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Significance of Bacteriophages for Controlling Bacterioplankton Growth in a Mesotrophic Lake

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Bacterium-specific viruses have attracted much interest in aquatic microbial ecology because they have been shown to be about 10 times more abundant than planktonic bacteria. So far most of the studies of interactions of planktonic bacteria and viruses have been done in marine environments, and very little is known about these interactions in lakes. Therefore, we studied phage proliferation in Lake Constance, a large mesotrophic lake in Germany. We enumerated bacteria and quantified the fraction of bacteria with mature intracellular phage particles and the number of free viruses by transmission electron microscopy. Between the end of March and early August 1992, peaks of bacterial abundance were followed in 1 to 2 weeks by peaks in the fraction of bacteria containing visible phage particles (0 to 1.7%) and in the number of free viruses (1×10^7 to 4×10^7 ml^{-1}). We estimated that 1 to 17% ± 12% of all bacteria were phage infected, implying that phage-induced mortality was <34% ± 24% of total mortality. A direct comparison between phage-induced mortality, the net decrease of bacterial numbers, and bacterial growth rates indicated that phage-induced mortality accounted for <11% of total bacterial mortality during the phytoplankton spring bloom and 18 to 21% following the bloom. Estimated burst sizes ranged from 21 to 121 phages. Phage production rates of 0.5×10^6 to 2.5×10^6 ml^{-1} day^{-1} accounted for 70 to 380% of the observed net increase rates of free phages, implying high rates of simultaneous phage decay. The cyclic dynamics between bacteria and phages and the varying size structure of the intracellular mature phage particles suggested that phage infection was important in structuring the bacterial host assemblage during the study period.

Studies of interactions between bacterium-specific viruses, bacteriophages, and planktonic bacteria in aquatic environments have drawn much attention in the recent past, since it has been shown that the abundance of free viruses can exceed that of planktonic bacteria by 1 to 2 orders of magnitude (1, 2, 8, 18, 31). Short-term dynamics of abundances of planktonic bacteria and free viruses, in fact, imply that bacteriophages can be important in controlling bacterial growth (4, 5). It has also been shown by transmission electron microscopy (TEM) that <1 to 5% of planktonic bacteria were visibly infected by phages, which implies that 7 to 10 times more than this proportion of bacteria were infected (18, 21). Estimates of bacterial mortality due to production of phages suggest that phages can be responsible for 1 to 100% of bacterial mortality, depending on the trophic state (4, 9, 18, 21, 28). In oligo- and mesotrophic marine environments, phage infection accounts for 1 to ca. 25% of bacterial mortality, and only in highly eutrophic environments does it appear that phage-induced mortality can account for >50% of bacterial mortality. Positive correlations between the abundances of planktonic bacteria and phages (6, 34) and between bacterial growth, phage production, and chlorophyll (6, 28) suggest that the significance of phages in pelagic environments is favored by increased productivity. The majority of studies of interactions between planktonic bacteria and bacteriophages has been carried out in marine environments, and little information is available on the abundances of free viruses, phage-infected planktonic bacteria, and interactions between bacteria and phages in lakes; such studies have been restricted to investigating the abundance and the morphology of viruses (1, 7, 9, 12). To better understand the role of phages in aquatic ecosystems, it is important also to examine in detail bacterium-phage interactions in lakes.

Although correlations between bacteriophages and bacterial parameters have been established (see above), very little is known about temporal interactions between phages and planktonic bacteria. Strong short-term fluctuations between abundances of planktonic bacteria and phages in a Norwegian fjord during a phytoplankton spring bloom have been found (4), indicating that two maxima of bacteria were followed by those of phages. On the other hand, Waterbury and Valois (32) found that large numbers of marine *Synechococcus* spp. and high titers of cyanophages persisted during the growing season. This suggests that resistance of a host to its phage can occur in the pelagic environment, as has been concluded from experiments with cultures of *Escherichia coli* (16). Kokjohn et al. (13) have suggested that changing substrate and growth conditions for bacteria in aquatic environments lead to cyclic episodes of phage proliferation. In order to better understand temporal interactions between phages and planktonic bacteria, including potential shifts in the structure of the phage-host assemblage, it is necessary to study not only dynamics of the abundances of planktonic bacteria and phages but also those of infected bacteria, together with the burst size and the size structure of free and intracellular phages.

We studied dynamics of the abundance of planktonic bacteria, the fraction of phage-infected bacteria, the burst size, and the size structure of free and intracellular phages in Lake Constance, a large mesotrophic lake in Germany. The main results indicate that phage infection can be responsible to a substantial extent for the breakdown of bacterial blooms and that temporal changes in the size structure of free and intra-

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cellular phages occur. Phage-induced mortality on average, however, does not exceed 20% of total bacterial mortality.

MATERIALS AND METHODS

Sample collection. Samples were collected weekly between 31 March and 4 August 1992 at a central station in the Überlinger See (maximum depth, 147 m), the northwestern part of Lake Constance. Lake Constance is a mesotrophic and warm monomictic prealpine lake (539 km^2; maximum depth, 252 m) which has been studied extensively during the last decade (25, 27, 33). For chlorophyll analyses and the microcosm experiments (see below), samples were collected at a depth of 3 m with a prerinsed 9-liter van Dorn bottle. For all other parameters, samples were collected by four subsequent hauls with a 2-m-long Plexiglas tube (4-liter volume) covering the water column from 0 to 8 m. The tube samples were pooled in a plastic jug from which subsamples were withdrawn into 100-ml polyethylene bottles. Samples for electron microscopy and epifluorescence microscopy were preserved immediately after collection with barbital-acetate-buffered glutaraldehyde (final concentration, 1%; pH 8.2) and stored at 4°C in the dark until processing.

