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Size-specific mortality of lake bacterioplankton by natural virus communities

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ABSTRACT: The potential effect that viral lysis has on the cell size distribution of bacterioplankton was investigated during late summer stratification in Lake Plußsee, Germany. Size-specific bacterial mortality due to viral lysis was estimated from *in situ* samples by a transmission electron microscopy based examination of visibly infected cells (VIC) and in an experiment with varying concentrations of the natural virus community. In all depth layers the highest percentage of cells was found in a cell length class that was smaller for the entire bacterial community (0.3–0.6 µm) than for VIC (0.6–0.9 µm). For cells <2.4 µm the highest frequency of VIC (FVIC) was detected in the size classes 0.6–0.9 and 0.9–1.2 µm, and the FVIC was high in the size classes 1.2–1.5 (all depth layers) and 1.5–1.8 µm (meta- and hypolimnion). The estimated mortality due to viral lysis in these size classes was significant with maxima of 29 to 55% in the epilimnion, 30 to 59% in the metalimnion and 56 to 107% in the hypolimnion. In all depth layers the FVIC of bacteria <0.3 µm in length was ca 30% of that averaged for the entire bacterial community, and in the experiment the percentage of cells <0.3 µm was highest in enclosures with high viral activity. In the experiment the average cell size was smaller in enclosures with high than in that with low viral activity. The data demonstrate that being small could be a strategy of cells to reduce mortality due to viral lysis probably by reducing the contact rates with viruses. Thus, viral lysis could be one of the mechanisms keeping the cell size small in aquatic ecosystems. In oxic water cells in the largest size class (>2.4 µm) were not infected with viruses, and in enclosures with epilimnetic lake water the percentage of cells >2.4 µm was highest in enclosures with highest viral abundance, suggesting that resistance against infection favored large cells. However, in the meta- and hypolimnion the FVIC was high for cells >2.4 µm and, since the burst size increased with bacterial cell size, lysis of large cells could contribute significantly to viral production. Also, a major portion of biomass was found in cells >2.4 µm. The finding that viral lysis is size-specific and can affect the cell size distribution of bacteria in lake water has important implications for our understanding of the mechanisms which regulate bacterial production and nutrient cycling in pelagic environments.

KEY WORDS: Bacterioplankton size structure · Size-specific viral lysis · Lake Plußsee

INTRODUCTION

It is well known that bacteria play a crucial role in the cycling of energy and matter in aquatic systems (Pomeroy 1974, Azam et al. 1983); however, there is still a controversy on the mechanisms regulating bacterial production. Two major mechanisms for controlling bacterial production have been proposed, grazing by protozoans (Fenchel 1982, Sherr & Sherr 1987) and availability of resources (Billen et al. 1990). Recent findings indicate that viral lysis is another key factor in the microbial food web (Fuhrman 1992, Fuhrman & Suttle 1993, Thingstad et al. 1993, Bratbak et al. 1994, Suttle 1994).

The concepts on the regulation of bacterial production typically consider heterotrophic bacterioplankton a 'black box'. In an attempt to open this black box hypotheses were developed to understand the cell size distribution of bacteria (Güde 1979, Jürgens & Güde 1994). The cell size of bacteria in natural systems is typically smaller than that of bacteria grown in culture. The small cell size was explained by the occurrence of starvation forms in low-nutrient environments or ultramicrobacteria with a genetically determined small cell size (Morita 1982, Kjelleberg et al. 1987), and by size-selective grazing, i.e. that protozoans typically show

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higher grazing rates on medium-size than on small cells (Andersson et al. 1986, González et al. 1990). It is also known that bacteria can show growth forms that prevent grazing by protozoans such as filamentous, spiral-shaped and star-shaped cells and cell aggregations (Güde 1979, 1988); however, the role of this fraction of bacterioplankton has been largely ignored (Jürgens & Güde 1994). Large grazing-resistant cells are typically low in abundance, but can contribute significantly to bacterial biomass (Jürgens & Güde 1994, Sommaruga & Psenner 1995, Pernthaler et al. 1996). In general, being very small or very large seems to be a strategy of bacteria to avoid mortality due to grazing. The majority of active cells are usually of medium size (Pernthaler et al. 1996, Posch et al. 1997), indicating that cells which escape grazing grow slowly. Increasing the growth rate might be another strategy to cope with grazing (Pernthaler et al. 1997, Simek et al. 1997).

Grazing can affect the taxonomic structure, the cell size distribution and the activity pattern of bacteria. Since viruses are usually species- or strain-specific (Børsheim 1993), they might influence the taxonomic structure of bacterioplankton by controlling those species or strains which become abundant (Fuhrman & Suttle 1993, Thingstad et al. 1993). However, evidence for this role of viruses is still sparse (Hennes et al. 1995). *In situ* studies and experiments demonstrated that viral infection frequencies can differ among bacterial morphotypes (Bratbak et al. 1990, Weinbauer & Peduzzi 1994, Weinbauer & Peduzzi 1995), but nothing is known about the effect of viruses on the cell size distribution. Since the fastest-growing cells may be restricted to certain cell sizes and viruses probably infect the most active fraction, an influence of viral lysis on the cell size of bacteria is conceivable. We investigated the effect of viral lysis on the cell size distribution of bacterioplankton in oxic and anoxic water layers of a eutrophic lake. Data from a depth profile and an experiment indicate that the mortality of bacteria due to viral lysis is size-specific and viral lysis can influence the cell size distribution of bacteria. We further argue that being small is a strategy to reduce viral infection and viral lysis could be an important mechanism for keeping cells small in natural systems.

