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Seasonal dynamics of viruses in an alpine lake: importance of filamentous forms

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ABSTRACT: Viruses are an important component of the planktonic food web in freshwater and marine systems, but most studies have been done in the ocean and in lowland lakes. In this work, the seasonal dynamics and structure of the virioplankton as well as their impact on bacteria during a day/night cycle were studied in an alpine lake located 2417 m above sea level. The abundance of virus-like particles (VLP) was determined at 5 discrete depths (0.5 to 8 m) by direct counts with a TEM in samples collected from May to November 1998 at weekly to bi-weekly intervals. Viruses reached the highest abundances under ice (4.6×10^6 VLP ml^{-1}) with a second maximum in autumn. After ice-break, the VLP abundance decreased to undetectable values ($<2 \times 10^4$ VLP ml^{-1}) probably because of the negative effect of solar radiation that was negatively correlated with the viral abundance in the upper 2 m of the water column (Spearman rank correlation, $r_s = -0.773$, $p < 0.01$). The virioplankton was morphologically diverse, consisting of forms commonly found in other aquatic systems, but unlike other studies, we found filamentous VLP (FVLP) 450 to 730 nm long that attained abundances of up to 1.3×10^6 ml^{-1} and accounted for 7 to 100% of the total viral abundance. These FVLP were found occasionally inside filamentous heterotrophic bacteria (>10 µm) and their respective abundances were positively correlated ($r_s = 0.728$, $p < 0.01$). The absence of these conspicuous forms in other aquatic ecosystems suggests that FVLP are well adapted to the harsh environmental conditions or are specific to bacterial hosts found in alpine lakes. Finally, between 5 and 28% of the newly produced bacteria were killed by non-filamentous viruses, which therefore are a modest cause of bacterial mortality in this lake.

KEY WORDS: Virus-like particles (VLP) · Bacteriophage · Viral lysis · Bacterioplankton · Solar UV radiation · Microbial food web · High-mountain lakes

INTRODUCTION

Viruses are now recognized as an important component of the planktonic food web in freshwater and marine systems (Bratbak et al. 1994, Fuhrman 1999, Wommack & Colwell 2000). Viral infection and lysis of aquatic microorganisms short circuits the microbial food web, creating a loop among bacteria, viruses and the DOM pool (Bratbak et al. 1990, 1992, Suttle et al. 1990, Fuhrman 1999). Hence, viruses have the potential to influence the pathways of matter and energy transfer in aquatic systems (Fuhrman 1999). Several studies have shown that besides grazing by protists, viruses are an important cause of mortality among bacteria and phytoplankton (Proctor & Fuhrman 1990, Suttle et al. 1990, Hennes & Simon 1995, Weinbauer & Höfle 1998).

Temporal changes in viral abundance indicate that they are a dynamic component of the microbial community (Bergh et al. 1989, Jiang & Paul 1994, Weinbauer et al. 1995). Viral numbers are influenced by changes in the productivity and abundance of hosts, but also by environmental factors that affect host populations such as temperature and nutrient concentration (Jiang & Paul 1994, Maranger & Bird 1995, Weinbauer et al. 1995). Moreover, viruses can be removed or inactivated by solar UV radiation, adsorption to

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particulate material and sedimentation, grazing by protists, exo-enzymes and virocidal substances released from aquatic organisms (Suttle & Chen 1992). The mechanisms influencing and controlling viral abundance, however, might be very different in diverse aquatic systems, e.g., depending on the trophic state, the concentration of suspended particles and external forces.

