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Comparing the effects of resource enrichment and grazing on viral production in a meso-eutrophic reservoir

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ABSTRACT: As viral production depends on bacteria, factors which influence bacterial production should also impact viral production. Likewise, viruses and heterotrophic nanoflagellates (HNF) both exploit bacterial prey, so HNF grazing could influence interactions between viruses and bacteria. To examine these relationships, we examined samples from experiments in which natural bacterial populations were subjected to relaxation of nutrient limitation and different levels of grazing pressure from HNF. We observed that stimulation of bacterial production and abundance with the relaxation of nutrient limitation resulted in a higher standing stock of viruses, higher viral production and also a higher virus-induced lysis rate of bacterioplankton. These relationships suggest that the relative effect of virus-induced mortality is higher in more productive environments. We found that viral abundance, viral production and virus-induced mortality of bacteria was highest in the treatments in which grazing rates on bacteria by HNF were highest, and lowest in the treatments where no eukaryotic predators were present. Thus, high grazing rates were associated with high virus production rates. The resource enrichment had a stronger effect on viral production and infection of bacteria than grazing. Averaged over time for single treatments, viruses lysed a significant portion (range, 18 to 66%) of the bacterial production per day.

KEY WORDS: Viral lysis · Bacterial production · System productivity · Dialysis bag incubation

INTRODUCTION

In aquatic systems, a significant amount of energy and matter flows through pelagic food webs via bacteria. Grazing by predators (mainly protists) and viral lysis are the 2 major known sources of mortality for bacterioplankton. On average, the 2 mechanisms seem to be responsible for an equal amount of bacterial mortality (Fuhrman & Noble 1995); however, the temporal and spatial variability of both are high (Wommack & Colwell 2000). Although enough information has been accumulated over the last 10 to 15 yr to state that viral lysis is a significant loss factor of bacterial production, comparatively few attempts have been made to separate the specific role of predation and viral lysis on bacteria and bacteria-mediated processes in pelagic food webs (Fuhrman & Noble 1995, Weinbauer & Höfle 1998, Guixa-Boixereu et al. 1999, Bettarel et al. 2002).

Viral lysis has a significant effect on the cycling of nutrients and organic matter (Bratbak et al. 1994, Middelboe et al. 1996, Fuhrman 1999, Noble & Fuhrman 1999, Wilhelm & Suttle 1999). These studies indicated that viral lysis of bacteria can increase bacterial production and respiration by reducing the carbon flow to bacterivorous protists, but we still lack comparative studies from different environments. It has also been suggested

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that virus-induced mortality of bacteria increases towards more productive systems (Steward et al. 1992, 1996, Weinbauer et al. 1993, Noble & Fuhrman 2000). Supporting this hypothesis, some mesocosm studies have shown that enrichment with inorganic and organic nutrients can enhance viral production (Tuomi et al. 1995, Wilson et al. 1996, Tuomi & Kuuppo 1999). However, no studies have been performed with enrichments of natural complex nutrient mixtures or dissolved organic matter (DOM). Also, the effect of higher nutrient concentrations on viral production has not been documented in grazer-free incubations.

Little is known on the interaction between bacterial viruses and protists and on the effect of this interaction on bacteria. Heterotrophic nanoflagellates (HNF), the most important group of protistan bacterivores, also ingest and digest viruses (González & Suttle 1993). This ingestion of viruses seems to be more a by-product of ingestion of bacteria than a specific uptake of viruses, although there are flagellates which specifically graze on the virus-size fraction of seawater (González & Suttle 1993). Grazing does not seem to be a significant loss factor for viruses. However, on occasion, uptake of viruses could contribute significantly to flagellate nutrition (Suttle & Chen 1992, González & Suttle 1993). Nonetheless, protists might control viral production by (1) grazing on infected cells, and (2) controlling host abundance. For example, selective feeding by HNF on bacteria can significantly alter both total abundance as well as relative contributions of different host populations within natural bacterial assemblages (Jürgens et al. 1999, Šimek et al. 1999, Suzuki 1999). This indicates competition between grazers and viruses for prey. However, there is some preliminary evidence that protistan grazing may increase viral infection, suggesting a synergy between grazing-induced and virus-induced mortality (Šimek et al. 2001).