Chlorophyll and bacterial abundance. Chlorophyll *a* concentration was determined after hot ethanol extraction (70°C, 90%) according to the method of Simon and Tilzer (26). Bacterial abundance was determined by standard epifluorescence microscopy after DAPI (4′,6-diamidino-2-phenylindole) staining (17).

Free viruses. Viruses were enumerated according to the method of Børsheim et al. (3). Subsamples (13 ml) were centrifuged onto formvar-coated copper grids at 100,000 × *g* for 2.5 h in an ultracentrifuge (L8-70M; Beckman Instruments) by using a Sorvall AH 627 swing-out rotor. The formvar grids had been coated with a 20-nm layer of carbon before use. After centrifugation, the grids with the viruses were stained for 2 s with filtered (0.2-μm-pore-size filter) 2% uranyl acetate. The number of viruses was counted directly on the screen of a Siemens transmission electron microscope (Elmiskop 101) at a magnification of ×50,000. Total counts usually exceeded 300 viruses per sample. However, >150 viruses were enumerated on 7 April, 13 May, 30 June, and 28 July 1992. The mean coefficient of variation (CV) (standard deviation/mean) was 0.29

Phage-infected bacteria and bacterial mortality. Twenty milliliters of a fixed sample was filtered through a 0.2-μm-pore-size cellulose nitrate filter at a pressure of 0.4 bar. The filtration area was restricted to 20 mm^2 to ensure a high density of bacterial cells. After filtration, the filter was covered with liquid and warm (25°C) ultra-low gelling agarose (Sigma catalog no. A-5030) and instantly transferred to a warm glass petri dish. The filter area with retentate was cut into small strips (0.5 mm in width) around which the agarose flowed. The agarose with the floating strips was hardened by putting the petri dish on ice. The agarose gel was cut into pieces such that each piece contained one embedded strip. The strips were air dried for 15 min to stabilize the agarose surface and rinsed twice with barbital-acetate buffer. Finally, the specimens were incubated in buffered osmium tetroxide (final concentration, 2%) for 2 h, rinsed again with buffer, and incubated in ruthenium red (final concentration, 0.1%) overnight. The specimens were dehydrated in an ethanol series and embedded in Spurr medium. Ultrathin sections of 60 nm, cut with a diamond knife, were stained with uranyl acetate and lead citrate. The sections containing the filter-agarose interface with bacteria were examined by TEM at 80 kV and a magnification of ×30,000. Bacteria containing three or more electron-dense, distinct, round or polyhedral structures of the same size (mature phages) were defined as visibly phage-infected bacteria (Fig. 1). In total, >900 cells per sample were examined. Cyanobacteria containing only polyhedral bodies were excluded from counting (14). Electron micrographs of phage-infected bacteria were taken at a magnification of ×60,000 with AGFA Scientia 100 film.

Since phage infection becomes visible by TEM only at the end of the latent period, the total fraction of phage-infected bacteria is larger than the number of bacteria with mature phages (18, 21). Hence, a correction factor has to be applied in order to determine the total number of infected bacteria. We used a factor of 10, which approximates the maximum total fraction of bacteria that is phage infected and assumes that most mature phages are visible after 90% of the latent period (21). This fraction equals phage-induced bacterial mortality provided that the latent period matches the generation time of uninfected bacteria (21). Phage-induced mortality (day^{-1}) was calculated as growth rate of planktonic bacteria (published in reference 23) × fraction of infected bacteria. Rates of phage production were calculated as mortality rate × burst size (see below).

Burst size and sizing of phages. To calculate the burst size (N), the partial volumes (V_p) of visibly phage-infected bacteria within the ultrathin sections were calculated as the area of each infected bacterium times the thickness of the ultrathin section (60 nm). The burst size was calculated as follows: $N = N_p/V_p \times V$, where N_p is the number of phages per V_p and V is the mean volume of planktonic bacteria in Lake Constance, 0.054 μm^3 (24). Since only 5 to 20 bacteria containing phage-like particles were detected in one sample, mean values for two sampling days were used to calculate the burst size. The mean CV of the estimates of the burst size was 0.76. These measurements and also the sizing of mature phages in bacterial cells and of free viruses were done by using enlarged electron micrographs with a semiautomatic image analysis system (VIDS VI; AI Tektron).

Femtoplankton-enriched microcosms. In order to examine the effect of phages on bacterial mortality, we added concentrated femtoplankton to microcosms obtained by using a 1-μm-pore-size filter (1-μm filtrates) and determined bacterial abundance and the number of free viruses over time relative to those for an enriched control. A subsample of 100 to 200 ml from a 3-m depth was filtered through a 1.0-μm-pore-size Nuclepore filter at a gentle pressure (0.1 bar). Fifteen liters of the same sample was screened by gravity through 100-, 30-, and 10-μm-mesh-size nylon gauze, filtered through a 0.2-μm-pore-size filter by a tangential flow system (Minitan; Millipore, Corp.), and enriched in the same device with a 30-kDa-cutoff polysulfon membrane to a final volume of 15 ml. Filtration was done at a maximum pressure of 0.2 bar. This high-molecular-weight concentrate was added to the 1-μm filtrate at a theoretical enrichment of viruses of 10- and 40-fold compared with a control without enrichment. The filtrates were incubated in 200-ml polyethylene bottles at ambient temperature in the dark and periodically subsampled for bacterial numbers and free viruses. The potential phage-induced mortality was calculated as the difference between the bacterial growth rates in the control and the enriched microcosms.