MATERIAL AND METHODS

Sampling and study site. The study site was Lake Plußsee (54° 10' N, 10° 23' E) near Plön in Schleswig-Holstein (northern Germany). Lake Plußsee is a eutrophic dimictic lake which is stratified during summer into the warm and oxic epilimnion and the cold and anoxic hypolimnion separated by the thermocline layer (metalimnion; Krambeck et al. 1994). A detailed characterization of the sampling site during the sampling period can be found in Weinbauer & Höfle (1998). On September 23, 1996, water samples were collected along a depth profile with a Ruttner sampler from a permanent platform mounted in the center of the lake. Subsamples were preserved in formaldehyde (2% final concentration) and kept at 4°C in the dark.

Enumeration of bacteria and viruses. Bacteria and viruses were stained with 4',6-diamidino-2-phenylindole (DAPI; final concentration 1 µg ml^{-1}) and enumerated by using epifluorescence microscopy (Zeiss; Axiovert model 135TV), slightly modifying the protocols described by Turley (1993) and Suttle (1993). Samples (1 ml) for bacteria and viruses were stained without DNase treatment for 30 min, filtered onto 0.02 µm pore-size Anodisc filters (Whatman) and enumerated as described in Weinbauer & Suttle (1997).

Determination of visibly infected bacteria, viral burst size and bacterial cell size. We used a modification of the transmission electron microscopy (TEM) based method originally published by Heldal & Bratbak (1991) to determine the number of visibly infected cells (VIC). Bacteria from a 10 ml sample were collected quantitatively onto Formvar-coated, 400-mesh electron microscope grids by centrifugation in a swinging-bucket rotor (Beckmann SW-41; 66000 × *g* for 20 min), stained for 30 s with 1% uranyl acetate and rinsed 3× with deonized distilled water. The chosen time and speed of centrifugation reduce disruption of infected cells and, as few viruses are pelleted, phages within cells are easily distinguished (Weinbauer & Suttle 1996). Grids were screened for VIC by using a TEM (Zeiss; model CEM 902) operated at an accelerating voltage of 80 kV. Between 200 and 2000 cells were examined for mature phages within the cells, in order to obtain at least 10 VIC per sample, corresponding to at least 40 VIC per depth layer. A minimum of 5 phages were observed in a VIC. Viruses inside cells were identified based on structure, size, intensity of staining and uniformity of structure, size and staining intensity. Viral burst size, i.e. the number of viruses produced within a cell, was estimated as the average number of viral particles in all VIC (minimum burst size) or as the average number of viruses in cells which were either completely filled with viral particles or were in the process of lysis (maximum burst size; Weinbauer & Peduzzi 1994, Weinbauer & Suttle 1996). According to the model of Proctor et al. (1993), bacterial mortality due to viral lysis was estimated by multiplying the frequency of VIC (FVIC) by the average (10.84) and the range of conversion factors (7.4 to 14.28).

Cell dimensions of the bacterial community were determined from at least 3 TEM micrographs made at a magnification of 3570× and 200 cells were sized per sample. VIC were also sized by using electron micro-

graphs made at the same magnification. The cell volume was calculated by using the following formula which is based on the assumption that bacteria are rods with hemispherical ends, but also works well for cocci (Bratbak 1993):

$$V = (\pi/4)W^2(L - W/3)$$

where V = cell volume, L = cell length and W = cell width. Bacterial biomass was calculated by multiplying bacterial abundance with average cell size to obtain the total cell volume, and a conversion factor of 350 fg C μm^{-3} (Lee & Fuhrman 1987) was used for relating bacterial biovolume to carbon content.

Contact rates. The rate of contact (R) between viruses and bacteria was calculated by using following formulae (Murray & Jackson 1992):

$$R = (Sh\, 2\pi\, w\, D_v)VP$$

where Sh is the Sherwood number (1.06 for a bacterial community with 10% motile cells; Wilhelm et al. 1998), w is the cell diameter (calculated from the mean bacterial cell volume assuming that the cells are spheres), V and P are the abundances of viruses and bacteria, and D_v is the diffusivity of viruses.

$$D_v = kT/3\pi\mu d_v$$

where k is the Boltzmann constant (1.38×10^{-23} J K^{-1}), T is the *in situ* temperature (K), μ is the viscosity of water [in Pa s^{-1}; μ was calculated from values given in Schwörbel (1987) for temperatures in the range of 4 to 15°C] and d_v is the diameter of the viral capsid (73 nm in Lake Plußsee; Demuth et al. 1993). Specific contact rates were calculated for different size classes by correcting for bacterial abundance in the different size classes to estimate the number of contacts per cell on a daily basis.

Experimental procedure. To test experimentally whether viral lysis can influence bacterial cell size, 100 l of lake water was collected on September 24, 1996, from 0.5 m depth by using a submersible pump and prefiltered through 3 µm pore-size filters (Nuclepore) to remove larger zoo- and phytoplankton and a part of the protozoan plankton. Subsamples were passed through filters of different pore size, in order to obtain dissolved organic matter (DOM) fractions with varying concentrations of the natural virus community. One subsample was filtered through Milli Q rinsed 0.2 µm pore-size polycarbonate filters (Nuclepore) to remove bacterioplankton and obtain DOM which contains the majority of the natural virus community (virus-rich fraction). Another subsample was passed through a 0.1 µm pore-size hollow-fiber filter using a tangential-flow ultrafiltration system (Amicon M12) to concentrate bacteria for later use as an inoculum. The concentration of viruses in the fraction of DOM passing this filter was reduced (virus-reduced fraction) compared to that in the 0.2 µm fraction, but the organic carbon concentration in the virus-reduced fraction was only ca 4% lower than that in the virus-rich fraction (data not shown). Aliquots of the bacterial concentrate were added to the 2 DOM fractions at a final concentration of 10%. Duplicate 10 l glass flasks were incubated at *in situ* temperatures (15°C) in the dark. Samples for microbial parameters were preserved in formaldehyde (2% final concentration) and kept at 4°C in the dark until analysis.