The aim of this study was to determine if a stimulating effect of grazing on viral production could be confirmed by analysing samples from experiments in which grazing pressure and nutrient limitation of natural bacterioplankton communities were manipulated. We found a stimulating effect of grazing on viral growth as well as the synergy between grazing-induced and virus-induced mortality. Moreover, an increased resource supply stimulated virus-induced mortality of bacteria.

MATERIALS AND METHODS

Study site and experimental design. The samples for the experiments were collected about 250 m off the dam (dam site) of the meso-eutrophic Rímov Reservoir, a 13.5 km long, canyon-shaped reservoir in South Bohemia, Czech Republic (for details see Šimek et al. 1999), during late clear-water phase on June 12, 2000. Common features and specific details of the experimental set-up are described in Šimek et al. (2003, this issue). Briefly, water samples were collected at the dam site of the reservoir and sequentially size-fractionated through 5 and 0.8 µm pore-size filters to produce samples containing only viruses, bacteria and HNF (<5 µm), containing viruses and bacteria only (<0.8 µm) and unfiltered samples (Unf treatments) containing the entire community. These samples were incubated in dialysis bags (diameter 75 mm, molecular weight cutoff 12 to 16 kDa) at the dam site in a depth of 0.5 m, oriented horizontally in open Plexiglas holders. The same set of samples containing different fractions of the dam community was also deployed in a part of the reservoir close to the river inflow (river site). The river site showed higher P concentrations and DOC concentrations (Šimek et al. 2003). Since inorganic nutrients and smaller components of the DOM pool can penetrate the dialysis bags, the transplantation of the dam community to the river site represented a nutrient enrichment experiment with *in situ* nutrient concentrations. Dialysis bags were incubated in duplicates and subsamples were removed from each dialysis bag every 24 to 48 h. In addition, we used published and unpublished data from a similar experiment performed in 1999 (Expt 1999) at the dam site (Šimek et al. 2001). This experiment was different from the Expt 2000 in that there was no Unf fraction, but samples were taken directly from the reservoir. Data are denoted as derived from either Dam-2000, River-2000 or Dam-1999.

Bacterial and flagellate parameters. Bacteria and HNF were stained with DAPI and enumerated by using epifluorescence microscopy. Bacteria were sized with an image analysis system. Bacterial production was estimated by the [^{3}H]-thymidine incorporation method using conversion factors for relating the thymidine incorporation to cell production rate, which were determined empirically for the reservoir bacterioplankton. Grazing of HNF on bacteria was estimated using fluorescently labelled bacteria made from reservoir bacterioplankton. For more details of these methods and details on the data see Šimek et al. 2003.

Viral abundance and viral mortality of bacteria. Viruses in formaldehyde (2% final concentration) preserved samples were filtered onto 0.02 µm pore-size filters (Anodisc, Whatman), stained with SYBR Green I (10 000× in DMSO; Molecular Probes) and enumerated by epifluorescence microscopy as described in Noble & Fuhrman (1998). Bacteria were collected quantitatively onto Formvar-coated, 400 mesh electron microscope grids by using a bench top centrifuge and a rotor with

swing-out buckets for 15 ml Falcon tubes, into which removable grid platforms were inserted. Bacteria were stained for 30 s with 1% uranyl acetate and rinsed 3× with deionized distilled water. The frequency of visibly infected bacteria (FVIC) and the burst size were determined as described in Weinbauer et al. (1993). Subsamples from each replicate were pooled yielding a single sample for each treatment for each time point. Duplicate grids were prepared and processed to estimate measurement error. The FVIC was related to the frequency of infected cells (FIC) using the average conversion factor of 7.11 determined for natural communities (Weinbauer et al. 2002). Virus-induced mortality of bacteria (VMB) was estimated from FIC following the model of Binder (1999) with:

$$VMB = (FIC + 0.6\ FIC^2)/(1-1.2\ FIC)$$

and is given as a percentage. In this equation, it is assumed that infected and uninfected bacteria are grazed at the same rate and that the latent period equals the bacterial generation time (Proctor et al. 1993, Guixa-Boixareu et al. 1996, Middelboe 2000). FIC and VMB for the experiment 1999 were recalculated using the conversion factor of 7.11. Burst size was estimated as the average number of viral particles found in visibly infected cells (VIC).