RESULTS

This study started at the end of March before the phytoplankton spring bloom. The peak in chlorophyll *a* occurred 4 weeks later (Fig. 2). Bacterial abundance peaked with the chlorophyll *a* concentration and oscillated thereafter, with further peaks at the end of May, June, and July (Fig. 2).

Bacteria with distinct intracellular phage-like particles were present in almost all samples that we examined (Fig. 1). However, only between <0.1% and 1.7% ± 1.2% of total bacteria contained phage-like particles (Fig. 2). The highest proportion of bacteria with mature phages occurred at the beginning of May, June, and July, with a time lag of 1 to 2 weeks after the maxima in bacterial abundance. Because of the small proportion of visibly phage-infected bacteria, the CV of the counts was quite high, 1.45, resulting in considerable uncertainty in terms of the actual proportion of bacteria that were infected. The mean fraction of bacteria with mature phages during the study period was 0.6%.

Assuming that mature phages become visible after 90% of the latent period, 10 times more bacteria were infected; hence, the proportion of infected bacteria ranged from <1% to 17% ± 12%. The multiplication of the growth rate of planktonic bacteria (0.06 to 0.35 day^{-1} [23]) by the fraction of phage-infected bacteria yielded a phage-induced mortality rate of 0.012 to 0.027 day^{-1} (Table 1).

The estimated mean diameter of intracellular phages varied between 46 ± 6 and 63 ± 28 nm but was not significantly different between sampling dates (Student's t test; $P < 0.05$). There was, however, a continuous increase in the mean size from April until mid-June. This was also reflected in the size distribution of the intracellular phages. In April and July, phages in the size range of 20 to 50 nm constituted >60% of total visible phages and those with sizes of >80 nm were rare or absent. In May, however, phages 100 to 110 nm in size made up about 20% of the total.

The inferred burst size ranged between 21 and 121 phages per bacterium (Fig. 2). In early May and June, large burst sizes coincided with high percentages of phage-infected bacteria. In July, however, when the largest burst size occurred, the peak of phage-infected bacteria preceeded that of the burst size by 2 weeks. The smallest burst size occurred in mid-June at the end of the clear water phase, when very low chlorophyll *a* concentrations and the smallest bacterial cells, with a volume of 0.032 μm^3, occurred (24). During this period, however, the size of intracellular phages was larger (see above).

Estimated rates of phage production ranged from 0.1×10^6 to 2.5×10^6 ml^{-1} day^{-1}. The highest rate occurred at the peak of the phytoplankton spring bloom at the end of April, and a second maximum occurred at the beginning of July (Fig. 2). The two peaks of phage production coincided with or were