Viruses have been shown to be abundant and ubiquitous in aquatic ecosystems; however, most studies have been done in the ocean (Wommack & Colwell 2000). Relatively little information is available on the virioplankton of freshwater lakes (Wommack & Colwell 2000 and references therein) and particularly of alpine lakes (Pina et al. 1998). Alpine lakes can be considered as harsh environments because their microorganisms have to cope with low nutrient conditions, low temperatures, extreme changes in light conditions between ice-covered and ice-free situations, and strong solar UV radiation. Consequently, viruses in these ecosystems are faced with low host densities, slow growth rates of hosts and potential UV damage (Pina et al. 1998, Sommaruga et al. 1999). Most alpine lakes are remote, i.e., they have no direct anthropogenic influence, and, therefore, viruses are expected to be mainly indigenous. Moreover, in alpine lakes without surface inflow, the availability of new hosts is restricted to natural populations growing in the lake and to those from groundwater and atmospheric input.

Pina et al. (1998) showed that viruses are numerically important in the plankton of alpine lakes (10^6 to 10^7 virus-like particles [VLP] ml^{-1}) but provided no information on the dynamics of virioplankton in these ecosystems. Consequently, the objectives of our study were (1) to investigate in an alpine lake the seasonal dynamics of VLP in the water column and the factors controlling their abundance, and (2) to assess the impact of viruses on bacterial mortality during a day/night cycle.

MATERIALS AND METHODS

Study site and sampling. The study was conducted in 1998 at Gossenköllesee, an oligotrophic lake situated above the treeline (2417 m above sea level) in the Central Alps, Austria (47° 13′ N, 11° 01′ E). Gossenköllesee is a small lake (area: 1.7 ha) with a maximum depth of 9.9 m that is usually ice covered between November and June.

Water samples were collected from a platform located over the deepest point of the lake with a modified opaque Schindler (5 l) sampler at 5 depths (0.5, 2, 4, 6 and 8 m) from May to November at weekly to biweekly intervals. Sub-samples for the determination of viruses and bacteria were fixed with formaldehyde (final concentration 2% v/v) and stored in the dark at 4°C. Samples for the determination of chlorophyll *a* (chl *a*) concentration were collected in opaque 5 l carboys.

Solar radiation and temperature. Global (i.e., diffuse plus direct) solar radiation (300 to 3000 nm) was measured at 15 min intervals with a star-pyranometer located in the same valley ca 1000 m distant from the lake. Values of solar radiation were integrated daily and summed for the 3 d before sampling.

Water temperature was measured with a thermometer (±0.1°C) placed inside the water sampler immediately after collection.

Chl *a*. Between 2 and 3 l of lake water was filtered onto Whatman GF/F filters (Whatman, Maidstone, UK) that were kept frozen (–20°C) until extraction within 2 wk. The filters were extracted in 90% alkaline acetone for 24 h in the dark at 4°C and afterwards sonicated for 1 min on an ice bath. The extracts were then filtered through an Anodisc filter of 0.1 µm pore size (Whatman), and the absorbance of the filtrate was measured in a spectrophotometer (Hitachi U-2000, scans between 400 and 750 nm) against an acetone blank using cells of 5 cm path length. The formulas of Jeffrey & Humphrey (1975) were used to calculate pigment concentration.

Heterotrophic bacteria. Bacteria were stained with the fluorochrome DAPI and counted in an epifluorescence microscope. For bacterial counts, 10 ml of formaldehyde-preserved sample was stained for 10 min with DAPI (final concentration 0.1% w/v) and filtered onto black polycarbonate membrane filters (Poretics, 0.2 µm pore size). At least 400 non-filamentous (<10 µm) bacteria and 200 filamentous bacteria (>10 µm) were counted at 1000 to 1600× magnification in a Zeiss Axioplan epifluorescence microscope equipped with a BP 365, FT 395 and LP 397 filter set.