Statistical analysis. Analyses of variance (ANOVA) and Fisher's Protected Least Significant Difference (PLSD) post-hoc tests (for pair-wise comparisons) were used to compare changes in viral abundance, virus-to-bacterium ratios and FVIC among filtration treatments and incubation sites, i.e. dam versus river, at times 48, 72 and 96 h. Changes in parameters were considered significant at $p < 0.05$. The relationship between FVIC and other parameters was tested using correlation analyses. Percent values were arcsine-transformed and other parameters were log-transformed before analyses.

RESULTS

Effect of nutrient enrichment on the dynamics of the viral community

Exposing the dam community to the higher nutrient levels of the river water resulted in marked differences in viral and bacterial parameters compared to the dam community deployed at the dam (Table 1). Viral abundance and net growth rate was stimulated 2- to 3-fold in the various treatments. Bacterial production lysed per day by viruses increased by ca. 70 to 90% and the daily lysis rate of the bacterial standing stock by 3- to 7-fold. Burst size did not change dramatically after the exposure of bacteria to higher nutrient levels. Bacterial growth rate and cell size (only for <0.8 µm) increased due to the exposure to river water (Šimek et al. 2003). Across treatments, viral abundance was significantly higher at the river than at the dam at t_{72} ($p < 0.05$) and t_{96} ($p < 0.005$). FVIC was significantly higher at t_{72} ($p < 0.05$), whereas the viruses to bacteria ratio (VBR) did not differ significantly between the dam and river sites.

Effect of grazing on the dynamics of the viral community

Viral abundance increased with time in all treatments in both Dam-2000 and River-2000 incubations (Fig. 1). In the Dam-2000 treatments, viral abundance increased from 15×10^6 to 37×10^6 particles ml^{-1} in the <0.8 µm treatment. In the <5 µm and Unf treatments, the development of viral abundance was similar with highest values of ca. 50×10^6 particles ml^{-1}, and a strong increase of viral abundance was observed after only 48 h of incubation. In the River-2000 treatments, the largest increase in viral abundance was between

Table 1. Summary of the effect of removal of grazers on viral and bacterial parameters in the experiments. Most data are averages of all data during the time course of the experiments except for the t_0 values. Viral net growth rates were calculated as increase of ln2-transformed viral abundance over time. Data on bacterial parameters from the Dam-1999 experiment are from Šimek et al. (2001), and bacterial stock and production lysed were recalculated using new conversion factors (Weinbauer et al. 2002). Bacterial growth rate was calculated from ^{3}H-thymidine incorporation converted to cell production divided by bacterial abundance. Res: reservoir, i.e. samples taken directly from the reservoir and not from dialysis bags; Unf: unfiltered

Parameter	Dam-1999			Dam-2000			River-2000		
	<0.8 µm	<5 µm	Res	<0.8 µm	<5 µm	Unf	<0.8 µm	<5 µm	Unf
Viral abundance (10^6 ml^{-1})	13	23	16	26	40	31	59	84	69
Viral net growth rate (d^{-1})	0.37	0.52	0.48	0.23	0.28	0.28	0.53	0.62	0.55
Bacterial standing stock lysed (% d^{-1})	14	36	29	3.0	7.1	4.5	9.2	38	31.0
Bacterial production lysed (% d^{-1})	20	30	32	18	39	24	30	66	46
Bacterial abundance (10^6 ml^{-1})	8.9	3.7	3.4	5.9	2.7	2.6	18	6.8	5.5
Bacterial growth rate (d^{-1})	0.72	1.2	0.88	0.14	0.23	0.25	0.27	0.48	0.73
Average bacterial cell volume (µm^3)	0.11	0.17	0.11	0.16	0.23	0.16	0.21	0.22	0.18
Burst size	23	28	19	33	39	22	40	35	31