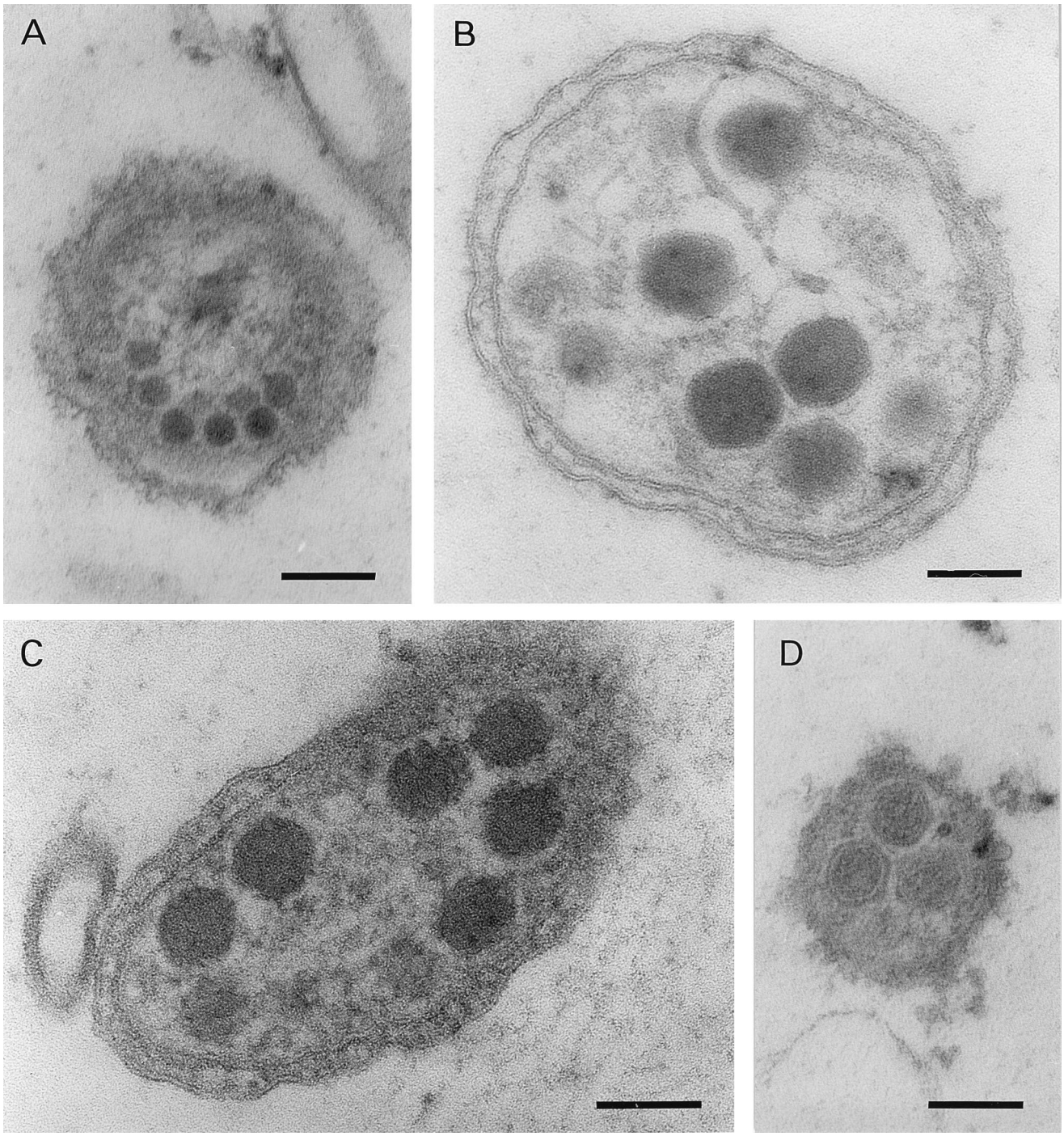

FIG. 1. Bacteria with intracellular phage particles from different samples (panels A through D, respectively) from Lake Constance. Shown are TEM micrographs of ultrathin sections of bacteria concentrated on a 0.2-μm-pore-size filter and embedded in an agarose gel. Scale bars = 100 nm.

followed by maxima of free viruses. This was also true for the slightly enhanced rate of phage production in early June.

Free viruses were abundant throughout the study. Their morphology and size were variable, but in most cases a distinct capsid and a tail were evident (Fig. 3). The abundance of free viruses in the size class 20 to 100 nm ranged between $<1 \times 10^6$ ml^{-1} in early April and 42×10^6 ml^{-1} in early June and July (Fig. 2). Peaks in abundance occurred at the beginning of May, June, July, and August, coinciding with maxima of bacteria containing phage particles and following peaks of bacterial numbers by 1 week. Numbers of free viruses <100 nm in diameter were significantly correlated with bacterial numbers if this 1-week time lag was taken into account ($r^2 = 0.57$; $P < 0.05$) but not if the two parameters were compared at the same time. Free viruses <60 nm in diameter were more abundant than those with sizes of 60 to 100 nm except at the end of June (Fig. 4). Seasonal dynamics of free viruses of the size classes <60 and 60 to 100 nm covaried ($r^2 = 0.57$; $P < 0.05$), although

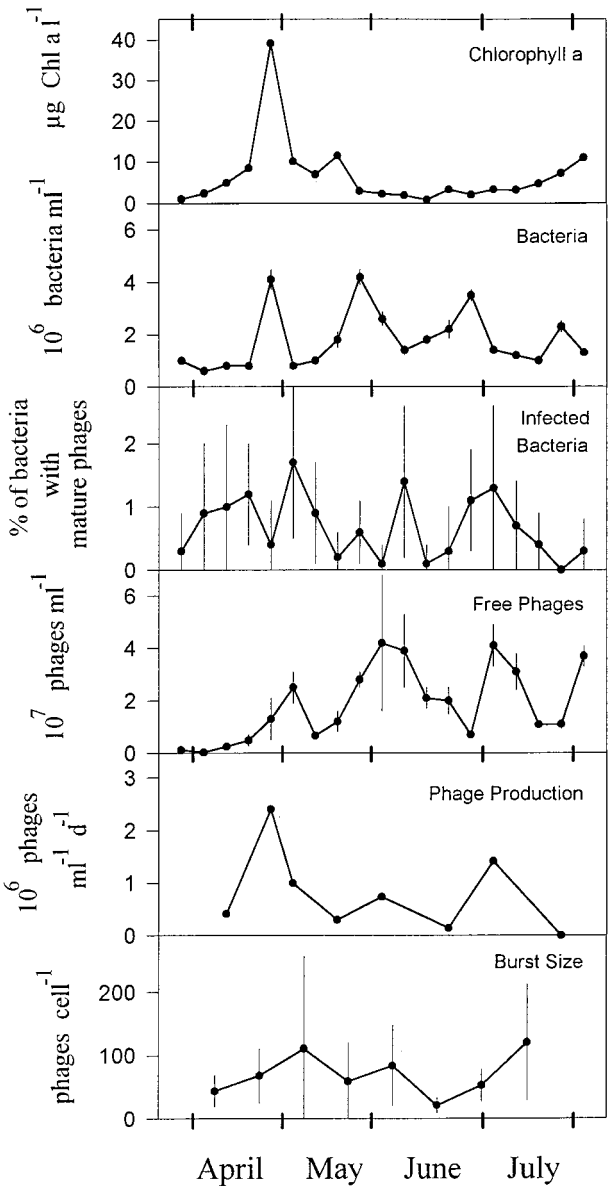

FIG. 2. Chlorophyll *a* (Chl a), bacterial abundance, bacteria with intracellular mature phage particles, free phages <100 nm in diameter, phage production, and burst size in Lake Constance during different seasons between late March and August 1992. Error bars indicate standard deviations.

fluctuations of those <60 nm in diameter were higher, particularly during June and July (Fig. 4). Numbers of free viruses >100 nm in diameter were much lower than those with sizes of <100 nm, always being below 3×10^6 ml^{-1} (Fig. 4). Their seasonal dynamics exhibited a pattern different from that of those <100 nm in diameter. Distinct maxima occurred only at the end of June and in mid-July.

