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Seasonal Variations of Virus Abundance and Viral Control of the Bacterial Production in a Backwater System of the Danube River

C. B. MATHIAS, A. K. T. KIRSCHNER, AND B. VELIMIROV*

Institut für Allgemeine Biologie, Abteilung Mikrobiologie, Universität Wien, A-1090 Vienna, Austria

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The abundance of virus-like particles in a backwater system of the Danube River covered a range of 1.2×10^7 to 6.1×10^7 ml^{-1} from 1992 to 1993. Measurements of head diameters for these particles, all of which were presumed to be viruses, led to four defined size classes, ranging from <60 nm to >150 nm. The 60- to <90-nm size class contained the largest fraction of total particles (41%), followed by the 90- to <150-nm size class (33%). The frequency of size classes was not significantly different between the two years. The frequency of bacteria with mature phages ranged from 1 to 4% over the seasons, with mean burst sizes ranging from 17 to 36 phage per host cell. Among the bacterial morphotypes, rods and vibrios were the major host systems for phages, while coccoid and filamentous cells were considered negligible. Counts from transmission electron microscopy and acridine orange direct counts confirmed that rods and vibrios accounted for 85 to 95% of the bacterial population over the seasons. Virus decay experiments showed lower decay rates for temperatures between 5 and 15°C (52 to 70% of the virus population remained) relative to 18 and 25°C (31 to 51% of the virus remained). Bacterial production measurements, performed at the same time and under the same conditions as decay experiments, allowed us to estimate virus-induced death rates, which ranged from 15.8 to 30.1% over the year, with an average of 20% viral control of the bacterial production. Considering that mature phage particles are visible only in the last phase of the latent period and using a mean conversion factor of 5.4 from the literature, based on descriptions of various phage host systems to relate the percentage of visibly infected cells to the total percentage of the bacterial community that is phage infected, we estimate that some 5.4 to 21.6% of the bacterial population is infected with viruses. This would imply that virus-induced death rates of bacteria range from 10.8 to 43.2%. The data on virus-induced bacterial mortality obtained by both the viral decay method and the determination of the frequency of infected cells are compared and discussed.

Aquatic bacterial production is thought to be largely controlled by predation, with microzooplankters as the major bacterivores (24, 26, 29). Although biometric studies, scaling arguments, and experiments (11, 22) have provided quantifiable evidence that zooflagellates and small ciliates are effective at grazing on free-living planktonic bacteria, there is still room for debate about the sink for the bacterial production (10, 19, 20). Recent observations concerning large numbers of viruses in both marine and freshwater systems, ranging from 10^4 to 10^7 ml^{-1} (3, 4, 8, 14, 27, 31, 34, 37), and the fact that up to 34% of all marine bacteria may contain mature phage particles (27) suggest that viral lysis may be a quantitatively important process in altering the carbon flow within the microbial loop (4). The observed coupling between viral abundance and bacterial dynamics over a spring phytoplankton bloom at the Norwegian coast (6) showed convincingly that viruses must be considered an active component of the microbial food web and led to the concept of the viral loop (7). However, only little quantitative information on the relative contribution of viruses in controlling either procaryotic or eucaryotic production is available (5, 7, 15, 25, 33). Enclosure experiments combining measurements of viral decay, observations concerning phage-producing bacteria, and bacterial production (7) showed a discrepancy in carbon balance calculations between production and loss estimates for bacteria, with viral lysis as the dominant mortality factor. These findings are in contrast to results from earlier studies (20, 29, 30, 38) indicating that microheterotrophic predators control the major part of the bacterial production.

Most of the research on interactions between free bacteria and bacteriophages has been performed in marine environments, and little information on viral dynamics in freshwater systems is available. To our knowledge, only two studies of the significance of phages in controlling bacterioplankton in a mesotrophic lake are available (16, 23). In the present study we investigated the variations in virus abundance in a freshwater system over a period of 2 years and tried to estimate the relative contribution of viruses in controlling bacterial production, using both the viral decay method (15) and the estimation of bacterial mortality via determination of the frequency of infected cells (27, 28). The investigated locality is within the extensive backwater system of the Danube River near Vienna and is a nature reserve.

MATERIALS AND METHODS

Sample collection. Water samples were taken with an acid-rinsed Ruttner sampler (1.5-liter volume) or an IRO bottle (1.4 liters) from a small boat at a constant station in the lower part of the Kühwörte backwater system (Fig. 1). We sampled on a monthly basis from January 1992 to December 1993 with the exception of February 1993, during which a 20- to 30-cm-thick ice layer covered the whole of the Kühwörte. The average depth of the Kühwörte is 1 m, the water covering a surface area of some 0.23 km^2. All samples were taken from 40 cm below the surface, between 9 and 11 a.m. The sampled water was immediately split into subsamples (by using acid-rinsed containers) for the determination of viral abundance, bacterial abundance, and production or for viral decay experiments. Temperature was measured at the sampling depth at 10 a.m. and 1 p.m. and prior to sampling.