Statistical analysis. All data were log transformed for statistical analyses. ANOVA and Fisher PLSD post-hoc tests (StatView D-4.5 program) were used to test whether parameters were significantly different between the depth layers. Paired t-tests were used to check for differences of parameters between the entire bacterial community and VIC. Multiple regression and correlation analyses allowed were used to the co-dependence of burst size with other parameters. A probability of <0.05 was considered as significant.

RESULTS

Size structure of the entire bacterial community

Bacterial and viral abundances varied significantly along the depth profile and were highest in the metalimnion and lowest in the epilimnion (Table 1). The average cell volume of bacterioplankton communities ranged from 0.04 to 0.24 μm^3. The cell volume increased significantly with depth, averaging 0.06 μm^3 in the epilimnion, 0.13 μm^3 in the metalimnion and 0.20 μm^3 in the hypolimnion (Table 1). The average cell length was significantly smaller in the epi- (0.8 µm) and metalimnion (0.9 µm) than in the hypolimnion (1.4 µm; Fig. 1). The cell length distribution was similar in all depth layers (Fig. 2), and most of the cells belonged to the size class 0.3–0.6 µm. However, the percentage of cells in this size class was higher in the epi- and metalimnion (ca 35%) than in the hypolimnion (28%). Also, more cells belonged to the smallest size class (<0.3 µm) in the meta- and hypolimnion (ca 20%) than in the epilimnion (ca 10%). The percentage of cells larger than 1.5 µm increased with depth, averaging 10% in the epilimnion, 11% in the metalimnion and 17% in the hypolimnion. The average length of cells >2.4 µm in size was 4.5 µm in the epilimnion, 6.6 µm in the metalimnion and 7.7 µm in the hypolimnion.

The average standing stock of bacterial carbon was significantly lower in the epilimnion (110 µg C l^{-1}) than in the 2 deeper water layers (ca 380 to 390 µg C l^{-1}; Table 1). In all depth layers most of the biomass was

Table 1. Abundance, cell volume and biomass of the entire bacterial community, frequency of visibly infected cells (FVIC) and viral abundance in different water layers of Lake Plußsee, Germany. Values are expressed as means calculated from 4 samples collected within a depth layer and ranges are given in parentheses. Analysis of variance (ANOVA) was used to test whether parameters were significantly different between depth layers

Depth layer	Entire bacterial community			FVIC (%)	Viral abundance (10^7 ml^{-1})
	Abundance (10^6 ml^{-1})	Cell volume (µm^3)	Biomass (µg C l^{-1})		
Epilimnion	4.6 (2.8–7.6)	0.06 (0.05–0.10)	110 (52–220)	1.2 (0.5–1.8)	1.3 (1.6–2.6)
Metalimnion	7.7 (5.5–10.8)	0.13 (0.12–0.16)	390 (236–527)	2.4 (1.4–3.4)	4.3 (2.2–8.8)
Hypolimnion	5.8 (4.9–8.0)	0.20 (0.14–0.29)	379 (353–416)	4.5 (2.5–6.4)	2.8 (1.1–5.3)
ANOVA	·	·	···	··	··

·$p < 0.05$, ··$p < 0.005$, ···$p < 0.001$

found in the size classes 0.6–0.9 and >2.4 µm (Fig. 3). However, the contribution of the size class 0.6–0.9 µm to total bacterial biomass decreased with depth from 28% in the epilimnion to 13% in the hypolimnion, whereas that in the size class >2.4 µm increased from ca 28–29% in the epi- and metalimnion to 43% in the hypolimnion.

Virus-infected bacteria

In all samples cells were observed which were visibly infected by viruses. The FVIC ranged from 0.5 to 6.4% of the total bacterial numbers and increased significantly with depth, averaging 1.2% in the epilimnion, 2.4% in the metalimnion and 4.5% in the hypolimnion (Table 1). Cell length of VIC increased significantly with depth (Table 2, Fig. 1) and was significantly larger (2-tailed paired *t*-test; $p = 0.0059$) than that of the entire bacterial community (Fig. 1). In all depth layers most VIC belonged to the size class 0.6–0.9 µm (ca 35–40%; Fig. 2). In the epilimnion we

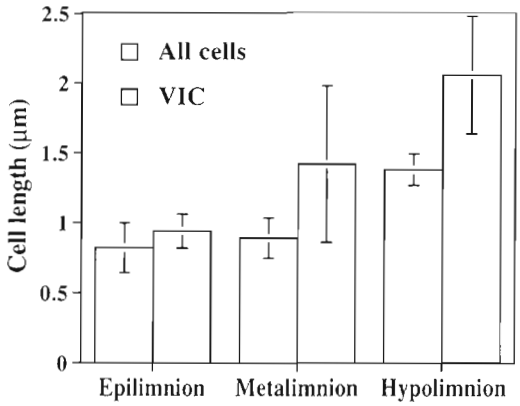

Fig. 1. Cell length of the entire bacterial community and visibly infected cells (VIC) in the 3 depth layers of Lake Plußsee, Germany, on September 23, 1996