Femtoplankton enrichments reduced the growth of bacterial assemblages in 10μm filtrates in two of three experiments (Fig. 5). There was only a slight difference in reduction of bacterial growth between 10- and 40-fold enrichments. On 9 June, bacterial growth was inhibited completely after 28 h but already substantially reduced after 16 h by the 40-fold enrichment (Fig. 5A). On 15 June, no clear-cut pattern of reduced bacterial growth occurred, presumably because even in the control experiment the bacterial assemblage did not grow continuously (data not shown). The experiment of 15 July showed a substantial reduction of bacterial growth during the initial 3 h (Fig. 5B). After 5 h, bacterial growth resumed until 15 h (data not shown). During the initial 2 h, the number of free viruses <100 nm in size increased from 35×10^6 to 95×10^6 ml^{-1}, whereas no increase occurred in the control (Fig. 5C). This increase equals a rate of phage production of 5.8×10^8 phages ml^{-1} day^{-1} or 15.6 day^{-1}, 2 orders of magnitude higher than those calculated for the in situ conditions (Fig. 2). No viruses >100 nm in diameter were observed in the 1-μm filtrate throughout the entire experiment. The experiment of 15 July revealed that the theoretical 10-fold enrichment of viruses yielded only a 5.4-fold-higher virus concentration than did the control.

TABLE 1. Growth rate and phage-induced and net mortality rates of bacteria during the breakdown of bacterioplankton blooms in Lake Constance[a]

Date (1992)	Growth rate[b] (day^{-1})	Mortality (day^{-1})		
		Phage induced[c]	Net[d]	Phage/net ratio
27 April–6 May	0.20	0.021	0.19	0.11
26 May–2 June	0.35	0.012	0.07	0.18
30 June–7 July	0.18	0.027	0.13	0.21

[a] Rates are mean values for the period indicated.
[b] Calculated according to the method of Schweitzer and Simon (23).
[c] Calculated as fraction of phage-infected bacteria × growth rate.
[d] Calculated as net decrease of bacteria numbers.

On the basis of the observed time period with reduced bacterial growth, bacterial mortality rates in the experiments of 9 June and 15 July were 0.1 and 8.8 day^{-1}, respectively. These rates are also 2 orders of magnitude higher than those calculated for planktonic bacteria in situ (Table 1).

DISCUSSION

Our results indicate that bacteriophages can be an important factor for controlling dynamics of planktonic bacterial assemblages in Lake Constance and periodically lead to the breakdown of bacterial blooms. From April until August, four maxima in bacterial numbers were followed in 1 to 2 weeks by maxima in the fraction of bacteria with intracellular phage-like particles and free viruses <100 nm in diameter. In ultrathin sections, the virus particles had distinct circular or polyhedral shapes which were always the same within a bacterium, strongly suggesting that the intracellular phage-like particles (Fig. 1) were indeed viruses. The mean diameters of these particles ranged between 46 and 63 nm and were always <105 nm, well within the size range for known bacteriophages (2). Phages produced in the femtoplankton-enriched microcosm were also of a similar size (see below). Ultrathin sections are far superior for identifying intracellular phages (18, 21) to the method of lysing bacterial cells prior to ultracentrifugation onto TEM grids following negative staining (5, 9).

It is surprising that <1.7% of all bacterial cells were visibly infected by phages, with the percentage often being <1%. Assuming that phage assembly becomes visible after 90% of the proliferation cycle, this translates to a maximum of 17% of bacteria being infected (21). According to the results of Proctor et al. (21), however, an estimate of 12% appears to be more realistic because mature phages can appear earlier during the proliferation cycle. The fraction of visibly phage-infected bacteria we found is somewhat lower than previously reported for free-living and particle-associated marine bacteria (18, 19). It