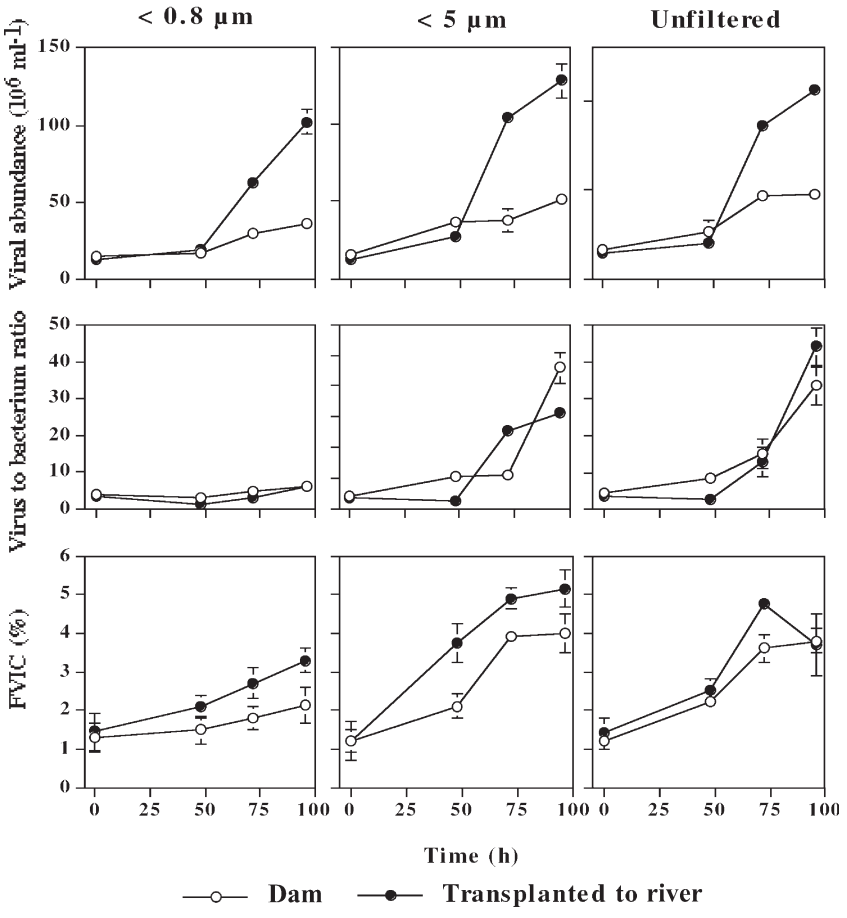

Fig. 1. Dynamics of viral abundance, virus-to-bacterium ratio (VBR) and the frequency of visibly infected cells (FVIC) in the dam viral community in the different filtration treatments exposed at the dam and the river site during Expt 2000. Data are presented as means ± ranges of duplicates

48 and 72 h. Viral abundance was highest in the <5 µm treatment with a maximum of 13 × 10^6 particles ml^{-1} and lowest in the 0.8 µm treatment with values of 10 × 10^6 particles ml^{-1}. In Dam-2000 incubations, viral abundance was 1.6 times higher in the <5 µm than in the <0.8 µm treatment compared to 1.4 times in the River-2000 incubations. In the River-2000 incubations, viral abundance at 2 time points was significantly higher in the <5 µm than in the <0.8 µm treatment, whereas a significant difference between the <0.8 µm and the Unf treatment was found for 1 time point (Table 2). Significant differences of viral abundance between <5 µm and Unf treatments were only found in the river incubations at t_{48} and t_{72}.

In both Dam-2000 and River-2000 incubations, the VBR increased only slightly in the <0.8 µm treatment, but markedly in the treatments with grazers (Fig. 1). VBR was similar in the 2 sets of incubations. FVIC ranged from 1.1 to 5.2% and increased with time in all experiments and treatments (Fig. 1). In the experiment performed in 2000, FVIC was typically highest in the <5 µm treatment and lowest in the <0.8 µm treatment. Similar data were reported for Expt 1999 (Šimek et al. 2001). In Expt 2000, FVIC was significantly higher in the <5 µm and the Unf than in the <0.8 µm treatment at t_{72}, whereas <5 µm and Unf did not differ significantly (Table 2). At at least 2 time points, VBR was higher in the <5 µm and Unf treatments than in the <0.8 µm treatment. A significant difference between <5 µm and Unf treatments was only found for the dam site at t_{48}.

In both experiments, bacterial abundance in the <0.8 µm treatment increased sharply due to the reduction in grazing pressure (Šimek et al. 2003). Between 24 and 48 h, bacterial abundance was roughly similar in the <5 µm and the Unf treatment and consistently lower than in the grazer-free <0.8 µm treatment (Table 1). In the <0.8 µm treatment, bacterial abundance was 3-fold higher in the River-2000 than in the Dam-2000 experiment. Overall, differential filtration had a similar effect on the dynamics of viral and bacterial abundance compared to the Dam-1999 experiment (Šimek et al. 2001).

