Significance of Viral Lysis and Flagellate Grazing as Factors Controlling Bacterioplankton Production in a Eutrophic Lake

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Received 19 August 1997/Accepted 14 November 1997

The effects of viral lysis and heterotrophic nanoflagellate (HNF) grazing on bacterial mortality were estimated in a eutrophic lake (Lake Plußsee in northern Germany) which was separated by a steep temperature and oxygen gradient into a warm and oxic epilimnion and a cold and anoxic hypolimnion. Two transmission electron microscopy-based methods (whole-cell examination and thin sections) were used to determine the frequency of visibly infected cells, and a model was used to estimate bacterial mortality due to viral lysis. Examination of thin sections also showed that between 20.2 and 29.2% (average, 26.1%) of the bacterial cells were empty (ghosts) and thus could not contribute to viral production. The most important finding was that the mechanism for regulating bacterial production shifted with depth from grazing control in the epilimnion to control due to viral lysis in the hypolimnion. We estimated that in the epilimnion viral lysis accounted on average for 8.4 to 41.8% of the summed mortality (calculated by determining the sum of the mortalities due to lysis and grazing), compared to 51.3 to 91.0% of the summed mortality in the metalimnion and 88.5 to 94.2% of the summed mortality in the hypolimnion. Estimates of summed mortality values indicated that bacterial production was controlled completely or almost completely in the epilimnion (summed mortality, 66.6 to 128.5%) and the hypolimnion (summed mortality, 43.4 to 103.3%), whereas in the metalimnion viral lysis and HNF grazing were not sufficient to control bacterial production (summed mortality, 22.4 to 56.7%). The estimated contribution of organic matter released by viral lysis of cells into the pool of dissolved organic matter (DOM) was low; however, since cell lysis products are very likely labile compared to the bulk DOM, they might stimulate bacterial production. The high mortality of bacterioplankton due to viral lysis in anoxic water indicates that a significant portion of bacterial production in the metalimnion and hypolimnion is cycled in the bacterium-virus-DOM loop. This finding has major implications for the fate and cycling of organic nutrients in lakes.

In a seminal paper, Pomeroy (47) showed that bacteria play a major role in the cycling of energy and matter in aquatic systems. The development of techniques which allowed quantification of bacterial abundance (31) and production (21) was a milestone in the investigation of the ecology of bacterioplankton. Later, Azam et al. (2) developed the concept of the “microbial loop,” in which bacteria recycle organic matter which otherwise would be lost from the food web. These findings have stimulated a large amount of research on the mechanisms which regulate bacterial biomass and processes in aquatic systems.

There is an ongoing debate about whether bacterial production and biomass are regulated by available resources (bottom-up control) or by predators (top-down control). On the basis of a cross-system survey, Billen et al. (8) argued that bacteria are controlled by resources. Similar conclusions were drawn from other cross-system investigations (9, 14), and Pace and Cole (44) found no evidence in experimental studies that protozoa effectively regulate bacterial abundance. Other workers have argued that bacterial mortality is largely due to protist grazing (19, 54), and after reviews of the literature, Sanders et al. (51) and Berninger et al. (6) described a strong relationship be-

tween bacterial abundance and heterotrophic nanoflagellate (HNF) abundance and suggested that significant predatory control of bacteria occurs. However, it has also been shown that bacteria and HNFs are not strongly coupled across systems, and, consequently, HNFs do not always control bacterial abundance (25), probably because of predatory control of HNFs by larger zooplankton (e.g., daphnids) (24). Ducklow and Carlson (18) have argued that the control mechanisms may change seasonally. The finding that the range of estimated clearance of bacteria in the water column due to HNF grazing is large, 5 to 250% per day (1), further supports the notion that the effect of grazing on the control of bacterioplankton changes with time and space. Thus, the key problem might be determining where and when protist grazing is important for regulating bacterioplankton.

In the late 1980s it was shown that in marine and limnetic systems viral particles occur in great numbers which usually exceed even the bacterial numbers (5, 48, 59). It was concluded that the majority of viruses are bacterial viruses (bacteriophages) and that viral lysis is a major cause of bacterial mortality. On average, ca. 10 to 20% of the bacterial production is lysed daily by viruses (58). Thus, viral lysis is an additional mechanism which may contribute to the regulation of bacterial production and processes. As viruses cause mortality of bacteria, they are responsible in part for the top-down type of control, as are the protists. The effect of viral lysis on bacterial mortality has been compared with the effect of flagellate grazing in various oceanic systems (22, 28, 55, 66), and the results

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have shown that the proportion of bacterial production that is removed by viral lysis can be as high as the proportion that is removed by grazing. A major consequence of these findings is that a significant fraction of bacterial carbon is probably not transferred to higher trophic levels, but is cycled in a bacterium-virus-dissolved organic matter (DOM) loop. Thus, this "viral loop" (11) might be a mechanism for controlling bacterial production.