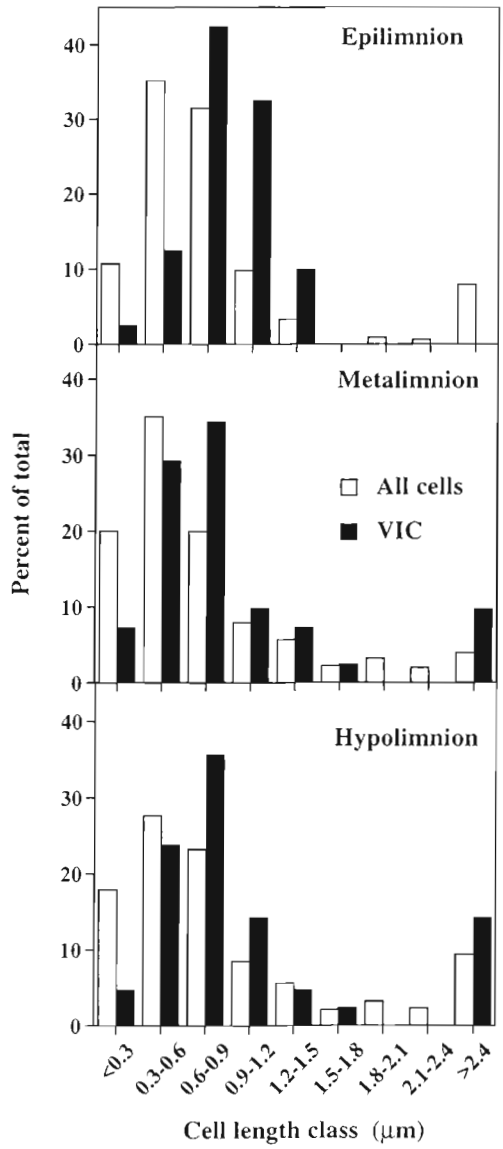

Fig. 2. Cell size distribution of the entire bacterial community and visibly infected cells (VIC) in 3 depth layers of Lake Plußsee on September 23, 1996

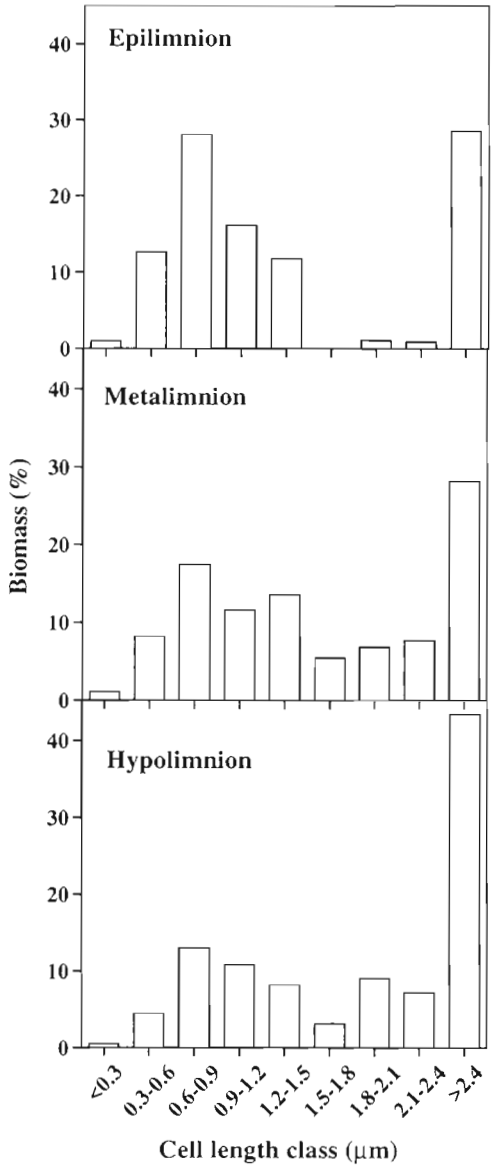

Fig. 3. Biomass distribution of the entire bacterial community in 3 depth layers of Lake Plußsee on September 23, 1996

found VIC in 5 out of 9 size classes (56%) compared to 7 (78%) in the meta- and hypolimnion. In the meta- and hypolimnion ca 5 to 7% of the VIC belonged to the smallest size class (<0.3 µm) compared to 2.5% in the epilimnion. Cells larger than 1.5 µm were not visibly infected in the epilimnion, whereas in the meta- and hypolimnion VIC were also found in the size classes 1.5–1.8 and >2.4 µm. Small (<0.3 µm in length), filamentous, spirochaete-like and dividing VIC are shown in Fig. 4.

Calculating the FVIC for single size classes showed that the FVIC increased with cell length in the epilimnion and was highest (ca 3.5%) between 0.9 and 1.5 µm (Fig. 5). In the meta- and hypolimnion we detected 2 maxima, one between 0.6 and 0.9 (4%; metalimnion) and 0.6 and 1.2 µm (ca 7%; hypolimnion), respectively, the other in cells larger than 2.4 µm (ca 6 to 7% in both depth layers). In the smallest size class (<0.3 µm) VIC were found in all depth layers; however, FVIC values were only 23 to 36% of the average FVIC. The highest estimated mortality in a single size class was 29 to 55% in the epilimnion, 44 to 84% in the metalimnion and 56 to 107% in the hypolimnion (Table 2).

The burst size of individual cells varied over 2 orders of magnitude and ranged from 5 to ca 500. Multiple correlation and regression analyses were performed to assess the co-dependence of burst size with temperature, oxygen and nutrient concentrations and microbial parameters. Using stepwise regression analysis, cell volume was the only measured parameter that was related to burst size and could explain 66% of the variability of the minimum burst size and 61% of the variability of the maximum burst size. The relationship of burst size with cell volume is shown in Fig. 6. Minimum and maximum burst size increased significantly with depth averaging, respectively, 34 and 51 in the epilimnion, 44 and 66, in the metalimnion and 63 and 94, in the hypolimnion.