Table 1 presents a summary of viral and bacterial parameters determined in the Dam-2000 and River-2000 populations, and also includes the Dam-1999 experiment data (Šimek et al. 2001) for comparison. For the viral parameters of the Dam-1999 experiment, only data on viral abundance and virus-induced mortality of bacteria were shown in the previous publication; however, data on virus-induced mortality were recalculated using new conversion factors. In both experiments, viral net growth rate, bacterial standing stock and production lysed by viruses on a daily basis were lower in the <0.8 µm treatment than in the <5 µm treatment. In the Unf treatment, most of these parameters were between values in the <0.8 µm and the <5 µm treatments. Burst size was lowest in the Unf treatments and roughly similar in the 2 other treatments; however, differences between treatments were rather small. Viral growth rates ranged from 0.37 to 0.48 d^{-1} in the Dam-1999 experiment, from 0.23 to 0.28 d^{-1} in the Dam-2000 incubations and from 0.53 to 0.62 d^{-1} in the River-2000

incubations. The averaged virus-induced removal rate of bacterial production ranged from 18 to 66% d^{-1} across treatments and experiments.

Statistical relationships

A correlation analysis of FVIC abundance with other parameters was used to reveal potential trends across experiments and treatments as well as across experiments in different size fractions (Table 3). Across experiments and treatments, FVIC was related best to HNF abundance, and grazing followed by average bacterial cell volume (r = 0.670 to 0.841, p > 0.0001). In the <0.8 µm treatment, FVIC was most significantly related to bacterial abundance (r = 0.766, p < 0.005). In the <5 µm treatment, FVIC was related best to HNF abundance and grazing (r > 0.9, p < 0.0001) and to cell volume (r = 0.841, p ≪ 0.0005).

Table 2. Results (p-values) of ANOVA and post-hoc tests of pair-wise comparisons between treatments at t_{48}, t_{72} and t_{96} incubated at the dam and river sites. Significant differences are shown in **bold**. Unf: unfiltered, ns: not significant

Parameter	Dam site			River site		
Treatments tested	t_{48}	t_{72}	t_{96}	t_{48}	t_{72}	t_{96}
Viral abundance						
<0.8 µm vs <5 µm	**<0.05**	ns	**<0.05**	**<0.05**	**<0.01**	ns
<0.8 µm vs UNF	ns	**<0.05**	ns	ns	**<0.01**	ns
<5 µm vs UNF	ns	ns	ns	**<0.05**	**<0.05**	ns
VBR						
<0.8 µm vs <5 µm	**<0.05**	ns	**<0.01**	**<0.01**	**<0.05**	**<0.01**
<0.8 µm vs UNF	**<0.05**	ns	**<0.05**	**<0.01**	ns	**<0.01**
<5 µm vs UNF	**<0.05**	ns	ns	ns	ns	ns
FVIC						
<0.8 µm vs <5 µm	ns	**<0.05**	ns	ns	**<0.05**	ns
<0.8 µm vs UNF	ns	**<0.05**	ns	ns	**<0.05**	ns
<5 µm vs UNF	ns	ns	ns	ns	ns	ns

DISCUSSION

The microbial community examined in this study came from a single environment, the dam site of the Rímov Reservoir, and filtration was used to manipulate grazing pressure. Within Expt 2000, we also transplanted the dam community to higher nutrient concentrations at the river site to increase the resource availability. Thus, we studied a community of microorganisms from a single environment under distinct nutrient and grazer control regimes. A pronounced increase of viral abundance and viral infection of bacteria due to nutrient enrichment was documented, which was stronger than the stimulation of viral production induced by grazing. The response of the viral community to grazer control was consistent for all experiments, resulting in a higher viral abundance and production in the presence of grazing.

Effect of resource enrichment on viral production and infection

The transplanted populations served as a case study to investigate the effect of increased inorganic nutrient and DOM concentration on viral activity. We found that relieving nutrient stress (probably P-limitation; Šimek et al. 2003) was associated not only with an enhanced bacterial growth rate, but was also coupled with an increased viral production and standing stock of viruses as well as an increased impact of viruses on bacterial mortality in all treatments. The higher percentage of bacteria detectable with fluorescent *in situ* hybridisation (FISH), and the