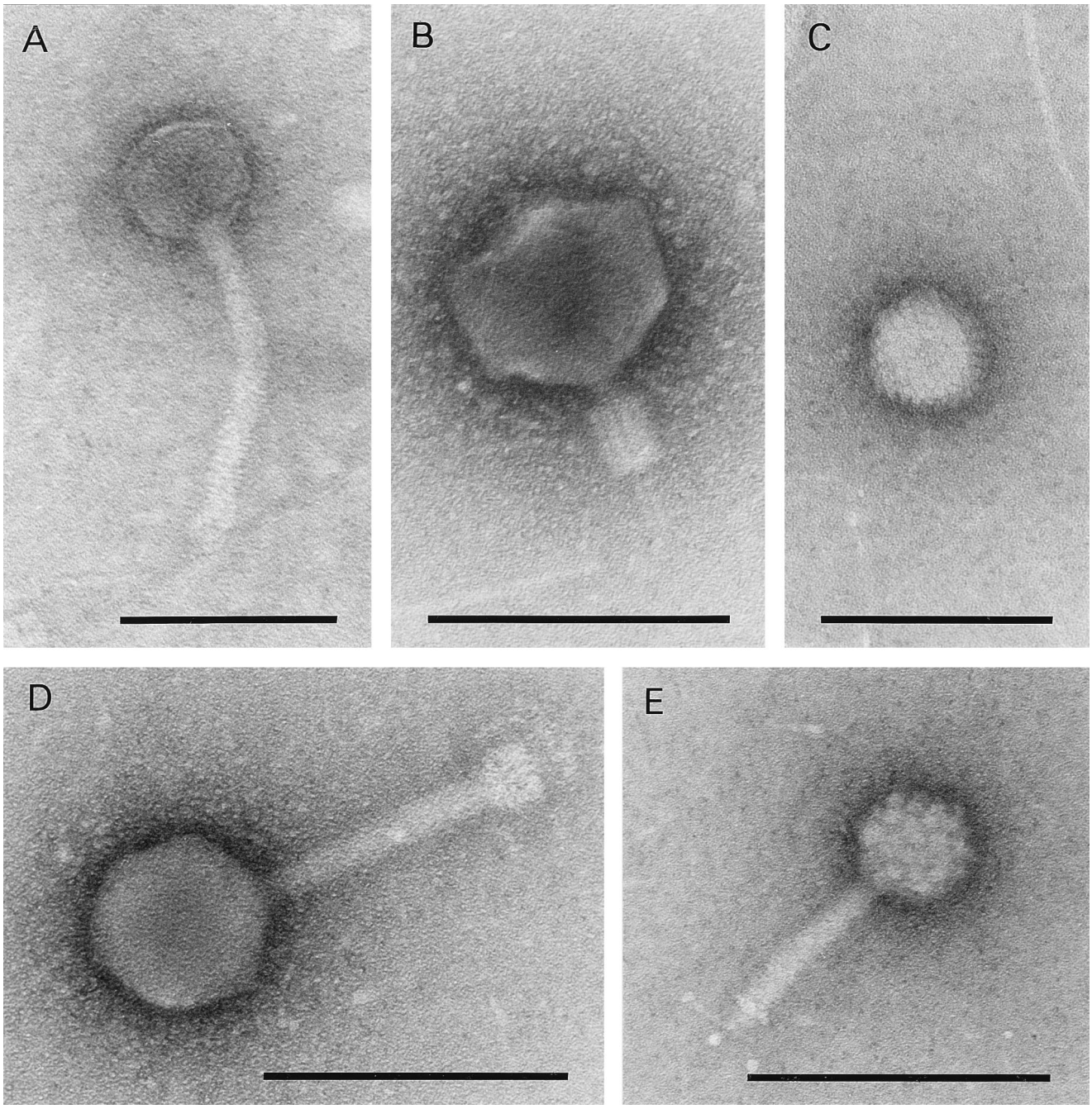

FIG. 3. Various morphotypes of free viruses in Lake Constance (panels A through E, respectively). Shown are TEM micrographs of negatively stained free viruses harvested by ultracentrifugation on carbon-coated grids. Scale bars = 100 nm.

is substantially lower than observed for coastal environments off Norway in experiments in which bacteria were lysed by using streptomycin (5, 9). Proctor et al. (21) illustrate that under steady-state conditions, only 50% of the bacterial standing stock has to be removed by virulent cell lysis to reach 100% mortality provided that the lengths of the generation time and the proliferation cycle are similar. Therefore, in our study a maximum of 34% but presumably a more realistic proportion of 24% of total mortality was due to phage infection. The mortality rates we estimated are in the same range as those obtained by Proctor and Fuhrman (18) and Proctor et al. (21) but substantially lower than those estimated by Heldal and Bratbak (9) and Bratbak et al. (5). However, they are in the same range as the minimum estimates published by Steward et al. (28) for mesotrophic waters of the Southern California Bight and also similar to estimates based on virus turnover rates (29).

The relative impact of phage-induced mortality on the breakdown of the bacterial blooms, e.g., under non-steady-state conditions, can be estimated by comparing phage-induced mortality rates with bacterial growth rates and the net decrease of bacterial numbers. During the first breakdown

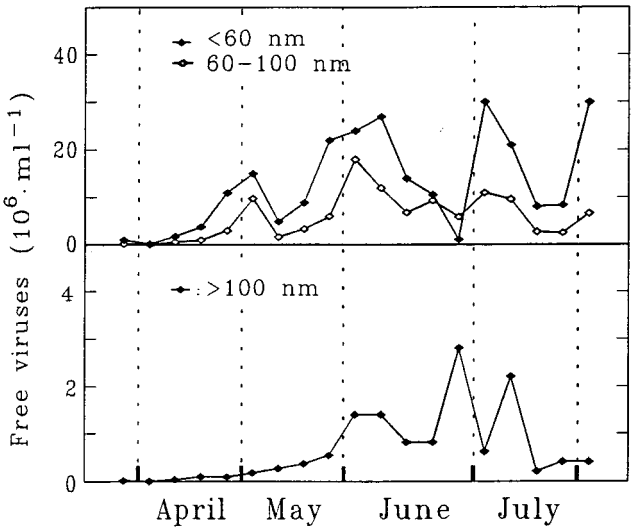

FIG. 4. Free viruses <60 and 60 to 100 nm in diameter (upper panel) and >100 nm in diameter (lower panel) between April and July 1992 in Lake Constance.

event, the maximum phage-induced mortality rate was about 10% of the bacterial growth rate. The latter was similar to the net decrease rate (Table 1). Here phage-induced mortality could have contributed about 11% to bacterial mortality on the basis of the net decrease of bacterial numbers. During the breakdown events at the end of May and June, phage-induced mortality contributed higher percentages to bacterial mortality on this basis, 18 and 21%, respectively. These values are close to the mean estimated on the basis of phage-infected bacteria, assuming steady-state conditions (see above). However, since bacterial growth rates at the end of May and June were higher than the net decrease rates of bacterial numbers, total bacterial mortality was higher than just the net decrease in abundance. This indicates that other factors of mortality also contributed to the decrease of bacterial numbers at the end of May and June. Thus, our results indicate that during the spring bloom, losses of bacterial production other than phage-induced mortality largely dominated. Thereafter, phage infection contributed substantially to the breakdown of the bacterial blooms.