VLP abundance. VLP were enumerated by TEM according to Bergh et al. (1989) and Suttle (1993). Briefly, 30 ml of fixed lake water was harvested directly onto electron microscope grids (400 mesh Cu grids) supported with a carbon-coated Formvar film, using a Sorvall OTD-2 ultracentrifuge (50 000 × *g* for 6 h at 20°C) with a swing-out rotor (AH627). After the removal of the supernatant, the grids were stained facedown with 2% (w/v) uranyl acetate for 30 s and then rinsed 3 times with distilled water (filtered through a 0.02 µm Anodisc filter). VLP were counted in view fields randomly selected until total numbers exceeded 200 using a Zeiss (CEM 902) TEM at 80 kV and 85 000× magnification. An ocular with additional magnification of 9× was used to look in detail at viruses in the TEM. Taper corrections were implemented into final calculations (Suttle 1993).

In a previous experiment, we tested the among- and within-sample variability of VLP abundance considering 5 independent samples collected from a 4 m depth and 2 replicates (grids) for each of them. A 2-way ANOVA indicated that there was no statistically significant difference among the independent samples ($p = 0.829$) or among the replicates ($p = 0.518$). Nevertheless, the abundance of VLP was estimated for 2 grids per sample.

Size of VLP. The size of VLP was determined with an image-analysis system. Images were recorded with a SIT camera (Model 66, Zeiss) on the TEM and stored on the hard disk of a personal computer. The length and width of the particles were measured with the image analysis program Lucia D (version 3.5, Laboratory Imaging, Prague, Czech Republic). The image-analysis system was calibrated with latex beads (1.0 and 0.2 µm) and catalase crystals (beef liver, lattice spacings of 6.85 and 8.75 nm).

Viral lysis. This experiment was done on samples collected from 0.5 and 8 m depths on September 10 at 05:00 and 16:00 h (local time). Samples for measurement of bacterial activity, VLP abundance, bacterial abundance and chl *a* were collected at the same time. Whole bacterial cells were examined in the TEM for visibly infected bacteria (VIB). Because of the low number of bacteria, 10 l of lake water at each sample occasion was concentrated by ultrafiltration to ~60 ml with an Amicon hollow fiber filter apparatus (CH2RSA; cut-off 0.1 µm). Samples were previously screened through a 5 µm net to eliminate larger cells. The samples were fixed with glutaraldehyde (electron microscope quality, final concentration 2% v/v) and stored in the dark at 4°C until further processing within 2 wk. The bacteria were harvested directly onto electron microscope grids in the same way as described above for VLP, but the ultracentrifuge was run for only 2 h at 4°C at 50 000 × *g*. The electron microscope grids were stained with 1% (w/v) uranyl acetate for 30 s. In the TEM (Zeiss CEM 902, 80 kV), at least 1000 bacteria per sample were inspected for VIB at 3000 to 12 000× magnification. Bacteria that contained at least 3 mature phages were considered to be visibly infected. The mature viral particles were identified based on the shape, size, staining intensity and uniformity of these parameters. Simultaneously, the burst size, i.e., the average number of mature viral particles inside of VIB, was determined. The model of Binder (1999; Eq. 23) was used to estimate the bacterial mortality due to viral lysis. The ratio of the latent period to the generation time of bacteria (γ) was considered to be 1, and the latent period elapsed before the visible appearance of intracellular viral particles (ε) was calculated with the conversion factors given by Proctor et al. (1993).

Bacterial activity was measured by the incorporation of [^{3}H]-thymidine (84 Ci mmol^{-1}, Amersham, UK), which was added at saturating concentrations (10 nM) to triplicate sub-samples (Fuhrman & Azam 1982). Incubations were done at *in situ* temperatures in the dark for 1 h and terminated by the addition of formaldehyde (final concentration 3% v/v). Bacteria were then collected by filtering samples through 0.2 µm pore size polycarbonate filters (Poretics). After extraction of the macromolecules with ice-cold 5% (w/v) TCA for 10 min, the filters were washed 10 times with 1 ml TCA and 2 times with 5 ml ice-cold 80% ethanol (v/v). The dry filters were placed into scintillation vials with 10 ml of scintillation cocktail (Ready Safe, Beckman, Fullerton, CA, USA). Disintegrations min^{-1} were determined in a Beckman LS 6000 IC scintillation counter after complete dissolution of the filters. All samples were corrected for abiotic incorporation by subtracting the radioactivity of 2 formaldehyde-killed controls. Incorporation rates of [^{3}H]-thymidine were converted to the number of cells produced using factors of 0.5×10^{18} (theoretical) and 2×10^{18} (most common empirical conversion factor found in the literature) bacteria mol^{-1} thymidine incorporated (Bell 1993).