Virus-like particle counts. Two subsamples of 100 ml were preserved immediately after collection with glutaraldehyde (1% final concentration) and stored

* Corresponding author. Mailing address: Institut für Allgemeine Biologie, Universität Wien, Abt. Mikrobiologie, Währingerstr. 17/2, A-1090 Vienna, Austria.

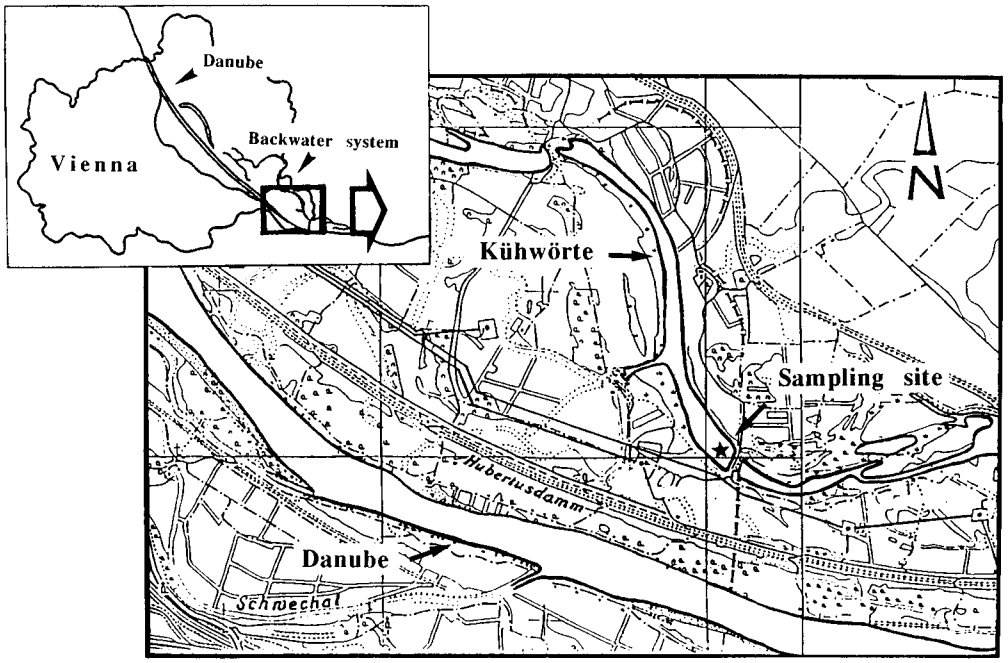

FIG. 1. Location of the Kühwörte backwater system and sampling site.

at 4°C in the dark until analysis. We used the ultracentrifugation methodology to harvest viruses and/or virus-like particles directly onto collodium-coated, 400-mesh Ni electron microscope grids by using a Beckman L8-80M centrifuge with an SW40 Ti swing rotor (1 h at 100,000 × g) according to the procedures of Borsheim et al. (4) and Bratbak et al. (6). For both samples duplicate grids were stained with uranyl acetate (1%). Virus abundance was determined with a Zeiss EM 902 transmission electron microscope operating at 80 kV and at magnifications between ×20,000 and ×85,000. Viruses and bacteria were counted in 25 to 35 randomly chosen eyefields per grid. Concentrations of viruses and bacteria were calculated as described by Mathews and Buthala (21). We sized head diameters of phages from photomicrographs generated at a magnification of ×50,000 under a dissecting lens (magnification, ×10). Our experimental setup did not allow us to test the observed particles for infectivity. In this text we assume that all virus-like particles which we observed were indeed viruses.

Number of bacteria with mature phages. Quantitatively collected bacteria on the electron microscope grids (4) and the use of a high acceleration voltage of 80 kV during the investigation allowed the identification and quantification of bacteria with mature phages (34, 35) on the basis of a clear recognition of the shapes and sizes of the phages within the bacterial cell. The number of mature phages was recorded for each infected bacterial morphotype and used for the estimation of an average burst size representative of the seasons.

Phage decay experiments. Water samples were immediately brought to the laboratory in acid-rinsed 5-liter bottles and incubated at the in situ temperature.

The first decay experiments were initiated in autumn (11 October 1992) at a water temperature of 18°C, and these experiments were repeated for all seasons, with the last experiment taking place in late summer (17 August 1993) at a mean temperature of 25°C (see the legend to Fig. 5). All experiments were sampled in triplicate. In accordance with the experimental approach of Heldal and Bratbak (15), production of new viruses was inhibited by adding KCN to a final concentration of 2 mM and adjusting the pH of the KCN stock solutions to 7.0 for freshwater. Since cyanide will stop virus production via inhibition of host respiration but without affecting enzyme activities, the method was considered adequate within our experimental design. Samples of 100 ml for the determination of virus and bacterium counts were withdrawn at time intervals down to 0.5 h over an experimental time of between 21 and 24 h and treated as described above.