Table 3. Correlation of the frequency of visibly infected cells (FVIC) with bacterial, viral and flagellate parameters across all data from Expts 1999 and 2000 as well as for all data from these experiments in size fractions <0.8 and <5 µm. FVIC data were arcsine-transformed and all other parameters were log-transformed. Significant differences are shown in **bold**. r: correlation coefficient; p: significance value; ns: not significant

Parameter	All			<0.8 µm			<5 µm		
	n	r	p	n	r	p	n	r	p
Bacterial abundance	41	−0.055	ns	14	0.766	**<0.005**	14	−0.048	ns
Average cell volume	41	0.670	**<0.0001**	14	0.517	ns	14	0.841	**<0.0005**
Bacterial production	41	0.206	ns	14	0.627	**<0.05**	14	0.112	ns
Bacterial growth rate	41	0.315	**<0.05**	14	0.197	ns	14	0.189	ns
Viral abundance	41	0.536	**<0.0005**	14	0.568	**<0.05**	14	0.468	ns
Viruses to bacteria ratio	41	0.457	**<0.005**	14	0.042	ns	14	0.385	ns
Heterotrophic nanoflagellates abundance	27	0.841	**<0.0001**	na	na	na	14	0.922	**<0.0001**
HNF grazing	27	0.789	**<0.0001**	na	na	na	14	0.901	**<0.0001**

higher hybridisation signal also implies a higher cell-specific activity of bacteria in the resource-enriched treatments (Šimek et al. 2003). Some studies performed along trophic gradients have reported that viral production or infection frequencies of bacteria increase towards more productive systems (Steward et al. 1992, 1996, Weinbauer et al. 1993). Mesocosm and batch culture experiments indicate that adding nutrients stimulates viral proliferation in natural communities (Tuomi et al. 1995, Wilson et al. 1996, Tuomi & Kuuppo 1999). P-limitation might be particularly important, since phosphorus is a significant component of DNA, and DNA comprises a large portion of the virus particles (Wilson et al. 1996). Our study is novel in experimentally confirming the stimulating effect of an increased availability of natural inorganic and organic nutrients on the virus-induced removal rate of bacterial production and demonstrating this also in the <0.8 µm treatment, where indirect effects by higher trophic levels can be excluded. Moreover, FVIC was related best to bacterial abundance in the <0.8 µm treatment (Table 3). It is noteworthy that the dialysis bag approach allowed for a constant supply of nutrients in contrast to the closed system of batch cultures with no exchange of nutrients with the surrounding environment. Since viruses hijack the metabolism of the hosts, they are clearly but indirectly influenced by nutrient limitation, which can directly control the resource for viruses, i.e. the hosts.

Transmission electron microscope-based estimates of virus-induced mortality of bacteria in freshwater systems ranged from 0 to 130% (reviewed in Weinbauer et al. 2002). With an average bacterial production lysed by viruses of ca. 18 to 66% d^{-1}, the values from the Rimov Reservoir experiment belong to the higher range of values reported in the literature and were only higher in a eutrophic lake of the Danube River (Fischer & Velimirov 2002). High lysis rates imply a significant influence on host diversity and food web processes and the cycling of elements.

Unfortunately, it is difficult to compare viral- and grazing-induced mortality in the experimental incubations. Grazing of protists on bacteria was estimated using fluorescently labelled small bacteria made at the start of the experiment (Šimek et al. 2003). The shift of the bacterial community towards larger filamentous bacteria and small flocs during the experiment has likely resulted in an overestimation of grazing because grazing rates on these grazing-resistant forms were likely lower or insignificant. Thus, we could not directly compare virus-induced and grazing-induced mortality.

Transplantation to the nutrient-rich river site had a stimulating effect on all viral parameters except burst size. Bacterial production lysed per day was stimulated by almost 2-fold in all treatments due to resource enrichment (Table 1). This effect was similar in all size fractions. In contrast, zooplankton could not cope with enhanced microbial growth (bacteria and HNF) at higher nutrient supply, suggesting an uncoupling of the top-down control by zooplankton (Šimek et al. 2003).