Data analysis. All statistical analyses were performed with the Sigma-Stat 2.03 software package (SPSS Inc., Chicago, IL, USA). After verification of normality and variance equality, the variance of parameters in different samples was tested by 1-way ANOVA. Spearman rank correlations (r_s) were used to investigate the relations among parameters. To limit the overall experimental error rate (type I error), we corrected the significance level according to the Bonferroni method as $\{\alpha/[n(n-1)/2]\}$, where α is the probability level and n the number of observations.

RESULTS

Solar radiation, temperature and chl *a* concentration

The highest value of insolation (sum of the daily integrals for the 3 d before sampling) was found on June 23 after the ice-break (Fig. 1a). The lowest temperature was measured under the ice-cover (0°C) and the highest (~15°C) on August 20 at the surface (Fig. 1b). The lake mixed shortly after ice-break on June 8. The lake was thermally stratified during July, August and the beginning of September, after which there was a long period of mixing until the ice-cover formed at the end of October (Fig. 1b).

Beneath the ice-cover, the maximum chl *a* concentration (2.4 µg l^{-1}) occurred at a 4 m depth (relative to the top ice surface) in May. During the ice-free period, the maximum chl *a* concentration was measured at 8 m

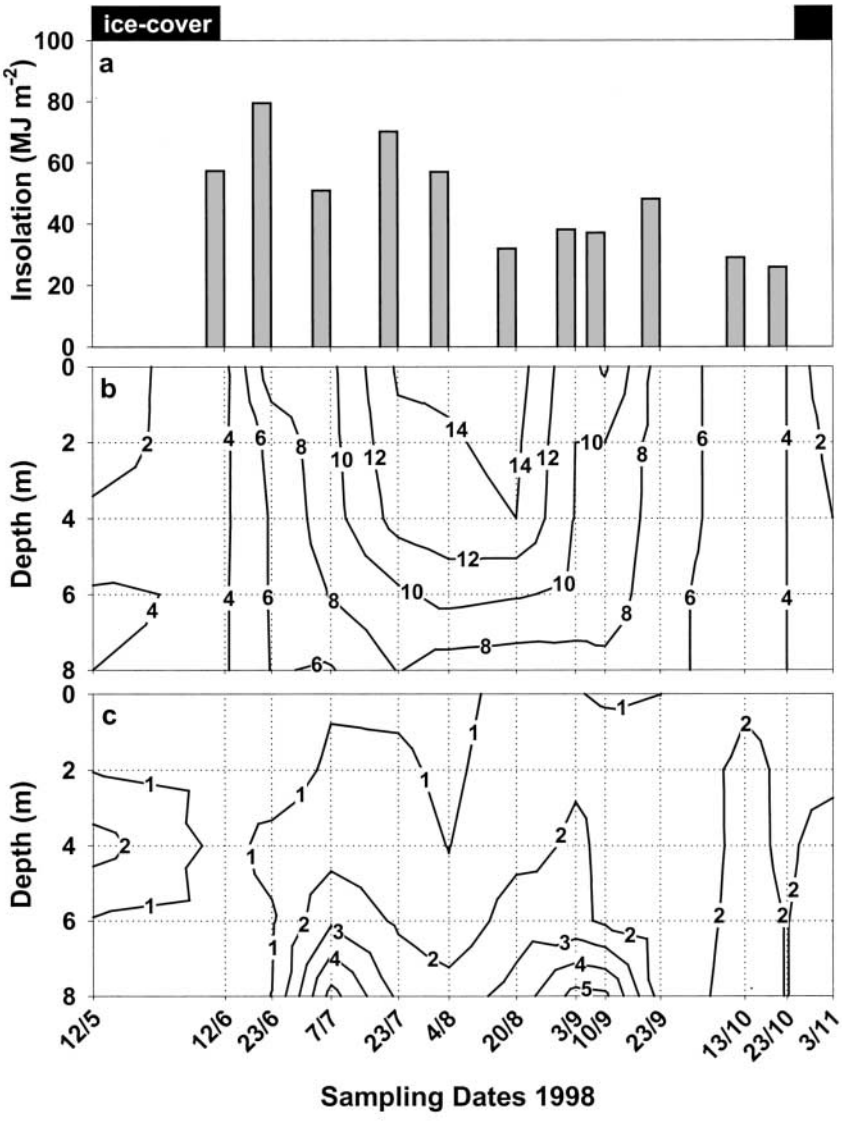

Fig. 1. (a) Sum of daily integrals of global solar radiation (insolation) for the 3 d before sampling; (b) isopleths of temperature; and (c) concentration of chlorophyll *a* in Gossenköllesee. The sampling points in (b) and (c) are indicated by the intersections of the dotted lines

(2.5 to 5.4 µg l^{-1}) when the lake was stratified (Fig. 1c). Surface chl *a* concentrations during summer stratification were always <1.4 µg l^{-1} (Fig. 1c).

Size structure of the virioplankton