Some information concerning the spatiotemporal distribution of viruses, the mechanisms controlling viral abundance and infectivity, and the role of viral lysis in regulating bacterial production in oceanic systems has been accumulated (12, 20, 23, 58, 61), whereas there have been only a few reports on limnetic systems (30, 37–39). The only two reports which have estimated the contribution of viral lysis to bacterial mortality in freshwater systems indicate that viral lysis can occasionally be an important factor in the control of bacterial production (30, 39). Our knowledge concerning the significance of viral lysis for bacterial production in oxic waters of limnetic systems is still sparse, and there is no information concerning the significance of viral lysis for bacterial production in anoxic waters. Since grazing rates are typically low in anoxic waters, other mechanisms, such as viral lysis, must be responsible for bacterial mortality. Thus, we quantified the roles of viral lysis and flagellate grazing in the control of bacterial production in the oxic and anoxic water layers of a stratified lake. Our data indicate that the rates of removal of bacterial production by flagellate grazing decreased with depth (i.e., with decreasing oxygen concentration), whereas viral lysis was predominant in anoxic waters.

MATERIALS AND METHODS

Sampling and study site. The study site was Lake Plußsee (10°23'E, 54°10'N) near Plön in Schleswig-Holstein in northern Germany. Protection against wind exposure, a lack of discharging rivers or creeks, and a comparatively small size result in stratification of the lake which is extremely stable and lasts from spring to fall (35). On 23 September 1996 water samples were collected along a depth profile with a Ruttner sampler from a permanent platform located in the center of the lake. Subsamples were preserved in formaldehyde (final concentration, 2%) and kept at 4°C in the dark. Oxygen and temperature profiles were determined with a microprocessor oximeter (model OX 196; Wissenschaftlich-Technische Werkstätten, Weilheim, Germany) equipped with a model 2BK 90190 oxygen detector and a thermometer.

Microbial cell counts and chlorophyll *a* concentrations. Bacteria and viruses were stained with 4',6-diamidino-2-phenylindole (DAPI) (final concentration, 1 µg ml⁻¹) and were enumerated by epifluorescence microscopy by using slight modifications of the protocols described by Turley (62) and Suttle (57). Samples (1 ml) examined for bacteria and viruses were stained without DNase treatment for 30 min, filtered onto 0.02-µm-pore-size Anodisc filters (Whatman), and enumerated by epifluorescence microscopy (Axiovert model 135TV microscope; Zeiss) as described by Weinbauer and Suttle (67). Samples (5 ml) examined for HNFs were stained with DAPI, filtered onto 0.2-µm-pore-size black polycarbonate filters (Nuclepore), and enumerated by epifluorescence microscopy as described by Sherr et al. (53). For two samples of every depth layer, bacterial counts obtained by using DAPI staining and epifluorescence microscopy were compared with bacterial counts obtained by transmission electron microscopy (TEM) (see below), and the values obtained by the two methods did not differ significantly (data not shown). Chlorophyll *a* concentrations were determined spectrophotometrically as described by Parsons et al. (45).

Bacterial production. Bacterial production was estimated by the [³H]thymidine (83.0 Ci mmol⁻¹; Amersham) incorporation method (21). Samples from various depths were placed in 100-ml acid-cleaned conical shoulder reagent bottles and spiked with [³H]thymidine at a final concentration of 20 nM. The rate of incorporation of [³H]thymidine into the trichloroacetic acid-insoluble macromolecular fraction is constant for bacterioplankton in Lake Plußsee at concentrations of ≥15 nM (13). The flasks were mounted on iron racks, protected from sunlight by polyvinyl chloride tubes, and deployed from a fixed platform to the depths from which the samples were collected previously. The samples were deployed for ca. 2 h, and the incorporation of label was stopped with formaldehyde (final concentration, 2%). Samples were deployed in duplicate, and duplicate formaldehyde-killed samples were used as controls. Five-milliliter subsamples were filtered onto cellulose nitrate filters (pore size, 0.22 µm; type GSWP; Millipore), and the [³H]thymidine was extracted by two 10-min incubations with 5% ice-cold trichloroacetic acid (Sigma Chemical Co.). The filters

were dissolved with a scintillation cocktail, and radioactivity was determined with a model 8500 Packard Tri-Carb scintillation counter. Conversion factors for relating thymidine incorporation to cell production were obtained from values determined in the fall during a seasonal study performed in Lake Plußsee, and these conversion factors were 1.92 × 10⁶ cells pmol⁻¹ for the euphotic zone and 1.57 × 10⁶ cells pmol⁻¹ for deeper water (13).