Bacterial abundance and production. Three subsamples (10 ml each) of the water used for the decay experiments were preserved with 0.2-μm-pore-size-filtered formaldehyde (final concentration, 4%) for the determination of bacterial numbers by epifluorescence microscopy and the acridine orange direct counting technique (17). An additional 80 ml was used to measure bacterial production by means of [^{3}H]thymidine incorporation into the DNA. All incorporation experiments were run at the same time and under the same temperature conditions as the virus decay experiments. We adopted the method of Fuhrman and Azam (12) as modified by Wicks and Robarts (36). Samples of 10 ml were incubated at the in situ temperature for 30 min with 15 to 25 nM [^{3}H]thymidine (80 μCi nmol^{-1}; NET-027Z thymidine, methyl-^{3}H; Dupont, NEN Research Products, Boston, Mass.). Each incorporation assay consisted of eight samples and three blanks, and the [^{3}H]thymidine added to each incubation tube gave final concentrations which should fully inhibit de novo thymidine synthesis (1, 2), as indicated by saturation experiments (three samples and two blanks) run at the same temperature. Blanks were prepared from samples treated with formaldehyde (2% in final solution) immediately before the addition of [^{3}H]thymidine. After incubation, the samples were killed with formalin, cooled to below 5°C in an ice water bath, precipitated with ice-cold trichloroacetic acid for 20 min, and filtered through 0.1-μm-pore-size filters (Sartorius; cellulose nitrate; 25-mm diameter). Subsequently, the filters were treated with 5 ml of buffered (50 mM Tris-Cl, pH 8) phenol-chloroform solution (50% [wt/vol]) for 10 min and washed with ice-cold ethanol (80%).

During the whole procedure, vacuum pressure remained below 20 kPa. Filters were left to dry overnight in scintillation vials and pretreated with 0.75 ml of Cellosolve (2-ethoxyethanol [99%]; Sigma) for 2 h to enhance filter disintegration. After the addition of 4.5 ml of scintillation cocktail (Filter Count; Packard Corp.), the filters were counted after 24 h of storage in a Packard 1900 TR scintillation counter. For the determination of the initial radioactivity added, 100-μl portions were withdrawn from the samples at the beginning of the assay and counted after the addition of 4.5 ml of Ultima Gold scintillation cocktail (24 h of storage) from Packard. Measured [^{3}H]thymidine incorporation into the DNA was converted into bacterial cell production according to an empirical conversion factor (see Discussion).

Statistical analysis. To meet requirements for parametric statistics (Student's *t* test), log transformation was applied for data not following normal distribution (39). We used Statview, Microsoft Excel, and Delta Graph software packages.

RESULTS

Viral and bacterial abundance. Virus numbers ranged from 1.2×10^7 to 6.1×10^7 particles ml^{-1} over the 2 years (Fig. 2 and 3). There was no statistically significant difference ($P > 0.05$) between the virus counts in 1992 (mean, 2.04×10^7 particles ml^{-1}) and those in 1993 (mean, 3.18×10^7 ml^{-1}). In 1992, the highest viral densities were recorded in October and November, with 3.78×10^7 and 3.54×10^7 virus particles ml^{-1}, respectively. In 1993, maximum viral densities could be observed in May and June, with 6.1×10^7 and 5.5×10^7 virus particles ml^{-1}, but virus numbers peaked again in October, with 4.6×10^7 virus particles ml^{-1}. Viral numbers increased and decreased with bacterial numbers from January to June 1992 and from October to the end of the year, but they decreased and remained low over the summer period with tem-

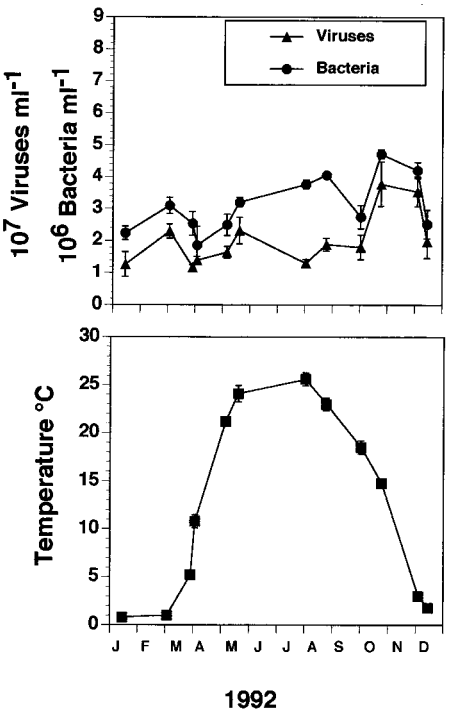

FIG. 2. Variations of viral and bacterial abundances and temperature in the Kühwörte for 1992. Error bars represent SDs.

peratures ranging from 24 to 26°C while bacteria increased in number. A different variation pattern was observed for 1993. Decreases for both viruses and bacteria were recorded only between May and June and between October and December.