Observations with the TEM indicated the existence of a morphologically diverse virioplankton, consisting of icosahedral VLP without tails with diameters between 20 and 300 nm, phages with rigid or contractile tails, and large flexible filamentous VLP (FVLP). These FVLP with lengths between 450 and 730 nm and widths between 30 and 40 nm had a remarkable morphology. The longest filamentous form presented a long electron-dense part at one end, a shorter one in the middle of the particle and a tail-like structure (Fig. 2a). FVLP 450 to 550 nm long presented the same long electron-dense part but lacked the tail-like structure (Fig. 2b). FVLP of the same morphology and length as those observed free in the plankton were found occasionally inside filamentous heterotrophic bacteria (Fig. 2c,d). Large icosahedral particles (head diameter ≈ 170 nm) with 4 µm long tails and star-shaped particles (diameter 70 nm) were also observed (the latter were not included in the counts). When the viral community was separated into size classes based on lengths of particles or viral heads, a bimodal distribution was observed due to the dominance of VLP <100 and >500 nm (Fig. 3).

VLP abundance and dynamics

The maximum VLP abundance (4.6×10^6 VLP ml^{-1}) occurred under ice (May) at 4 m (Fig. 4), coinciding with the chl *a* maximum (Fig. 1c). After ice-break (June and July), the VLP abundance decreased at times below the detection limit of our TEM method ($<2 \times 10^4$ VLP ml^{-1}). The second maximum of viral abundance was recorded in September and October at different depths, with concentrations ranging between 1 and 2×10^6 VLP ml^{-1} (Fig. 4).

The abundance of VLP varied down the water column (Fig. 4), except during the 2 mixing periods of the lake in June and October. However, considering the whole sampling period, there were no statistically significant differences between VLP abundances at different depths (ANOVA, $p = 0.687$).

FVLP (Fig. 2a) were detected for the first time 4 wk after ice-break, with an abundance of 10^4 to 10^5 ml^{-1} (value for all filamentous forms) in the upper 6 m of the water column (Fig. 5). From August onward, they were present in the whole water column, with abundances ranging from 2×10^4 (August) to 1.3×10^6 ml^{-1} (September). In this period, the FVLP accounted for 7 to 85% of the total viral abundance, with the exception of the 4 m depth on August 20, when 100% of VLP were FVLP (Fig. 5).