Determination of visibly infected bacteria, viral burst size, and cell integrity. A TEM-based method was used to determine the number of visibly infected cells (64). In the modified method used, samples were not incubated with streptomycin, and a lower centrifugation speed and less time were used. Bacteria from 10-ml samples were collected quantitatively onto Formvar-coated, 400-mesh electron microscope grids by centrifugation at 66,000 × *g* for 20 min in a swinging-bucket rotor (Beckmann type SW-41), stained for 30 s with 1% uranyl acetate, and rinsed three times with deionized distilled water. The time and speed of centrifugation used reduced the disruption of infected bacteria, and, as few viruses were pelleted, phage in bacteria were easily distinguished. Grids were examined for visibly infected cells by using a TEM (model CEM 902; Zeiss) operating at an accelerating voltage of 80 kV. Between 200 and 2,000 cells per sample were examined for mature phages inside the cells in order to obtain at least 10 visibly infected cells, and a minimum of five phage were observed in each visibly infected cell. Viruses inside cells were identified based on structure, size, intensity of staining, and uniformity of structure, size, and staining intensity. The viral burst size (i.e., the number of viruses produced in a cell) was estimated from all of the visibly infected cells in a sample as described previously (64). Total bacterial abundance values were also obtained from the TEM grids (67).

To estimate the frequency of visibly infected cells (FVIC) and the cell integrity from thin sections, glutaraldehyde (final concentration, 2%)-preserved bacterioplankton from ca. 1 liter were collected on a 0.2-µm-pore-size polycarbonate filter (Nuclepore) by using the protocol of Hennes and Simon (30). The bacteria were immobilized in 2% agar, postfixed with 1% osmium tetroxide, dehydrated with acetone, and embedded in standard Spurr medium. Ultrathin sections were stained with uranyl acetate and lead citrate. The visibly infected cells in the thin sections were identified as described by Proctor et al. (50), and a minimum of three phage were observed in each cell. The integrity of cells was determined from thin sections by using the conservative criteria described by Heissenberger et al. (29). Only those cells which were completely empty or contained only amorphous cytoplasmic structures in combination with a detached inner membrane were considered dead (ghosts).

Flagellate grazing. Grazing rates were estimated as described by Steward et al. (55) as the product of the bacterial abundance and the HNF abundance determined along the depth profile and clearance rates obtained from previously published papers. The average clearance rates (± standard error) of HNFs in Lake Plußsee during the entire stratification period were 0.60 ± 0.38 nl cell⁻¹ h⁻¹ in the epilimnion, 0.60 ± 0.40 nl cell⁻¹ h⁻¹ in the metalimnion, and 0.27 ± 0.13 nl cell⁻¹ h⁻¹ in the hypolimnion (calculated from the data in reference 40).

Bacterial mortality and viral production. A model was used to estimate bacterial mortality due to viral lysis (50). This model assumed that the proportion of bacterial mortality due to viral lysis was about equal to the FVIC multiplied by conversion factors (average, 10.84; range, 7.4 to 14.28). Bacterial mortality due to flagellate grazing was calculated by dividing the grazing rate by the bacterial production rate. Summed bacterial mortality was calculated by determining the sum of mortality due to grazing and mortality due to viral lysis. In a steady-state system bacterial mortality due to viral lysis matches the bacterial production which is removed by lysis (58). Thus, multiplying the lysed bacterial production by the burst size yielded viral production. The concentration of carbon released by viral lysis of bacteria was calculated from the viral lysis rate of bacteria, the cell volumes determined in Lake Plußsee along the depth profile on 23 September 1996 (63), and a conversion factor of 350 fg of C µm⁻³ (36) for relating the bacterial biovolume to the carbon content. The release of nitrogen and phosphorus was estimated by using an N/C ratio of 0.26 (10) and a P/C ratio of 0.04 (15). We further assumed that all biomass was released as dissolved compounds.