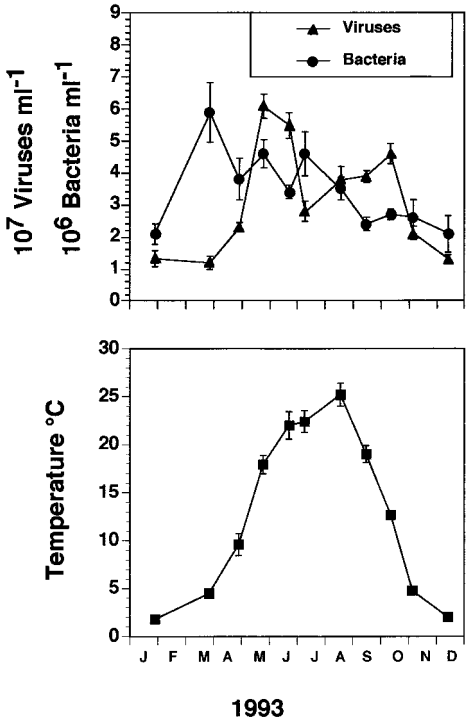

FIG. 3. Variations of viral and bacterial abundances and temperature in the Kühwörte for 1993. Error bars represent SDs.

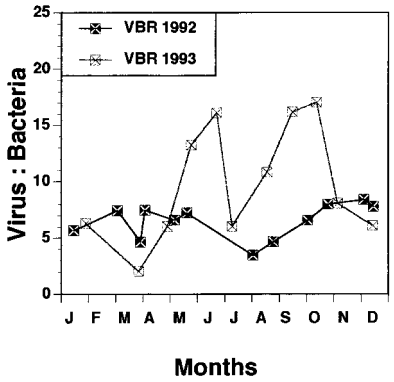

FIG. 4. Variations of the VBR in the Kühwörte for 1992 and 1993.

For the rest of the year increases in numbers of bacteria were inversely related to the number of virus particles.

VBR. For 1992 the virus particle-to-bacterium ratio (VBR) ranged from 3.4 to 8.4. Higher VBR values, ranging from 2.0 to 17.0, were recorded for 1993. Figure 4 shows two peaks in 1993, for the end of spring and for the beginning of autumn, while no clear variation pattern could be detected for 1992.

Distribution of virus head diameters. We defined four classes of head diameters (Table 1), the lowest class grouping all phage heads below 60 nm. This size class was represented at frequencies of 13.5% of the virus population in 1992 and 17.6% of the virus population in 1993. The size class 60 to <90 nm was represented at frequencies of 40.9 and 41.6%, i.e., it was the most frequent virus head diameter range in both years. The size class 90 to <150 nm was represented at frequencies of 35.9 and 31.1% for the two years, respectively, while the size class with the lowest frequencies, about 9%, grouped the largest head diameters (>150 nm). Within the years, the frequency of viruses in the smallest size class was always significantly lower than the frequency in the size class 60 to <90 nm ($P < 0.0001$ for 1992 and 1993) and the size class 90 to <150 nm ($P < 0.001$ for 1992 and 1993), but it was not significantly different from the size class >150 nm ($P > 0.05$ for 1992 and 1993). The size class 60 to <90 nm was significantly more frequent than the size class 90 to <150 nm in 1993 ($P < 0.05$) but not in 1992. Between the years, no significant differences ($P > 0.05$ for all tests) were found for the frequencies of the four size classes.

Burst sizes and relative abundance of bacterial morphotypes. Despite a total of over 8,000 bacterial cells inspected for mature phages over 2 years, only three infected coccoid bacteria could be detected in monthly samples, with burst sizes ranging from 14 to 20 phage per host cell. On one occasion we found two filamentous bacteria with mature phages (burst sizes of 140 and 160 phage per host cell), a phenomenon restricted to April 1993, which was the only month of the year in which this bacterial morphotype occurred at densities above or equal to 10^5 ml^{-1}. The burst size of infected bacteria ranged from 7 to 110 phage per host cell for rods and from 5 to 120 for

TABLE 1. Class distribution of virus head diameters in 1992 and 1993

Yr	% of total in size class (SD):			
	<60 nm	60 to <90 nm	90 to <150 nm	>150 nm
1992	13.5 (8.1)	40.9 (15.7)	35.9 (11.0)	9.5 (8.4)
1993	17.6 (15.2)	41.6 (16.0)	31.1 (14.1)	9.6 (7.6)

TABLE 2. Cell-specific burst sizes, mean burst sizes, relative abundances of bacterial morphotypes, and percentages of bacteria with mature phages for five temperature ranges obtained from the seasonal study[a]

