stability of bacterial community composition is assumed.

to removal rates of bacterioplankton. The 'balance' between grazing and production could be altered by shift in the input of organic substrates (e.g., decay of a phytoplankton bloom or polluted water inflow). On a smaller spatial scale, a mismatch between growth and mortality might occur during the initial colonization of organic particles: bacteria on aggregates showed enhanced growth rates because of high substrate availability (Grossart & Ploug 2000) while profiting at least temporarily from low grazing. A recent observation of shifts in BCC during the aging of aggregates (change of substrate quality and concomitant growth of attached bacterivores, Ploug & Grossart 2000) is in agreement with our hypothesis. Another instance of a rapid change in the grazer community is the diurnal vertical migration of large daphnids that can decimate the epilimnetic protistan populations during the night hours, thus temporarily relieving freshwater bacterioplankton from selective protistan bacterivory (Šimek & Chrzanowski 1992; Jürgens & Güde 1994).

However, in our studies we totally ignored another important factor – host-specific viral infection of bacterial cells, which may influence BCC as well. Since virus production and infection rates are directly affected by BP (e.g., Fuhrman 1999) and BP is stimulated by protistan grazing (e.g., Sanders et al. 1992; Posch et al. 1999), we cannot reject the possibility that

the highly significant relationship was strengthened by a synergistic effect of two factors affecting BCC – prey-selective protistan bacterivory and host-specific viral lysis. Such a synergistic effect with protistan bacterivory indirectly stimulating viral-induced bacterial mortality has been reported for our manipulation experiment, EXP-III (for details see Šimek et al. 2001). It would imply that protistan bacterivory is the dominant factor.

We suggest that the combination of imbalance between bacterioplankton growth and loss rates on the one hand and prey-selective protistan bacterivory on the other hand (e.g. Šimek & Chrzanowski 1992; Posch et al. 1999) are among 'keystone' factors controlling BCC of natural bacterial assemblages. For example, a shift towards increased protistan bacterivory will favor only those strains capable of balancing their grazing loses with enhanced growth rate (Pernthaler et al. 1997b; Posch et al. 1999), or those able to develop some grazing-resistant morphotypes (Jürgens & Güde 1994; Hahn & Höfle 2001), such forms will maintain or even enhance their proportions within the assemblage. Potentially, even normally rare community members such as the filamentous *Flectobacillus* might be favored (Šimek et al. 2001). We assume that eventually a new assortment will result in renewed community stability and a re-establishment of the balance between bacterial growth and loss processes. Equally, a sudden relief from grazing pressure (i.e., bacterivore-free treatments) can yield significant BCC shifts (cf. Šimek et al. 1999, 2001; Suzuki 1999), favoring the dominance of rapidly growing strains that were formerly selectively grazed by protists, or result in the disappearance of those strains which depended on the positive feedback of bacterivory (i.e., enhanced substrate and nutrient availability, see e.g., Sherr et al. 1982).

However, our results may not be totally relevant in all aquatic environments, for example, oligotrophic marine systems in which grazing pressure is low. For instance, our data revealed relatively smaller changes in the Δ BCC parameter for the no-grazing treatments (Fig. 1) while in contrast Suzuki (1999) observed a rather opposite effect for marine bacterioplankton: rarer groups grew up in < 0.8 μm treatments, while in treatments with grazers, the relative proportions of major phylogenetic groups showed much less changes during incubations. In fact, besides an apparent differences in substrate availability between the studied systems, there do appear to be more general differences in community response to grazing for freshwater

compared to marine bacteria, e.g. in marine systems, filamentous or floc-forming bacteria do not routinely grow up in the presence of protistan grazers, as is common for freshwater bacteria.

Our intention was to manipulate only bacterivore communities via size-fractionation under the same or very similar bottom-up control (a relatively free exchange of limiting solutes between the content of dialysis bags and the surrounding water). Thus, though we found a highly significant relationship between the ratio of production to consumption and genetic shifts, we are aware of other important factors, e.g. substrate quality and quantity (see also Figure 2), viral lysis, temperature, etc. that can significantly contribute to the magnitude of BCC shifts in freshwaters.

For instance, Gasol et al. (2002) performed a transplant experiment in the highly eutrophic, canyon-shape Sau reservoir, a longitudinally heterogeneous system, to assess the relative importance of substrate supply vs. grazing in the control of bacterial dynamics and community composition. Water samples from the lacustrine and the riverine ends of the reservoir were size-fractionated and incubated in dialysis bags placed *in situ* and transplanted at the other site of the reservoir. At both sites, bacterial production appeared to be regulated by nutrient supply, however, bacterial abundance and activity rates were regulated differently at the two sites. The riverine site bacteria were limited by predator activity while the lacustrine sample was regulated by a combination of both predator activity and nutrient supply rates. Therefore, even in the same freshwater environment different modes of control can act simultaneously suggesting that a complex interplay of both types of control could occur in a single heterogeneous plankton system.

Conclusions

Our approach can not predict the nature of BCC shifts, i.e., which bacterial groups will increase or decrease their relative proportions in the community, as a response to sudden changes in the balance between bacterial production and protist-induced mortality rates. Nevertheless, our metric of change in the balance between overall community growth and loss rate parameter, Δ (TGR/BP), appears directly linked to a parameter quantitatively characterizing shifts in BCC. Since a continuous monitoring of BCC in natural systems is presently very time-consuming and costly, more readily produced information on TGR and BP

can be utilized to specifically predict the timing of such shifts prior to BCC analysis. We, therefore, hope that our results (Figure 1) and the general interpretation (Figure 2) will bring ecologically relevant and important information.

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