Contact rates. The rate of contact (*R*) between viruses and bacteria was calculated by using the following formulae (43): $R = (Sh2\pi wD_v)VP$, where *Sh* is the Sherwood number (1.06 for a bacterial community with 10% motile cells [68]), *w* is the cell diameter (calculated from the mean bacterial cell volume determined at each depth [63], assuming that the cells are spheres), *V* and *P* are the abundance of viruses and the abundance of undamaged cells, respectively, and *D_v* is the diffusivity of viruses, and $D_v = kT/3\pi\mu d_v$, where *k* is the Boltzmann constant (1.38 × 10⁻²³ J K⁻¹), *T* is the in situ temperature (in degrees Kelvin), *μ* is the viscosity of water (in pascals per second; *μ* was calculated from values given by Schwörbel [52] for temperatures in the range from 4 to 15°C), and *d_v* is the diameter of the viral capsid (73 nm in Lake Plußsee [16]). The contact rate was corrected for bacterial abundance to estimate the number of contacts per cell on a daily basis.

Carbon, phosphorus, and nitrogen concentrations. On 23 September 1996, soluble reactive phosphorus (SRP), NO₃, and NH₄ concentrations were determined along the depth profile by routine limnological methods (27) and were provided by the Max Planck Institute for Limnology, Plön, Germany. Dissolved organic carbon (DOC) concentrations were determined from water samples passed through 0.2-µm-pore-size polycarbonate filters (Nuclepore) that were rinsed with Milli Q water. DOC concentrations were measured by the high-temperature combustion method (56) by using a Shimadzu model TOC-5000

FIG. 1. Depth profiles for temperature and oxygen, DOC, and inorganic nitrogen and phosphorus (SRP) concentrations in Lake Plüßsee on 23 September 1996.

analyzer with a platinum catalyst on quartz and performing regular blank monitoring (4). The contamination of samples by leaching of carbon from the polycarbonate filters was less than 5% of the DOC concentration (data not shown).

Statistical analyses. All data were log transformed for statistical analyses. Analysis of variance and Fisher PLSD post hoc tests (StatView D-4.5 program) were used to determine whether parameters differed significantly between depth layers. A probability of <0.05 was considered significant.

RESULTS

Characterization of study site. Temperature and oxygen profiles showed that the pelagic zone of Lake Plüßsee was stratified and separated into three distinct layers, the oxic epilimnion, the thermocline layer (metalimnion), and the anoxic hypolimnion (Fig. 1). As determined by an analysis of variance, the SRP and NH_4 concentrations increased significantly with depth, whereas the DOC and NO_3 concentrations decreased significantly with depth. The DOC concentrations ranged from 9.2 to 14.5 mg liter^{-1} .

Bacterial production and abundance of microorganisms. All microbial parameters differed along the depth profile (Fig. 2). Bacterial production was significantly higher in the metalimnion than in the other layers, which is consistent with the high bacterial numbers in this layer. Also, the viral abundance values and chlorophyll *a* concentrations were significantly higher in the metalimnion than in the epilimnion and the hypolimnion, whereas the HNF abundance values decreased significantly with depth. For one depth of every depth layer thin sections and TEM were used to check for cell integrity. We found that between 70.8 and 79.1% (average, 73.9%) of the cells were intact (Table 1).

Visibly infected bacteria. For one depth of every depth layer we determined the FVIC by two methods, whole-cell examination and thin-section examination (Table 1). The FVIC obtained in the whole-cell examination were between 74.8 and 85.0% (average, 78.7%) of the FVIC obtained in the thin-

FIG. 2. Depth profiles for microbial parameters in Lake Plüßsee on 23 September 1996. Chl *a*, chlorophyll *a*.

TABLE 1. Frequency of intact bacteria and FVIC in different depth layers of Lake Plußsee

Layer	Depth (m)	% Intact cells	FVIC (%) as determined from examination of:		Whole-cell/thin-section ratio
			Thin sections	Whole cells ^a	
Epilimnion	1.0	70.8	1.53	1.30	0.850
Metalimnion	6.5	79.8	2.86	2.14	0.748
Hypolimnion	15.0	71.1	3.30	2.52	0.764

^a The FVIC was not corrected for ghost cells (see text).

section examination. In all samples we observed bacterial cells which were visibly infected with viruses. When corrected for the average percentage of nonintact cells, the FVIC ranged from 0.7 to 9.0% of the total bacterial numbers, and the FVIC increased significantly with depth (Fig. 2), averaging 1.6% in the epilimnion, 3.0% in the metalimnion, and 6.3% in the hypolimnion.