Temp range (°C)	Burst size[b]		Mean burst size	% of total bacterial population represented by:		% BMP (rod + vibrio)[c]
	Rod	Vibrio		Rod-shaped organisms	Vibrio-shaped organisms	
0–5	22.7 (17.7)	25.6 (16.5)	23.7 (17.2)	51.9 (7.6)	37.6 (8.1)	2.4 (0.2)
6–11	38.0 (19.1)	ND[d]	38.0 (19.1)	46.8 (6.0)	42.6 (12.0)	4.0 (1.9)
12–18	27.3 (25.3)	37.3 (21.3)	29.4 (23.4)	44.5 (2.7)	47.6 (1.0)	3.3 (0.4)
19–22	19.2 (11.5)	15.5 (10.8)	17.4 (11.0)	43.5 (2.0)	47.4 (1.3)	3.9 (0.3)
23–26	17.0 (10.4)	27.5 (14.9)	20.1 (12.2)	50.6 (11.8)	44.7 (9.3)	1.0 (0.6)

[a] Values in parentheses are SDs.
[b] Phage per host cell.
[c] BMP, bacteria with mature phages.
[d] ND, not determined.

vibrios. Grouping of average burst sizes according to temperature (Table 2) revealed significantly higher burst sizes for the rod morphotype only at 12 to 18°C, compared with rod morphotypes sampled at 19 to 22°C ($P < 0.05$) and at 23 to 25°C ($P < 0.05$). For infected vibrios a similar trend was observed, showing that the only difference was for burst sizes at 12 to 18°C, which were significantly higher than those at 19 to 22°C ($P < 0.05$). The test of the average burst sizes of rods against vibrios by pooling of data from all temperatures gave no significant difference ($P > 0.6$). Rods and vibrios, as assessed by transmission electron microscopy, represented 88 to 95% of the total bacterial population (Table 2) over all temperatures, with a mean of 91.4% (standard deviation [SD] = 2.43%). The frequency of bacteria with mature phages ranged from 0 to 4%.

Viral decay experiments. Decay patterns for all experiments were similar, with a fast decrease in viral numbers during the first 3 to 4 h and decay rates ranging from 0.06 to 0.08 h^{-1} for the experiments at 5, 10, and 15°C (Fig. 5). After 5 h, this decrease slowed down, leading to lower decay rates ranging from 0.02 to 0.05 h^{-1}. In the following samples and until the end of the experiment no significant changes in viral numbers occurred, with decay rates tending to zero. For the 5°C experiments 70% of the initial viral population remained, while 55 and 52% remained for the 10 and 15°C experiments, respectively. A different decay dynamic was noticed for the experiments at temperatures between 18 and 25°C. Decay rates for the first 4 h were higher than those for the experiments at lower temperatures, ranging from 0.08 to 0.15 h^{-1}. Between 5 and 10 h, the decay rates decreased, ranging from 0.02 to 0.04 h^{-1}, and these rates tended again towards zero after 10 h. For these experiments, between 31 and 51% of the initial viral population remained, indicating a greater loss over the observation time for summer and autumn samples compared with the samples taken in late winter and spring for the experiments at lower temperatures. For both low- and high-temperature experiments most of the virus losses occurred in the size class <60 nm, which was reduced to 28 and 30% of its initial abundance, respectively. Losses decreased with the relative increase of the bigger size classes, with no losses occurring in the size class >150 nm.

Bacterial abundance and production. Over all experiments, thymidine incorporation into the DNA (Fig. 6) ranged from 8.2 to 173.0 pmol $liter^{-1}$ h^{-1}, indicating high replication rates at and above 18°C and lowest replication rates at 5°C. No significant difference in mean thymidine incorporation was found for the experiments run at 18, 20, and 25°C ($P > 0.5$) or for those run at 5 and 10°C ($P > 0.1$). In both low-temperature experiments significantly less thymidine was incorporated ($P <$