Contact rates

Contact rates were not calculated for cells longer than 1.5 µm, since the length to width ratio of these cells is significantly higher than 1 which would seriously violate the assumption of the contact model that cells are spheres. In all depth layers the highest total contact rate between bacteria and viruses was found in the size classes 0.3–0.6 and 0.6–0.9 µm (Fig. 7). In all size classes total and specific contact rates were highest

Table 2. Size-specific mortality of bacteria due to viral lysis in different depth layers of Lake Plußsee. Mortality was calculated from the frequency of visibly infected cells (FVIC) using the average and the range (in parentheses) of conversion factors for relating the FVIC to mortality (Proctor et al. 1993). ND: not detectable

Cell length class (µm)	Mortality (%) Epilimnion	Metalimnion	Hypolimnion
<0.3	3 (2–4)	9 (6–12)	13 (9–17)
0.3–0.6	5 (3–6)	22 (15–28)	42 (29–55)
0.6–0.9	17 (12–23)	44 (30–59)	75 (51–98)
0.9–1.2	42 (29–55)	31 (22–42)	81 (56–107)
1.2–1.5	38 (26–50)	33 (23–44)	41 (28–54)
1.5–1.8	ND	28 (19–37)	56 (38–74)
1.8–2.1	ND	ND	ND
2.1–2.4	ND	ND	ND
>2.4	ND	64 (44–84)	74 (50–97)

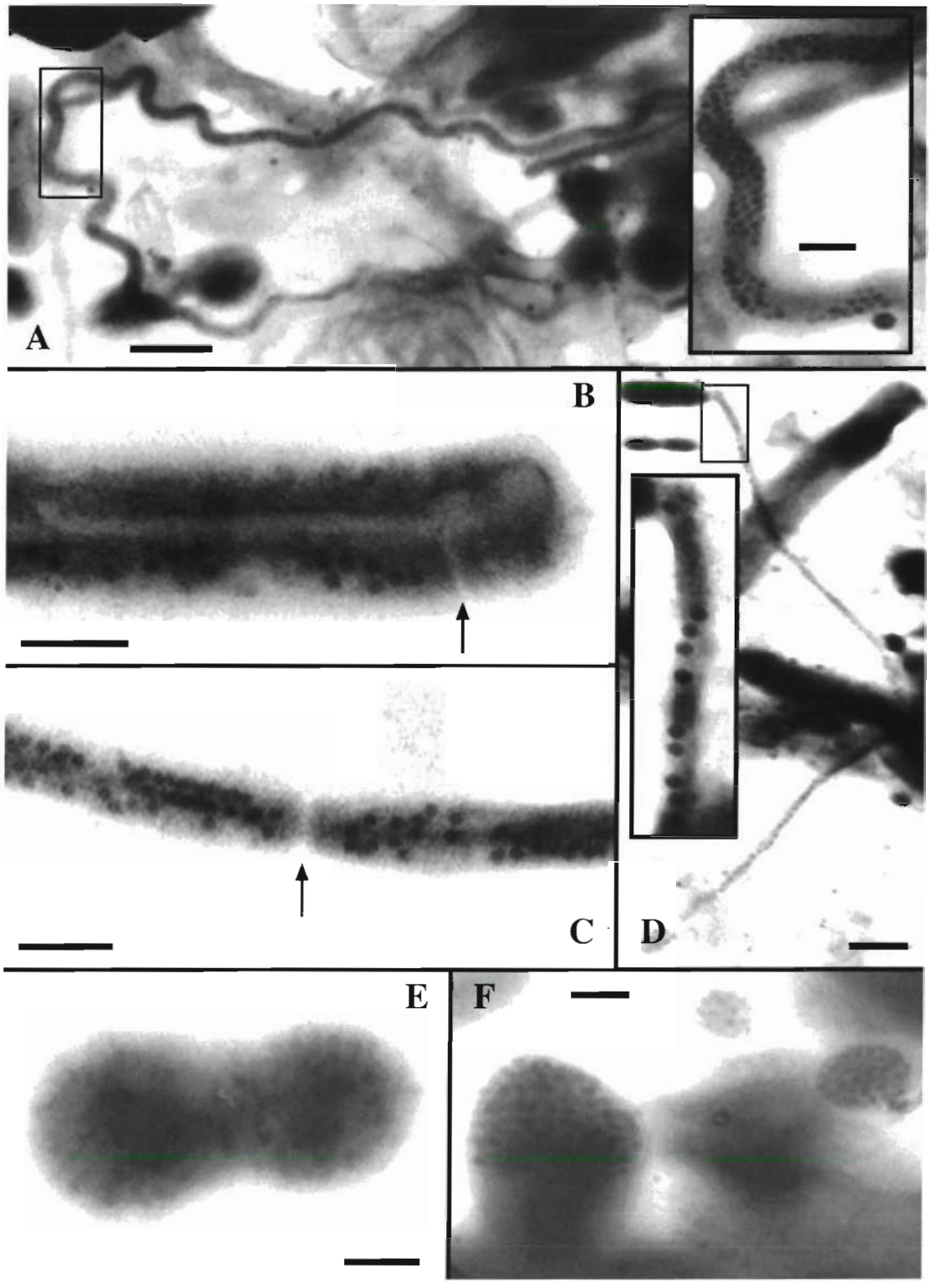

Fig. 4. Electron micrographs of visibly infected bacteria. (A) Spirochaete-like cell. Insert shows mature viruses within the cell. (B) Cells 1 and 2 of a colony consisting of 4 filamentous bacteria. Only the lower cell contains mature viruses. (C) Cells 3 and 4 of the cell colony shown in (B). Both cells are visibly infected. (D) Filamentous cell. Insert shows mature viruses within the cell. (E) Dividing visibly infected cell. (F) Three visibly infected cells. The cell in the middle is smaller than 0.3 µm in length. Rectangles show the approximate area of the inserts and arrows the septae in the colony. Scale bars: (A, D) 500 nm, (B, C, E, F, insert in A) 200 nm