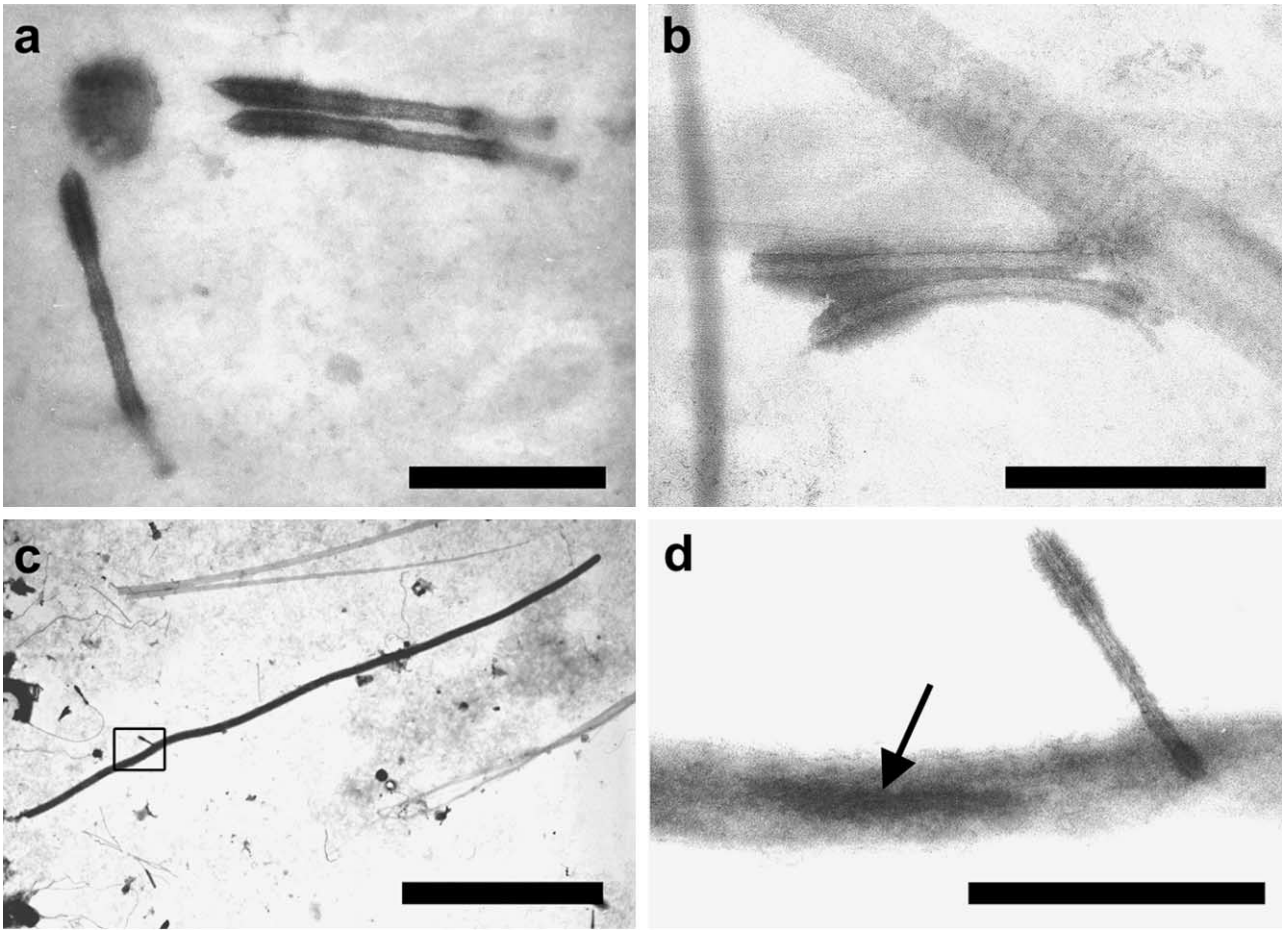

Fig. 2. TEM micrographs of filamentous virus-like particles (VLP) observed in Gossenköllesee. (a) Detail of the filamentous VLP showing the electron-dense and tail-like structures; (b) filamentous VLP without tail-like structures; (c) visibly infected filamentous bacterium and filamentous virus; and (d) enlargement of the area within the frame shown in (c) showing a detail with the intracellular localization of a filamentous VLP in the bacterium. Scale bars in (a), (b) = 400 nm; in (c) = 5 µm; and in (d) = 500 nm