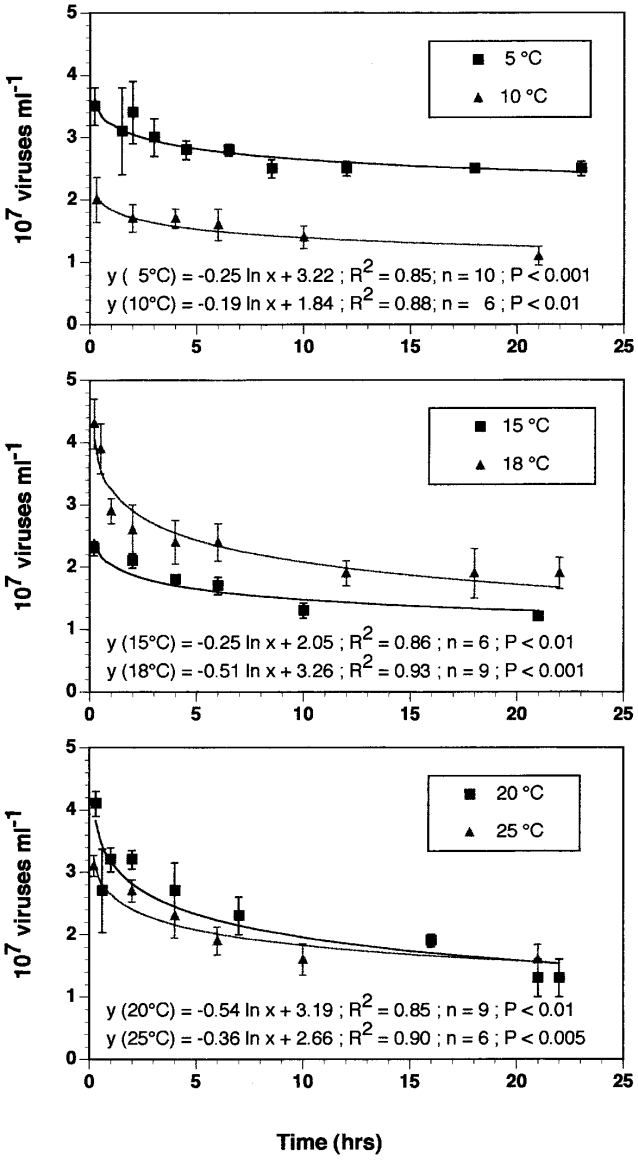

FIG. 5. Decay of viruses in mesocosms treated with KCN at 5°C (20 March 1993), 10°C (22 April 1993), 15°C (8 May 1993), 18°C (11 October 1992), 20°C (15 June 1993), and 25°C (17 August 1993). Each value is the mean for triplicate samples, and error bars represent SDs.

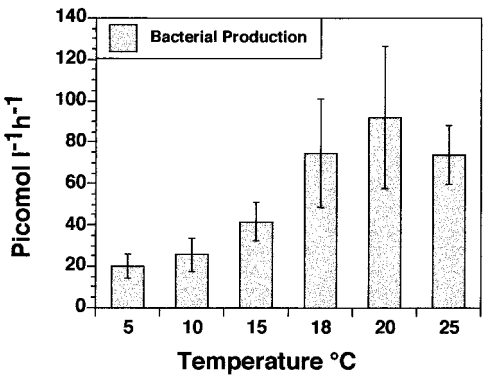

FIG. 6. [^{3}H]thymidine incorporation into bacterial DNA in relation to temperature. Error bars represent SDs.

0.01 in all cases) than was incorporated in the experiments at 18°C and above, while incorporation rates at 15°C were significantly lower only than those at 20 and 25°C ($P < 0.05$ in both cases). Numbers of bacteria counted by acridine orange direct counting ranged from 2.83×10^6 to 6.24×10^6 ml^{-1} (Table 3). At 5 and 10°C no significant difference was found between cell numbers ($P > 0.8$), but they were significantly lower than those at higher temperatures ($P < 0.005$ in all cases). The relative abundances of the bacterial morphotypes indicate that rods and vibrios account for 85 to 94% of the bacterial population, while cocci range from 5 to 10% and filamentous forms range from 1 to 5%. Estimations of cell-specific growth rates, expressed as the ratios between cell production and cell numbers at the beginning of the experiments (Table 3), showed a tendency to increase with temperature until 20°C, whereas a decrease was noticed for the experiment with the highest temperature.

DISCUSSION

The VBRs for our mesotrophic system from 1992 to 1993 are within the range of those reported by Hara et al. (14) for Japanese coastal and offshore waters, ranging from 2.3 to 15, and those reported by Wommack et al. (37), with mean values between 3.2 and 25.6 from April to October, but below mean values of Weinbauer et al. (34), ranging from 16.9 for the mesotrophic part of the northern Adriatic Sea to 35.9 under eutrophic conditions. Also, the VBR values from 1992 are close to those of Jiang and Paul (18), which ranged from 0.9 to 9.1, while our values from 1993 are clearly higher, with ratios ranging from 2 to 17. We obtained only small variations from January to June 1992, suggesting that viral and bacterial production occurred at similar rates. The decrease of the VBR in June-July, and the subsequent small but constant increase, contrasts with values in 1993. The different variation pattern of the VBR values in 1993 is attributable to peaks of viral abundance in May and October. Both VBR peaks were observed at temperatures around 18°C or above.

It is not obvious why our analysis revealed three small virus peaks in 1992 while the data for 1993 displayed two marked peaks within the year. One may only speculate that the two virus peaks may have resulted from a slight shift towards higher burst sizes or simply that a higher level of bacterial production led to proportionally higher virus release rates, since it was shown that viral production and the frequency of phage-infected cells increase with bacterial densities (31, 34). Also, a change in the resistance of viruses against decay may have taken place, leading to smaller losses of virus particles per day. However, it should also be pointed out that there was a slight shift in the sampling frequency between 1992 and 1993 due to the formation of a thick ice layer in February 1993, which may account for the missing peak at the end of the winter in 1993 and the above-mentioned peak differences between the two years. Therefore, the actual state of the information does not allow us to decide about the importance of the various possibilities which may determine changes in virus abundances in our system.