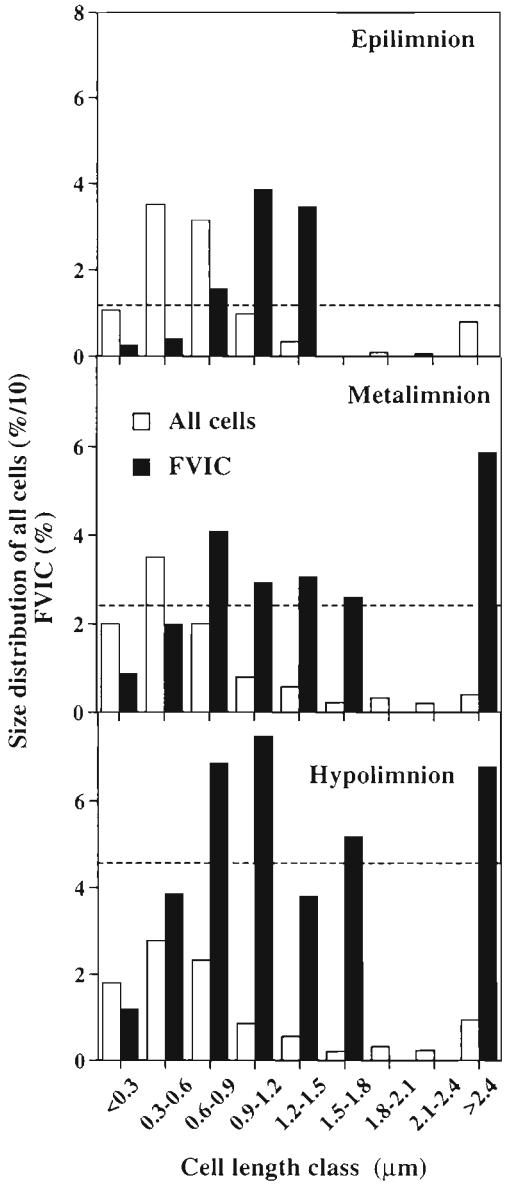

Fig. 5. Frequency of visibly infected cells (FVIC) and cell size distribution of the entire bacterial community in different size classes in 3 depth layers of Lake Plußsee on September 23, 1996. The cell size distribution of entire bacterial communities from Fig. 1 was shown to allow for a better comparison with the FVIC in different size classes. Dotted lines indicate the FVIC averaged for the entire bacterial community

in the metalimnion and lowest in the epilimnion. As a consequence of the contact model the specific contact rate increased with cell size.

Experiments

Lake water was passed through filters of different pore size to obtain DOM fractions with varying concentrations of the natural virus community and test the

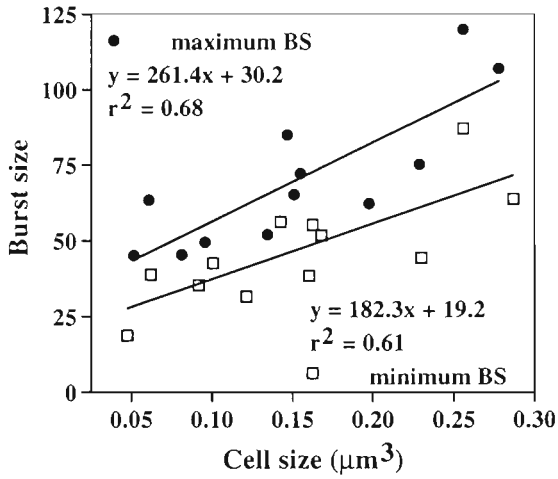

Fig. 6. Relationship between cell size and burst size (BS) of bacteria in Lake Plußsee on September 23, 1996. Data points are means obtained from all visibly infected cells per sample

hypothesis that viral lysis affects the cell size of bacterioplankton. Due to the treatment, viral abundance in the virus-reduced fraction was only 18% of that in the

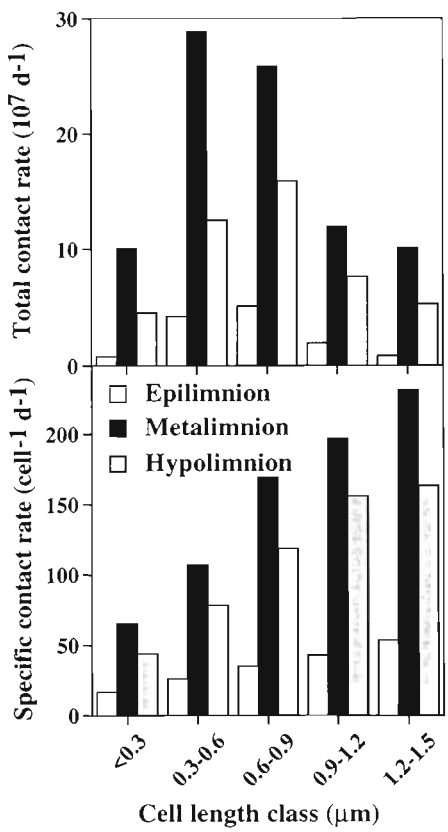

Fig. 7. Total and specific contact rates of viruses and bacteria in different size classes in 3 depth layers of Lake Plußsee on September 23, 1996. Viral contact rates were calculated by using the model of Murray & Jackson (1992)

Table 3. Viral abundance, frequency of visibly infected cells (FVIC) and length of the entire bacterial community and VIC in different fractions of dissolved organic matter at the end of the experiments (after 41 h). Values are expressed as means calculated from duplicate enclosures and ranges are given in parentheses