It has been shown that during the spring bloom in Lake Constance, grazing by heterotrophic nanoflagellates is a substantial sink of bacterial production (11, 33). Grazing rates of nanoflagellates, however, account always for <50% and often for <20% of bacterial production (11) and thus leave much room for an additional sink of bacterial production. In contrast to our estimate, Bratbak et al. (5) could not accommodate losses of bacterial production by both flagellate grazing and phage-induced mortality, mainly because the latter exceeded bacterial production about sixfold.

Simultaneously with the maximum proportion of phage-infected bacteria and maximum numbers of free bacteriophages in early May and June, the burst size also exhibited maxima. Its maximum in July, however, was not reflected by enhanced numbers of free bacteriophages. Hence, at the breakdown events in early May and June, the conditions for phage proliferation were favored by both an enhanced fraction of phage-infected bacteria and a larger burst size. A larger burst size suggests more favorable physiological conditions for growth of the host bacteria and thus for phage proliferation (13, 35) than do lower burst sizes, which occur, for example, during the clear water phase in mid-June. At this time, the fraction of phage-

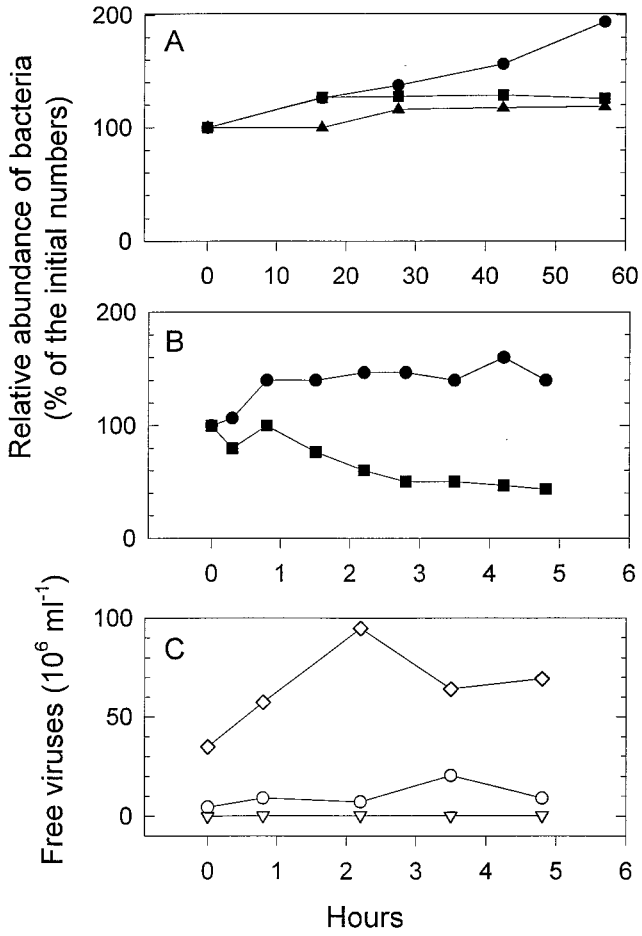

FIG. 5. Numbers of bacteria and free viruses in 1-μm filtrates enriched with femtoplankton concentrate. (A) Experiment of 9 June 1992. ■, 10× enrichment; ▲, 40× enrichment; ●, control. (B) Experiment of 15 July 1992. ■, 10× enrichment; ●, control. Given are abundances normalized to the initial number of bacteria. (C) Experiment of 15 July 1992; shown are free viruses <100 nm in diameter in the 10× enrichment (◇) and the control (○) and >100 nm in diameter in the 10× enrichment (△). Note the different time scales of the two experiments.

infected bacteria, the number of free phages, and the phage production rates were very low (Fig. 2). The range of burst sizes we calculated is similar to those estimated for marine bacterial assemblages (4, 9) and, at the lower end, for isolated marine bacteriophages (2).

Free viruses which were <100 nm in diameter showed maximum abundances 1 week after the peaks in bacterial numbers (Fig. 2). This also suggests that infection by bacteriophages was at least partially responsible for the breakdown of the bacterial blooms. Free viruses >100 nm in diameter exhibited a different pattern (Fig. 4), indicating that their seasonal dynamics were controlled differently. Similar observations have been made by Bratbak et al. (4). We compared the net increases of free phages at breakdown events with the phage production rates that we estimated. Therefore, we calculated the daily production rate of free phages as the mean for the time period before the maximum and compared it with the net increase of free phages during this time. We note that phage production rates calculated on the basis of phage-induced mortality do not account for possible simultaneous decay of phages (29). At the beginning of May, the production rate was 3.8 times higher than the net increase; at the beginning of June, the value was

TABLE 2. Production and net increase rates of bacteriophages during breakdown of bacterioplankton blooms in Lake Constance[a]

Date (1992)	Rate (10^6 ml^{-1} day^{-1}) of:	
	Production[b]	Net increase
27 April–6 May	4.6	1.2
26 May–2 June	2.9	2.0
30 June–7 July	3.5	4.9

[a] Values are means for the time period indicated.
[b] Calculated as bacterial abundance × mortality rate × burst size.