Burst sizes of bacterial morphotypes and number of infected cells. It was remarkable that the number of recognizable phage particles in cocci was extremely small in our system, a phenomenon which was so far not reported for other systems. If we assume that ultrathin sections are superior for identifying intracellular phages (27, 28), this would imply that we clearly underestimated the number of phage-infected bacteria. However, because this lack of infected cocci was noticed early in 1992, we looked specifically for mature phages in the coccoid bacteria without noticeably increasing our frequency of observations. This lack of infected cells was also confirmed by our diel study (unpublished data). Since cocci represent only 7.2% (SD = 1.4%) of the bacterial population and cocci were shown to have usually small burst sizes compared with rods, vibrios, or spirillae (34, 35), this possible underestimation will not drastically change our mortality estimations, which were based on rod and vibrio morphotypes, which represent between 85 and 95% of the population.

Viral decay and refractory virus size classes. It is noteworthy that we had much lower phage decay rates and a larger percentage of refractory phages in our system than were evidenced in the experiments of Heldal and Bratbak (15) and Bratbak et al. (7), which are representative of northern aquatic systems. It was observed (15) that over 60% of the viral population has a relatively short turnover time and that less than

TABLE 3. Total bacterial numbers, relative abundances of bacterial morphotypes, and specific bacterial growth rates at various temperatures[a]

Expt temp (°C)	TBN[b] (10^6 ml^{-1})	% of bacterial population represented by:				BSP[c]/TBN (10^{-2})
		Rod-shaped organisms	Vibrio-shaped organisms	Coccus-shaped organisms	Filamentous-shaped organisms	
5	2.83 (0.69)	60.9 (6.4)	29.4 (8.8)	7.1 (2.4)	1.8 (1.2)	2.07
10	2.90 (0.34)	60.7 (5.6)	26.8 (7.3)	7.3 (1.0)	4.9 (2.8)	2.46
15	4.40 (1.15)	64.8 (9.9)	20.5 (8.1)	9.6 (2.6)	5.1 (4.3)	2.65
18	4.05 (0.50)	64.0 (5.3)	25.8 (6.7)	7.2 (1.1)	2.9 (3.7)	5.28
20	4.51 (0.67)	63.9 (4.2)	27.6 (3.6)	7.2 (1.4)	1.2 (0.6)	5.69
25	6.24 (1.46)	53.9 (6.9)	39.7 (7.5)	5.0 (1.1)	1.2 (0.9)	2.36

[a] Values in parentheses are SDs.
[b] TBN, total bacterial number, as determined by acridine orange direct counting.
[c] BSP, bacterial secondary production (cells ml^{-1} h^{-1}), derived from [^{3}H]thymidine incorporation experiments (see Table 4).

TABLE 4. Combination of viral and bacterial parameters and calculations of viral impact on bacterial production[a]

Expt temp (°C)	Viral concentration (10^7 particles ml^{-1})	Viral decay rate (10^5 particles ml^{-1} h^{-1})	Bacterial lysis rate (10^4 cells ml^{-1} h^{-1})	Bacterial production rate (10^4 cells ml^{-1} h^{-1})	Viral control (%)[b]
5	3.5	4.20	1.77	5.88	30.1
10	2.0	4.28	1.13	7.14	15.8
15	2.3	5.23	1.78	11.67	15.2
18	4.3	13.00	4.42	21.40	20.6
20	3.2	9.50	5.45	25.70	21.2
25	3.1	7.38	3.69	20.69	17.8

[a] Bacterial cell production was determined by the formula moles of [^{3}H]thymidine incorporated · (2.8×10^{18}).
[b] Virus-induced death rate within the bacterial population, expressed as a percentage of the bacterial production rate.