Bacterial mortality and viral production. The bacterial mortality due to viral lysis and flagellate grazing was estimated along the depth profile in Lake Plußsee (Fig. 3) and was expressed as a ratio of the rate of removal (by lysis or grazing) to the bacterial production rate. We estimated that viral lysis removed on average 7.7 to 27.8% of the bacterial production in the epilimnion, 19.6 to 46.8% of the bacterial production in the metalimnion, and 38.4 to 97.3% of the bacterial production in the hypolimnion. Bacterial mortality due to flagellate grazing showed a trend opposite that found for viral lysis. While flagellate grazing removed on average 81.8 to 108.0% of the bacterial production in the epilimnion, the levels of mortality due to grazing were 2.9 to 27.6% in the metalimnion and 5.0 to 8.9% in the hypolimnion. In the epilimnion the summed mortality (calculated by determining the sum of the average mortality due to viral lysis and the average mortality due to grazing) was 66.6 to 128.5%, compared to 22.4 to 56.7% in the metalimnion and 43.4 to 103.3% in the hypolimnion. In the epilimnion 8.4

TABLE 2. Ratio of bacterial mortality due to viral lysis to bacterial mortality due to flagellate grazing and viral production in different depth layers of Lake Plußsee

Layer	Depth (m)	Viral lysis/grazing ratio ^a	Burst size ^a	Viral production (10 ¹⁰ viruses liter ⁻¹ day ⁻¹) ^{a,b}
Epilimnion	0–5	0.3 (0.1–0.7)	33.9 (18.9–35.3)	0.15 (0.10–0.20)
Metalimnion	5.5–9	6.0 (1.1–10.1)	44.3 (31.7–55.2)	1.89 (1.29–2.49)
Hypolimnion	10–25	10.6 (7.7–16.2)	62.8 (44.3–87.0)	1.20 (0.82–1.59)

^a Values are means calculated from three or four samples. The values in parentheses are ranges.

^b Bacterial production was considered constant throughout the hypolimnion (13).

to 41.8% of the summed mortality could be ascribed to viral lysis, compared to 51.3 to 91.0% in the metalimnion and 88.5 to 94.2% in the hypolimnion. The average ratios of viral lysis to grazing were 0.3 in the epilimnion, 6.0 in the metalimnion, and 10.6 in the hypolimnion (Table 2).

In the epilimnion on average 0.15×10^{10} viruses liter⁻¹ day⁻¹ were produced, whereas in the deeper water layers viral production was ca. 1 order of magnitude higher, averaging 1.9×10^{10} viruses liter⁻¹ day⁻¹ in the metalimnion and 1.2×10^{10} viruses liter⁻¹ day⁻¹ in the hypolimnion (Table 2).

Contact model. In the epilimnion we calculated an average contact rate of 49.0 viral contacts cell⁻¹ day⁻¹, compared to 180.4 viral contacts cell⁻¹ day⁻¹ in the metalimnion and 141.8 viral contacts cell⁻¹ day⁻¹ in the hypolimnion (Fig. 4).

Organic matter set free during viral lysis of bacteria. The estimated concentrations of organic carbon, nitrogen, and phosphorus released during viral lysis of bacterioplankton were more than 1 order of magnitude lower in the epilimnion than in the two deeper water layers (Table 3). The highest release rates were found in the hypolimnion. We estimated that the contribution of organic carbon released by viral lysis to the standing stock of DOC was <0.1% per day.

DISCUSSION

The most significant finding of this study was the shift in the mechanisms controlling bacterial production along the depth profile. Our data indicate that grazing was the main cause of bacterial mortality in the oxic epilimnion, whereas viral lysis predominated in the anoxic hypolimnion. This shift may have major implications for the fate and cycling of organic matter in stratified lakes.

FIG. 3. Depth profile for bacterial mortality due to viral lysis and grazing in Lake Plußsee on 23 September 1996. The summed mortality was calculated by determining the sum of the mortality due to viral lysis and the mortality due to grazing. Error bars indicate the ranges of conversion factors for viral lysis and from the standard error for grazing.

FIG. 4. Viral contact rates in different depth layers of Lake Plußsee on 23 September 1996. Contact rates were estimated from a contact model for viruses and bacteria (43). The values are the means $\pm$ standard deviations from four samples.

TABLE 3. Estimates of carbon, nitrogen, and phosphorus concentrations released by viral lysis of the bacterial community in different depth layers of Lake Plußsee

Layer	Depth (m)	Concn ($\mu\text{g liter}^{-1} \text{ day}^{-1}$) of ^a :			Contribution of lysis to DOC (% day^{-1})
		C	N	P	
Epilimnion	0–5.3	0.36 (0.25–0.48)	0.09 (0.06–0.12)	0.014 (0.010–0.019)	0.003
Metalimnion	5.5–9	5.92 (4.04–7.79)	1.54 (1.05–2.03)	0.237 (0.162–0.312)	0.056
Hypolimnion	10–25	8.08 (6.13–11.82)	2.33 (1.59–3.07)	0.359 (0.245–0.473)	0.091

^a Values are means calculated from three or four samples. The values in parentheses are ranges.