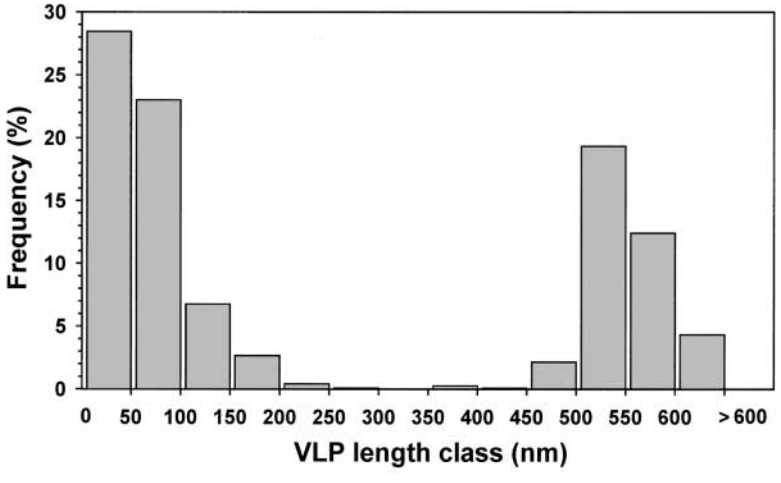

Fig. 3. Length class distribution of VLP for the whole sampling period (n = 1199)

Virus to bacterium ratio and correlations of viral numbers with other parameters

The virus to bacterium ratio ranged between 0.1 and 10.8 (Fig. 4); however, no significant correlation between bacterial and viral numbers was found (Table 1). On the other hand, the abundance of FVLP was positively correlated with the abundance of filamentous bacteria (r_s = 0.728, $p < 0.01$; Table 1), which ranged from 8.2×10^2 to 2.3×10^4 ml^{-1} (0.2 to 6.8% of the total bacterial abundance; Fig. 5). The ratio of FVLP to filamentous bacteria was between 3 and 121. During the ice-free season, the insolation summed for the 3 d before sampling was negatively correlated with the VLP abundance for the upper 2 m (r_s = –0.773, $p < 0.01$;