40% is refractory, in contrast to the 52 to 70% remaining virus particles for experiments run at 5 to 15°C and the 31 to 51% remaining particles for experiments run at 18 to 25°C in the present study. However, it was also the fraction <60 nm which had the fastest turnover. In this context, it should be mentioned that our observed decay rates may be underestimations. Assuming that bacteria play an active role in phage decay via ectoenzymes and extracellular enzymes, this process will be inhibited by cyanide inactivating the bacterial metabolism. Although it was shown earlier (15) that cyanide had no significant effect on viral decay rates compared with the decay rates for experimental systems in which bacteria were removed by centrifugation, we believe that the experimentation time of 2 h (15) was too short to be representative of our decay experiments lasting 24 h. Also, it should be pointed out that adsorption of viruses to the walls of the incubation bottle (5 liters; radius, 17 cm) could have been an additional source of error in the estimation of viral decay rates. By using the upper range of the Brownian displacement for viruses (7), amounting to 0.4 mm h^{-1}, and assuming that all virus particles within a distance of 0.4 mm of the vessel wall adsorb to the wall of the container, a disappearance rate of viruses from the water phase of 0.004 particles h^{-1} can be calculated for a viral density of 3.0×10^7 particles ml^{-1} (the mean of the initial viral concentrations of all decay experiments [Table 4]). Thus, wall adsorption is negligible and does not enhance the potential underestimation of the viral decay rates, and this is in agreement with the findings of Bratbak et al. (7). We have no method to distinguish bacteriophages from plant viruses by transmission electron microscopy, and we speculate that most of the virus-like particles that we counted and measured, up to the size class 90 to <150 nm, are bacteriophages. This is in agreement with a study of the morphological diversity of freshwater bacteriophages (9), in which head diameters of up to 117 nm were measured. We assume that viruses with head diameters of over 120 nm which belong to the refractile part of the population in our system are not only bacteriophages, implying that some of the viruses in the size classes 90 to <150 nm and >150 nm could be plant viruses.

Viral control of bacterial production. By compiling information about burst sizes, decay of viral particles, and bacterial secondary production, we attempted to estimate the impact of virus-induced cell lysis of heterotrophic procaryotes in controlling the bacterial compartment. We used the approach of Heldal and Bratbak (15) and Bratbak et al. (7), adopting the conservative assumption that the viral density in the system is maintained and that phages lost by decay are replaced by phages from lysed bacterial cells.

Obviously, the assessment of the factor for conversion of thymidine incorporation into cell production will be of major importance to describe a representative system model. We determined an empirical conversion factor for our system under several temperature conditions, during isotype saturation, and during measurement of [^{3}H]thymidine triphosphate in the DNA extract (unpublished data). The obtained values ranged from 2.7×10^{18} to 2.9×10^{18} cells mol^{-1} for the Kühwörte backwater system. Considering the theoretical conversion factors from the recent literature (1, 2, 12, 13, 22), a range of 2.5×10^{17} to 2.4×10^{18} cells mol of incorporated thymidine^{-1} is reported, indicating that our empirical values are rather in agreement with the upper range of the theoretical factors. Compared with the empirical conversion factors ranging from 1×10^{18} to 17×10^{18} cells mol^{-1} (1, 22), our values are situated near the lower end of the scale. Assuming that a mean of 2.8×10^{18} (SD = 0.1), which is close to the factor 2×10^{18} recommended by Bell (2), would be a representative conversion factor, Table 4 indicates that virus-induced cell lysis controls on average some 20% (SD = 5.4) of the bacterial production. The greatest relative contribution of viruses in lysing produced bacterial cells was obtained for the experiments at 5°C. This was mainly due to a low level of bacterial cell production, since the average burst size was not much greater than those for the higher temperatures and viral decay rates were comparable to those at 10 and 15°C. Our estimations are lower than those of Proctor and Fuhrman (27) for marine phage host systems, the latter researchers expecting a mortality level of 34% of the bacterial production and assuming that production is balanced by removal. In a more recent study Proctor et al. (28) converted the number of bacteria containing mature phages to a total number of phage-infected bacteria within the population by correcting for the number of cells which were in an earlier stage of the latent phase and in which no mature phage particles could be recognized when they were inspected by transmission electron microscopy. The conversion factor is based on the description of various phage-host systems, and the values range from 3.7 to 7.14. Adopting a mean factor of 5.4 (32) suggests that over a year some 15.7% of the bacterial population in our system (average of 2.92% bacteria with mature phages, calculated from Table 2), with a range of 5.4 to 21.6%, are infected by viruses. We assumed that the lengths of the latent periods are equivalent to the lengths of uninfected bacterial generation times (28) and that the linear relationship found between latent periods and generation times of marine bacteria in culture is also applicable to our system. If growth is balanced by mortality, viruses would account for 10.8 to 43.2% of the total bacterial mortality. This range, however, is much larger than the bacterial mortality range that we obtained by direct comparison of cell lysis estimated from decay experiments and bacterial production, i.e., 15.2 to 30.1%. If we use the factor of 3.7 from the lower end of the scale (28), it leads to 3.7 to 14.8% of the total infected bacteria and a range of 7.4 to 29.6% for bacterial mortality. Although the upper range value is close to our maximum values, our lower range values are nearly twice as high as those obtained from calculations

with the factor 3.7. This may be an indication that the established conversion factors from Proctor et al. (28) for estimation of the total number of infected cells and the consequent mortality in bacterial populations, based on observations of strains of marine vibrio cultures, cannot be applied to the phage-host systems of Danubian backwater environments. Nonetheless, it is obvious from our experiments that viruses control at least 15 to 30% of the bacterial production in the backwater systems, which implies that up to one-third of the bacterial carbon is not transferred to micropredators but cycles within the microbial loop.

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