Critical assumptions in estimation of bacterial mortality due to viral lysis. Conversion factors were used to relate the FVIC to bacterial mortality due to viral lysis. These conversion factors were derived from thin sections of isolated virus-host systems (50), whereas we examined whole cells. However, previously we have argued that the conversion factors are also applicable to whole cells (64). A study has shown that the FVIC determined by using whole-cell examination was much lower than the FVIC obtained by using thin sections (22). The high centrifugation speeds and the times usually used for the whole-cell method can disrupt infected cells (unpublished data) and might have resulted in the lower FVIC obtained with this method by Fuhrman and Noble (22). In our study we reduced the centrifugation speed and time, which avoided disruption of visibly infected cells, and we found that the values obtained from the whole-cell examination averaged 78% of the values obtained by using thin sections (Table 1). Thus, the whole-cell method systematically underestimated the FVIC; however, the differences were moderate and similar at all depths. The difference between the two methods might be due to pigmentation of cells, which prevented examination by the whole-cell method, although viruses were detected within pigmented *Synechococcus* sp. cells in the Gulf of Mexico (60). Also, cells might have lysed during centrifugation, although we reduced the centrifugation speed and time. Moreover, viruses within cells might be more easily detected in thin sections (detection limit, three viruses) than in whole cells (detection limit, five viruses). Overall, our data indicate that the whole-cell examination resulted in a conservative estimate of the effect of viral lysis on bacteria.

Frequency of visibly infected bacteria. FVIC data are available for a variety of freshwater and marine environments. In marine waters, the FVIC ranged from 0 to 7% in free-living and attached bacteria from various environments (48, 49, 55, 66). Along a salinity gradient in solar salterns, the FVIC were between <0.04 and 3.76% (28). In Lake Constance, the FVIC ranged from <0.1% to $1.7\% \pm 1.2\%$ (30), and the FVIC in a backwater system of the River Danube were between 0 and 4% (39). In Lake Plußsee the FVIC ranged from 0.7 to 9.0% (Fig. 2). The value obtained for the anoxic hypolimnion (9.0%) is the highest FVIC published so far for natural bacterial communities (when the FVIC was not corrected for damaged cells, a maximum value of 6.4% was observed). For an as-yet-undiscovered archaeal species in a solar saltern a maximum FVIC of 6.7% was observed (28). Bratbak et al. (12) obtained values of up to 40% by using whole-cell examination after incubation of the entire bacterial community with streptomycin, which supports lysis of the cells. It is not clear why the streptomycin treatment resulted in those high FVIC. However, since streptomycin can induce the lytic cycle in lysogenized bacteria (7), induction of lysogenized cells could have contributed to the high FVIC.

Frequency of intact cells. It has been known for a long time that only a fraction of the bacterioplankton community is ac-

tive or alive. The data of Zweifel and Hagström (69) indicated that only 2 to 32% of the members of natural marine bacterial communities collected in the Baltic Sea, the North Sea, and the Mediterranean Sea contained a nucleoid, and these authors assumed that the non-nucleoid-containing bacteria (ghosts) (i.e., the majority of bacterioplankton) are inactive or even dead. However, this method resulted in an underestimate of the living fraction of bacterioplankton in samples from the coastal Pacific Ocean off California when the data were compared with data obtained by autoradiography and with universal 16S rRNA-targeted probes (34). Using electron microscopy, Heissenberger et al. (29) demonstrated that on average ca. 25% of bacteria from the Mediterranean Sea were empty, ca. 25% were damaged (i.e., they had a shrunken cytoplasmic membrane or only remnants of plasma), and ca. 50% were intact. We found that between 70 and 80% of the bacterial cells were intact in Lake Plußsee. Although it is not certain that all intact cells were alive, it can be assumed that viruses can replicate only in undamaged bacteria. Thus, the FVIC was calculated by determining the percentage of intact cells. Since previously published data on the FVIC in aquatic systems were not corrected for damaged cells, they are probably underestimates of the effect of viral lysis on bacterial mortality. However, data on cell integrity should be viewed with some caution, since it is not possible to completely exclude preparation artifacts.