1.5 times higher; and at the beginning of July, it was 1.4 times lower (Table 2). This comparison indicates that during the spring bloom, the decay of phages was fairly fast or that phage production rates were overestimated. Such high phage decay rates have been observed to occur in coastal waters of Texas (29). During the other two events of breakdown of bacterial populations, our phage production rates and the net increase of phage numbers agreed within a factor of 2, suggesting that production and decay of phages were uncoupled.

Abundance of free phages was in the range of other values found for various marine waters (2) and for an oligotrophic lake in Norway (9). Our phage production rates are similar to the minimum estimates determined by Steward et al. (28) for offshore waters in the Southern California Bight but 5 to 10 times lower than those for nearshore waters. They are at least 1 order of magnitude lower than those estimated by Heldal and Bratbak (9) for a fjord and an oligotrophic lake.

After the breakdown of the bacterial blooms, the number of free bacteriophages also decreased quite rapidly. The net decrease rates were three to five times higher than phage production rates during the same time periods (Table 3). The decrease rates are two to seven times lower than rates determined experimentally for virus decay in coastal waters of Texas (29) and 2 orders of magnitude lower than rates estimated for mesocosms of waters from a fjord and an oligotrophic lake in Norway (9).

In the femtoplankton enrichments, bacterial growth was significantly reduced by the addition of virus concentrates. We cannot rule out the possibility that bacterial growth was reduced because of concentrated growth-inhibiting substances in the high-molecular-weight fraction. The increase of bacteriophages simultaneously with decreasing bacterial numbers, however, strongly suggests that reduced bacterial growth was predominantly caused by phage-induced lysis. The increase in phage abundance and decrease in bacterial abundance during the first 3 h yields a theoretical burst size of 30 ± 24 phages per bacterium, compared with an estimated burst size in situ of 121 ± 92 (Fig. 2). The phage production rates were about 1 to 2 orders of magnitude higher than those we determined for the in situ conditions. Therefore, we consider them potential rates which presumably are not realized in situ in Lake Constance

TABLE 3. Production and net decrease rates of free bacteriophages during periods of phage disappearance in Lake Constance

Date (1992)	Rate (day^{-1}) of:	
	Production	Decrease
6–13 May	0.04	0.19
10–16 June	0.02	0.10
7–21 July	0.03	0.10

because the phage bacterium ratio is at least 5-fold but often 10-fold lower, and presumably the infection rate is as well. Similar results were obtained by Proctor and Fuhrman (20) for marine bacterial assemblages and by Suttle et al. (30) for natural phytoplankton communities. Interestingly, our potential rates of phage production are similar to those determined by Heldal and Bratbak (9) in mesocosm experiments.

The femtoplankton enrichment experiment of 15 July showed that after a 5-h period of decreasing bacterial numbers, growth resumed, suggesting that bacteria resistant to phage infection became predominant after this time. Lenski (15) demonstrated that a bacterial host species can quickly become resistant to a specific virulent phage if the selection pressure is high. In the femtoplankton enrichment, the selection pressure for resistance or immunity against phage infection was at least 10 times higher than under natural conditions. For this reason, and also because bacterial growth rates in situ are much lower, it is unlikely that in the natural environment host resistance to specific phages occurs as quickly. Therefore, on the time scale of short-term events such as the breakdown of bacterial populations, it is more likely that bacterial species with a lower adsorption efficiency of phages or resistant to phage infection successfully compete against susceptible species. As discussed by Waterbury and Valois (32) and below, there is some evidence that host resistance occurs in natural waters.

Our results show dynamic interactions between phages and planktonic bacteria, with periodical decreases in bacterial abundance. Even though we do not have direct evidence, the changing size structure of the intracellular bacteriophages suggests that after every decline in bacterial abundance, a different assemblage of bacteriophages proliferated and/or a different assemblage of host bacteria evolved. Grazing by nanoflagellates also may have caused a shift in the structure of the bacterial assemblage after the first breakdown event in early May, when phage-induced mortality was relatively unimportant. The size structure of intracellular phages differed between April and May as well. This is consistent with results of Höfle (10), who found that grazing changed the structure of the bacterial assemblage in a eutrophic lake.

We cannot say if bacteria became resistant to specific phages and enabled the assemblage to recover and be infected by other phages. Alternatively, a newly structured bacterial assemblage, susceptible to other phages, may have evolved after a decline in bacterial abundance. A host bacterium will become resistant to a specific phage fairly quickly (15, 16), leading to cooccurring large numbers of host bacteria and phages if the selection pressure is high. We do not know if the selection pressure on bacterial species in Lake Constance for resistance to specific phages is high enough to result in resistant host assemblages of the same species composition. Growth rates of planktonic bacteria in Lake Constance are fairly low, ranging from 0.1 to 0.25 day^{-1} during breakdown events of bacterial assemblages (23). Presumably, growth rates are too slow to allow the development of an abundant and resistant type of a given host assemblage within the given time. Environmental conditions and availabilities of organic substrates (22, 25) and growth-limiting nutrients of planktonic bacteria in Lake Constance (23) vary substantially during different seasons. This suggests that newly structured bacterial assemblages developed in the lake rather than that one assemblage persisted during the growing season. Moreover, we did not observe fairly constant large numbers of bacteria and phages during longer time periods. In contrast, we found fairly variable but cyclic events of phage-host interactions which seem to be typical for changing rates of phage proliferation (13). In any case, phage infection, together with grazing by nanoflagellates, appears to be

important in structuring the bacterial assemblage during the growing season. This obviously occurs in cyclic episodes of enhanced and reduced rates of phage proliferation.

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