Stimulation of viral production by grazing

Based on the reasonable assumption that flagellates also graze on infected cells (Binder 1999) and remove non-infected potential hosts, it could be assumed that grazing pressure should result in reduced viral production. However, viral abundance was consistently highest in the <5 µm treatments in all experiments and lowest in the grazer-free treatment, suggesting that the presence of grazers stimulated viral production. This is consistent with a preliminary study performed in the same environment (Šimek et al. 2001). Burst size changes cannot explain the increase of viral abundance in the <5 µm treatment, since the burst size differed only moderately between treatments and the lowest burst size was typically found in the Unf treatment. A higher infection frequency and a higher viral proliferation rate as indicated by the higher FVIC and net viral growth rate values (Fig. 1, Table 1) are a likely explanation for the higher viral abundance in the <5 µm treatments. Also, the finding that FVIC was better related to HNF abundance and grazing than to bacterial abundance and production across experiments and treatments (Table 2) suggests a significant effect of HNF on viral infection in the performed experiments. In addition, viruses infecting HNFs might have contributed to the high viral abundance in the <5 µm treatments.

A variety of reasons, not mutually exclusive, might be responsible for enhanced viral production and viral infection. First, it has been argued that prophage induction could be a result of increased growth rates of the lysogenic hosts (Wilson & Mann 1997). This has been documented for isolated virus-host systems (Calendar 1970, Gottesman & Oppenheim 1994). Moreover, Ripp & Miller (1998, 1997) have suggested that pseudolysogeny can be regarded as the phenomenon where there is not enough energy available in starved cells for the phage to enter either the lytic or the lysogenic life cycle. Supplying nutrients could then be the trigger for forcing viruses to enter 1 of the 2 cycles. Thus, the stimulation of bacterial growth rates by flagellate grazing (Posch et al. 1999, Šimek et al. 2001), which was also shown in the present study (Table 1) (Šimek et al. 2003), could be an inducing mechanism or

force pseudolysogens in the lytic cycle, and thus explain the increase of viral growth in the <5 µm treatment. Although there is no evidence at the community level that an increased growth rate is an inducing mechanism in bacterioplankton communities (Wilcox & Fuhrman 1994, Weinbauer & Suttle 1996) or that pseudolysogeny is significantly involved in viral production (Weinbauer et al. 2002), these possibilities cannot be excluded.

Second, changes in the bacterial community composition due to grazing favour species which are either capable of rapid growth or resistant to grazing, either of which may be less resistant to viral infection. Indeed, incorporating grazers into modelling the effect of viral infection on resistant and susceptible cells has shown that grazing of resistant cells may provide a refuge for virus-sensitive cells, which can support viral infection (Middelboe et al. 2001). Grazing had an effect on bacterial community composition in the performed experiments as determined by FISH (Šimek et al. 2001, 2003). For example, a taxonomically narrow group of bacteria detected with the specific probe R-BT065 targeted against one of the lineages of β-proteobacteria increased in the grazer-free treatments and never formed grazing-resistant flocs or filaments in other treatments. In contrast, bacteria detected by BET42a targeted against the entire β-proteobacteria group increased in the presence of grazers and were mostly found in the outer parts of large grazing resistant flocs (see Fig. 5 in Šimek et al. 2001). It is tempting to assume that these grazing-resistant cells proliferating and dominating in the <5 µm treatment were more sensitive to viral infection and responsible for the higher FIC values in this treatment. Thus, shifts in the community composition could be responsible for the higher virus-induced mortality of bacteria.

Third, grazing usually stimulates bacterial growth rates, e.g. by regenerating nutrients such as phosphorus and releasing easily degradable organic material such as free amino acids (Andersson et al. 1985), and a stimulation of bacterial growth was also observed in the experiments (Table 1) (Šimek et al. 2001, 2003). A general stimulation of bacterial growth may result in enhanced viral production and infection cycles, thus causing the higher virus-induced mortality of bacteria and viral abundance found in the presence of grazers.

Overall, the reason for the higher viral abundance and viral infection of bacterioplankton in the presence of grazing remains obscure. However, there is no evidence that this stimulation of viral production and viral infection of bacteria was affected by relieving nutrient limitation (Fig. 1), suggesting a potential independence of this interaction from the trophic status.

CONCLUSIONS

Our data show that grazing did not reduce viral attack on bacteria but increased the proportion of bacterial production removed by viral lysis by 35 to 55%. This study underlines the need to study both grazing and viral infection as factors causing cell death and influencing nutrient cycling and bacterial community composition. It also shows that even at the community level, interactions between the 'black boxes' bacterioplankton, viruses and grazers are more complex than hitherto assumed. Moreover, the transplantation experiment indicates that the viral control of bacterial production increases at a higher supply rate of nutrients.

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