Viral production. In Lake Constance the estimated production of viruses ranged from 0.1×10^9 to 2.5×10^9 viruses $\text{liter}^{-1} \text{ day}^{-1}$ (30), whereas the estimated production of viruses in a backwater system of the River Danube ranged from 1.0×10^{10} to 3.0×10^{10} viruses $\text{liter}^{-1} \text{ day}^{-1}$ (calculated from the bacterial lysis rate and burst sizes given by Mathias et al. [39]). The average levels of viral production in the different water layers of Lake Plußsee ranged from 0.2×10^{10} to 1.9×10^{10} viruses $\text{liter}^{-1} \text{ day}^{-1}$ (Table 3) and thus were higher than the viral production rates in Lake Constance but slightly lower than the viral production rates found in the River Danube backwater system. Although the FVIC detected in our study occasionally exceeded the values determined by Mathias et al. (39), the viral production rates were lower. This can be explained by the fact that the bacterial production rates were higher in the backwater system (range, 1.4×10^9 to 6.2×10^9 cells $\text{liter}^{-1} \text{ day}^{-1}$) than in Lake Plußsee (range, 0.2×10^9 to 1.5×10^9 cells $\text{liter}^{-1} \text{ day}^{-1}$) and thus sustained higher rates of viral production. Also, differences in the methods used to assess viral production can account for some of the differences between the studies.

Bacterial mortality. When we used the model of Proctor et al. (50) and the average conversion factor, the bacterial mortality due to viral lysis ranged from 7.7 to 27.8% in the epilimnion, from 19.6 to 46.8% in the metalimnion, and from 38.4 to 97.3% in the hypolimnion of Lake Plußsee. When the same approach was used, the bacterial mortality due to viral lysis ranged from less than 0.11 to 18.4% in Lake Constance (cal-

culated from the data in reference 30) and from 10.8 to 43.2% in a backwater system of the River Danube (39). Thus, the metalimnion and hypolimnion of Lake Plußsee exhibited the greatest viral control of bacterial production found so far for limnetic systems.

Studies investigating the contribution of grazing and viral lysis to bacterial mortality have shown that the effect of viral lysis on bacterial mortality varies widely, but can be as large as the effect of grazing (22, 28, 55, 66). In this study we showed that the mechanism for regulating bacterial production changed with depth from grazing control in the epilimnion to control due to viral lysis in the hypolimnion. It has been speculated previously that the role that viruses play in bacterial mortality increases in situations in which flagellate grazing is reduced (66) (e.g., by top-down control of flagellates by larger protists or daphnids [24]). Here, we showed that in anoxic waters, where grazing rates are low, viral lysis is the major factor controlling bacterial production. A lack of control of hypolimnetic bacteria by grazing was also observed in another study of Lake Plußsee (40), and this lack is probably due to a low number of anaerobic protozoan species or to low grazing rates of these species. The bacterial community in the epilimnion and metalimnion is ca. twice as diverse as the bacterial community in the hypolimnion (17, 32). Thus, in addition to the lack of significant grazing, the low diversity of the bacterial community (i.e., high levels of a few host cells) might explain the high mortality due to viral lysis found in this depth layer. The low diversity in the hypolimnion might also explain why bacterial mortality due to viral lysis was higher in the hypolimnion than in the metalimnion, although the contact rates per cell were lower. Low microbial diversity in anoxic waters compared to oxic waters was also detected in the Baltic Sea (33). Additional studies will have to show whether the observed change with depth in the mechanisms that control bacterial production also occurs in other seasons or other systems.

Chróst and Rai (13) found no significant correlation between bacterial biomass and bacterial production in the euphotic zone of Lake Plußsee and argued that this could be the result of strong grazing pressure. In the aphotic zone the strong correlation found between bacterial biomass and bacterial production indicates that there is a low level of predatory control (13). Our data showed that the summed mortality of bacteria is ca. 100% in the epilimnion (Fig. 3), indicating that top-down control of bacterial production occurs in this water layer. In the metalimnion the summed mortality averaged 22.4 to 56.7%, showing that viral lysis and grazing could not control bacterial production. Other predators, such as cladocerans (3), might have been responsible for the remaining mortality. Alternatively, a lack of complete top-down control might have resulted in the high bacterial abundance and production in this water layer. In the hypolimnion most of the mortality could be ascribed to viral lysis and flagellate grazing, since the summed mortality averaged 74.7%. A seasonal study performed in Lake Plußsee showed that cladocerans and protists other than flagellates are rare in the hypolimnion (40) and thus could not contribute significantly to bacterial mortality.