DOM fraction	Viral abundance (10^7 ml^{-1})	FVIC (%)	Length (µm) of cells <2.4 µm	
			Entire community	VIC
Virus-reduced	1.2 (0.7–1.6)	0.2 (0.1–0.4)	0.92 (0.90–0.94)	1.2 (1.2–1.3)
Virus-rich	6.4 (4.5–8.3)	1.8 (1.4–2.1)	0.80 (0.79–0.81)	1.2 (1.1–1.2)

virus-rich fraction and the FVIC at the end of the experiments was higher in the virus-rich than in the virus-reduced fraction (Table 3). The cell length of VIC did not differ between the 2 DOM fractions, but average cell length was larger in VIC than in the entire bacterial community. The average cell length of the bacterial community was smaller in the virus-rich than in the virus-reduced fraction. Moreover, the percentage of cells <0.3 and >2.4 µm was higher in the virus-rich than in the virus-reduced fraction (Fig. 8), indicating that viral lysis had influenced the cell size of bacteria.

DISCUSSION

Bacterial cell volumes observed in this study (0.04 to 0.24 µm^3 cell^{-1}) were higher than those reported by Cole et al. (1993) for oxic and anoxic waters in 20 North American lakes (0.01 to 0.2 µm^3). Average cell volumes of bacterial communities from lakes are typically smaller than 0.15 µm^3 cell^{-1}; however, cell volumes larger than 0.4 µm^3 have also been reported (e.g. Riemann et al. 1986, Sime-Ngando et al. 1991). During a seasonal study in Lake Plußsee, bacterial cell volumes

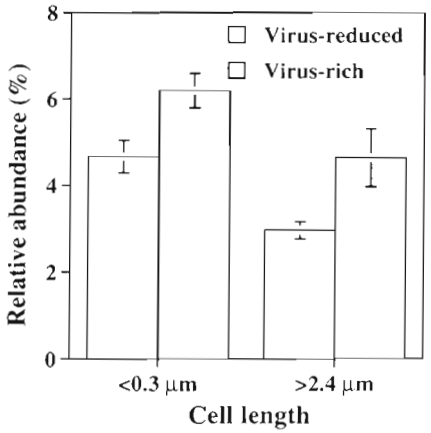

Fig. 8. Frequency of cells <0.3 and >2.4 µm in different fractions of dissolved organic matter from the epilimnion at the end of the experiment (41 h). Values are expressed as the means of duplicate enclosures and the error bars represent the ranges

ranged from 0.02 to 0.13 µm^3 (Krambeck et al. 1981) and were thus considerably lower than our values, but it is not clear from which depth layer Krambeck et al. (1981) collected the samples. Assuming that the samples were from the epilimnion, our values from this depth layer (0.04 to 0.08 µm^3) would fall within their range. Bemmer & Overbeck (1994) found a value of 0.086 µm^3 for epilimnetic bacteria in Lake Plußsee, which is close to our values from the epilimnion. Differences between studies could also result from the different methods used for sizing bacterioplankton. Bacterial biomass and cell size were larger in anoxic than in oxic water and thus support previous findings (Cole et al. 1993); however, our data do not provide new insight into the mechanisms which are responsible for this observation.

Burst size

Burst size is a crucial parameter for the estimation of viral production, since viral production depends on the number of lysed cells and the number of viruses set free per lysed cell. Burst sizes of bacteria in aquatic systems show a wide range from ca 5 to 300 (Børsheim 1993). In limnetic systems the burst size of individual cells ranged from 21 to 121 in Lake Constance (Hennes & Simon 1995), from 5 to 160 in a backwater system of the River Danube (Mathias et al. 1995), and from 5 to ca 500 in Lake Plußsee (this study). Only once did we determine a burst size of more than 300 phages in a long spirochaete-like cell (Fig. 4). Burst sizes of ca 120 viruses or more were typically found in filamentous bacteria (Fig. 4; Mathias et al. 1995). Studies with bacteriophage isolates showed that a variety of factors such as temperature, growth rate of the host and taxonomy can influence the burst size. Data from Mathias et al. (1995) suggest that the burst size of bacterioplankton is high at low temperatures. In our study the burst size increased with depth, i.e. with decreasing temperature. The higher burst sizes in colder water layers coincided with larger cells (Table 1) and the burst size generally increased with cell size (Fig. 6). Moreover, using stepwise multiple regression analysis the cell size was the only measured parameter that

could explain burst size. Thus, the differences in burst size with temperature found by Mathias et al. (1995) might have been influenced by larger cells in colder water. A positive relationship between burst size and cell volume was also reported from the Mediterranean Sea (Weinbauer & Peduzzi 1994).

Effect of viral lysis on cell size

In all depth layers we found VIC in the smallest size class (<0.3 µm; Figs. 1 & 4); however, the FVIC in this size class was only 23 to 36% of the average FVIC (Fig. 5), indicating that the mortality due to viral lysis in this size class was lower than the overall viral control. This is supported by the finding that the percentage of cells <0.3 µm was highest in enclosures with the highest viral activity (Fig. 8). The percentage of cells <0.3 µm might increase when a significant portion of larger cells is removed by viral lysis. Thus, being small might be a strategy to reduce not only grazing pressure (González et al. 1990) but also viral infection. A potential explanation for this is provided by the fact that the specific contact rate increases with cell size (compare also Fig. 7). Assuming that the contact success does not differ between size classes, i.e. the contact rate is directly related to mortality, the mortality due to viral lysis in the size class <0.3 µm was 56 to 64% of that in the size class 0.3–0.6 µm and 37 to 47% of that in the size class 0.6–0.9 µm. Moreover, small cells are frequently less active than medium-size cells (Pernthaler et al. 1996, Posch et al. 1997) and cells in the stationary growth phase are usually less susceptible to infection than those in the exponential growth phase (Lenski 1988). A low expression of receptors per surface area due to slow growth in combination with low contact rates could be responsible for the low infection frequencies in the size class <0.3 µm.