Since we used previously published clearance rates, we might have either underestimated or overestimated grazing, if the actual clearance rates were higher or lower. Thus, the estimated mortality due to grazing should be considered with caution. An underestimate of the clearance rates could have caused a low estimate for summed mortality (e.g., in the metalimnion). However, even if we used the upper ranges of the clearance rates (defined by the standard error) reported for the metalimnion and hypolimnion, the bacterial mortality values due to grazing were only 4.7 to 45.5% and 7.4 to 13.2%,

respectively. Since the whole-cell examination underestimated the FVIC by ca. 20% (Table 1), we might have underestimated the effect of viral lysis. This could explain in part why the summed mortality was not always 100% in the metalimnion and hypolimnion. In the present study predatory control was calculated by dividing grazing rates by bacterial production. However, flagellates might also prey on bacterial ghosts, which do not contribute to bacterial production. Although grazing rates on these cells might be lower than grazing rates on active or living cells, grazing on ghost cells might have resulted in an overestimate of predatory control of bacteria by flagellates.

Organic matter released by viral lysis of bacteria. DOC concentrations decreased slightly with depth, whereas SRP and NH_4 concentrations increased with depth. The high chlorophyll *a* concentrations in the photic zone (Fig. 3) might sustain high concentrations of organic carbon (e.g., concentrations due to photosynthetic extracellular release of carbon-rich substances). This depth distribution is typical for late summer stratification (42) and could indicate that phosphorus or (less likely) nitrogen is the limiting nutrient in the epilimnion, whereas in the hypolimnion bacteria were limited by the supply of organic carbon. More detailed studies have shown that the DOM in the hypolimnion of Lake Plußsee consists of refractory carbon skeletons depleted of nitrogen and phosphorus (42). The estimated contribution of organic carbon released by viral lysis of bacteria to the DOC pool was small ($<0.2\%$ day⁻¹) (Table 3). Since the DOM in Lake Plußsee is depleted with respect to phosphorus and nitrogen compared to bacteria (42), the contribution of organic phosphorus and nitrogen released by viral lysis into the pool of dissolved organic nitrogen and phosphorus is probably greater than the contribution of organic carbon. Overall, viral lysis of bacteria did not seem to contribute significantly to the DOM pool. However, releases of even small amounts of organic phosphorus and nitrogen in the epilimnion and organic carbon in the hypolimnion might stimulate bacterial growth, since bacterial lysis products are very likely readily utilizable by bacteria. Stimulation of bacterial growth by lysis products was also observed in a marine environment (41). Considering the fact that a cell is encountered by ca. 50 to 180 viruses per day, it is also possible that viruses are an important diet for bacteria. Also, viruses might be a diet for HNFs. Support for this hypothesis comes from estimates that viruses can be an important source of carbon, nitrogen, and phosphorus when there are 10^6 bacteria ml⁻¹ and 10^7 to 10^8 viruses ml⁻¹ (26), as in our study (Fig. 2).

Implications. Our data indicate that in oxic waters of Lake Plußsee the majority of the bacterial production was removed by HNFs and thus could be transferred to higher trophic levels of the food web. The high mortality of bacteria due to viruses in anoxic waters indicates that an important fraction of bacterial production remains in the viral loop. This is supported by the finding based on models and experiments that viral lysis and lysis products can strongly stimulate bacterial production and carbon uptake (20, 22, 41). Also, the addition of concentrates of the natural virus community to seawater incubation mixtures resulted in a higher FVIC and in increased concentrations of dissolved amino acids and carbohydrates (65). Thus, a significant portion of organic matter in anoxic water could be cycled several times in the viral loop before it gets mineralized. On the other hand, Proctor and Fuhrman (49) argued that the cell lysis products could act as glue for the formation of organic aggregates, and Peduzzi and Weinbauer (46) found that the addition of the virus size fraction of DOM to seawater resulted in an increase in the size of organic aggregates. Thus, the high lysis rates of bacteria in anoxic water could cause higher rates of sinking of aggregates and higher rates of incorporation of

organic matter into the sediment. Although additional studies will have to show whether viral lysis increases the mineralization of DOM or the rates of incorporation of organic matter in the sediments, it is clear that viral lysis is an important mechanism for regulation of organic matter cycling in lakes.

ACKNOWLEDGMENTS

We thank W. Lampert and D. Albrecht of the Max Planck Institute for Limnology in Plön, Germany, for providing lab space and data on nitrogen and phosphorus and ballerina K. Dominik for help during field and lab work. The help of H. Lünsdorf with electron microscopy is acknowledged. We also thank G. J. Herndl and I. Kolar for the organic carbon analyses and S. W. Wilhelm for comments on the manuscript. The comments of two reviewers improved the manuscript.

This work was supported by grant BEO-0319433B from the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie.

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