Although the average mortality due to viral lysis was low in the epilimnion (average 13%), bacterial mortality in the size classes 0.9–1.2 and 1.2–1.5 µm was comparatively high (ca 40%). Thus, viral lysis may even influence the cell size distribution in systems in which the overall impact of viruses on bacteria is low. This is supported by experimental data with epilimnetic water demonstrating that the cell size of the bacterial community was smaller in the virus-rich than in the virus-reduced fraction (Table 3). Overall, viral lysis might be one of the mechanisms keeping the cell size small in natural systems.

The finding that the average cell length in the size class >2.4 µm was low in the epilimnion (4.5 µm) compared to the meta- (6.6 µm) and the hypolimnion (7.7 µm) was surprising, since grazing on bacteria by protozoans decreased with depth during the investigation period (Weinbauer & Höfle 1998). Cladocerans which were present in the epi- and the upper part of the metalimnion might have fed on large cells and therefore reduced the cell length in the size class >2.4 µm. However, the concept of size-selective grazing cannot explain the high number of large cells in the hypolimnion, since grazing rates are low in this depth layer. Since the width of cells in the entire hypolimnetic bacterial community was not significantly different from that in cells >2.4 µm (data not shown), disadvantages of nutrient uptake due to the lower surface-to-volume ratio in large cells might be somewhat offset in cells >2.4 µm. This might be a reason for the occurrence of a high percentage of cells >2.4 µm in the hypolimnion.

In the epilimnion no VIC were found in cells >2.4 µm, whereas in the meta- and hypolimnion a high FVIC (6 to 7%) was found in this size class (Fig. 5). In the entire epilimnion ca 640 cells >2.4 µm were examined for mature viruses, indicating that the FVIC was less than 0.2% in this size class. Also, we did not find VIC >2.4 µm in the experiment which was performed with epilimnetic water. Moreover, the percentage of bacteria >2.4 µm was higher in the virus-rich than in the virus-reduced fraction (Fig. 8), indicating that viruses might influence the proportion of large cells in the bacterioplankton community. However, the reason for this finding is unclear. Bacteria were inoculated at a final concentration of 10% which resulted in reduced contact rates between bacteria and flagellates, and flagellate abundance was only ca 2×10^2 cells l^{-1} at the end of the experiments and did not differ between the treatments. Thus, it is unlikely that size-selective grazing influenced the percentage of cells >2.4 µm in the experiment.

It is possible that the cell division of the host is inhibited during viral infection, while the cell continues to grow. This would result in cells which are larger than those of the non-infected community and thus bias our findings. However, this is unlikely, since dividing VIC were found in Lake Plußsee (Fig. 4) and other freshwater systems (Velimirov pers. comm.). Since the latent period of infected cells is comparable to the generation time of the uninfected hosts (Proctor et al. 1993, Guixa-Boixareu et al. 1996), there is no indication that viral infection significantly inhibits the cell division. The highest percentage of cells in the entire bacterial community was found in the size class 0.3–0.6 µm, whereas a high FVIC was still detected in cells >0.9 µm. Thus, even an overestimation of the size of VIC by an entire size class would not have influenced our overall conclusions. Also, the data on the effect of viral lysis on the cell size distribution of bacteria obtained from the experiments would not be affected by changes of cell size due to infection.

Implications

Our data indicate that not only size-selective grazing but also size-specific lysis could influence the cell size distribution of bacterioplankton. While size-selective grazing is a function of the size preferences of the protozoan community, viral infection is not per se size-specific, but depends on the presence of viruses and their hosts. Since the FVIC increases with bacterial production (Steward et al. 1996), fast growth of cells in specific size classes very likely also results in high viral infection, e.g. due to a high number of receptors on the cell surface of fast growing cells. Not only changing the cell size, but also increasing the cell division rates might be a strategy of bacteria to cope with grazing (Pernthaler et al. 1997), and protozoans typically graze on dividing, i.e. active, cells (Sherr et al. 1992). Thus, the effect of viral lysis on the cell size distribution might be tightly linked to size-selective grazing, i.e. a growth stimulation of bacteria due to grazing might increase viral infection rates. However, it is also possible that medium-size cells generally grow faster, e.g. due to their optimum surface-to-volume ratio, and thus are predominantly infected by viruses.

Since the mortality due to grazing and viral lysis was low in the smallest bacteria, being small could be an important strategy to avoid mortality. However, based on the low growth rate (Pernthaler et al. 1996, Posch et al. 1997) and low biomass of small cells, it is unlikely that they are important for bacterial carbon cycling. Since grazing rates of protozoans on large cells are low, a significant portion of bacterial carbon is probably stored in large cells and only transferred to higher trophic levels during periods of strong grazing by cladocerans. The high mortality of large cells due to viral lysis in the meta- and hypolimnion implies that a significant amount of organic matter could be released and become available to the entire bacterial community. This indicates that—at least in the meta- and hypolimnion—a significant portion of carbon is recycled in the bacteria-virus-DOM loop. Also, the size fraction >2.4 µm might contribute significantly to viral production in the meta- and hypolimnion, since bacterial mortality due to viral lysis is high and the burst size increases with the cell size. Overall, we have provided circumstantial and experimental evidence that viral lysis can be an important factor determining the cell size distribution of bacterioplankton. This may have implications for our understanding of the effect of viral lysis on bacteria and the mechanisms regulating bacterial biomass and nutrient cycling.

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