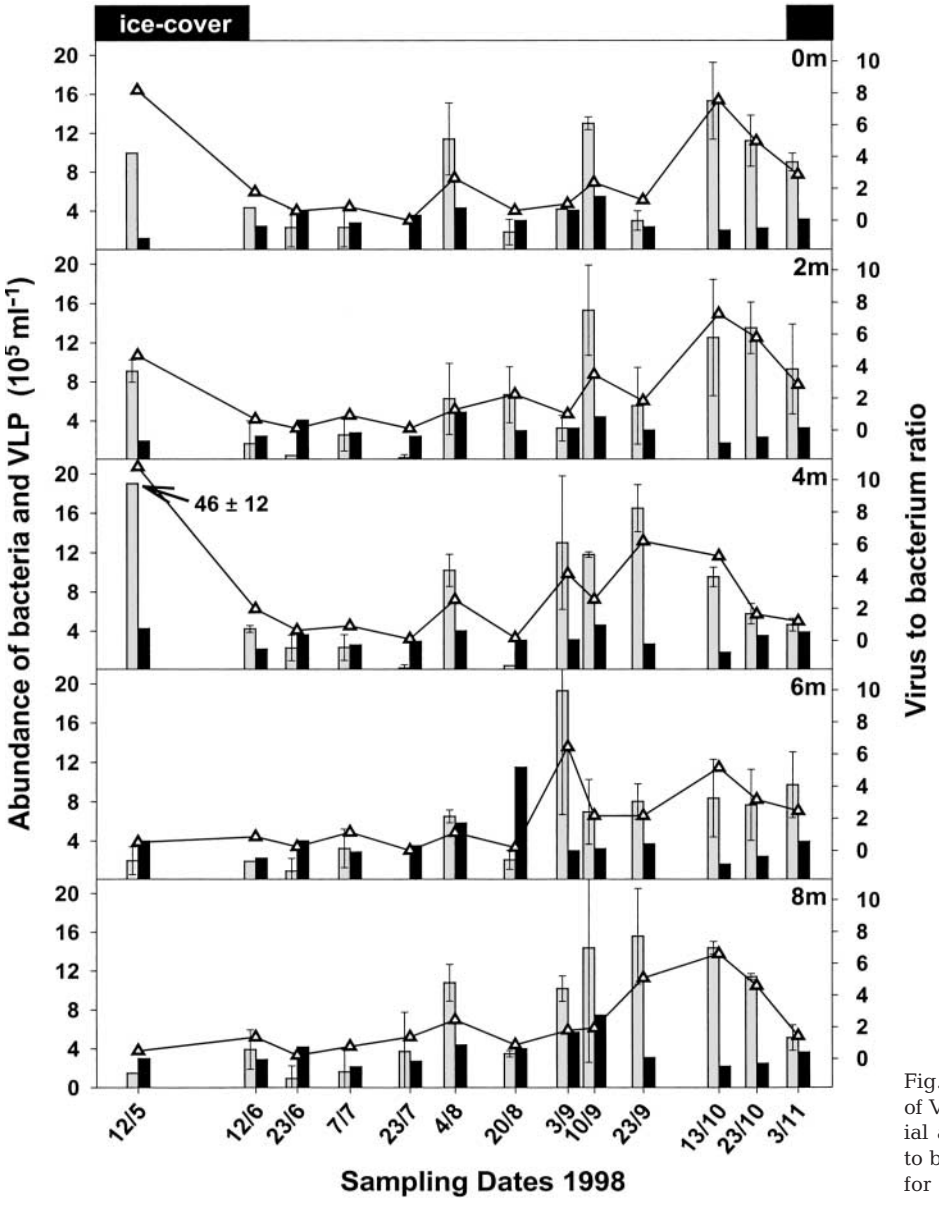

Fig. 4. Temporal and depth variation of VLP abundance (gray bars), bacterial abundance (black bars) and virus to bacteria ratio (triangles). Error bars for the VLP abundance are ±1 SD

Fig. 6). No significant correlation between these parameters was found for deeper water layers. When the insolation was summed for 2 or 4 d before sampling, the same trend was observed, but the correlation was highest for 3 d.

Viral lysis

On September 10, the lake was stratified with temperatures between 6.8 and 12.3°C, and the chl *a* maximum was found at an 8 m depth (Table 2). The bacterial abundance varied between 3.4 and 7.5×10^5 ml^{-1}, was higher by day than by night and was always higher at the lake bottom than at the surface. Simi-

Table 1. Spearman rank correlations between water temperature (Temp), chlorophyll *a* concentration (Chl *a*), bacterial abundance (BAC), virus-like particle abundance (VLP), abundance of filamentous VLP (FVLP) and abundance of filamentous bacteria (FBAC) for the whole data set (n = 65). The significance level was corrected according to Bonferroni; $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$

	Chl *a*	BAC	VLP	FVLP	FBAC
Temp	−0.056	0.375	−0.176	0.071	0.184
Chl *a*		0.045	0.350	0.406*	0.213
BAC			0.013	0.004	0.069
VLP				0.767**	0.425*
FVLP					0.728**

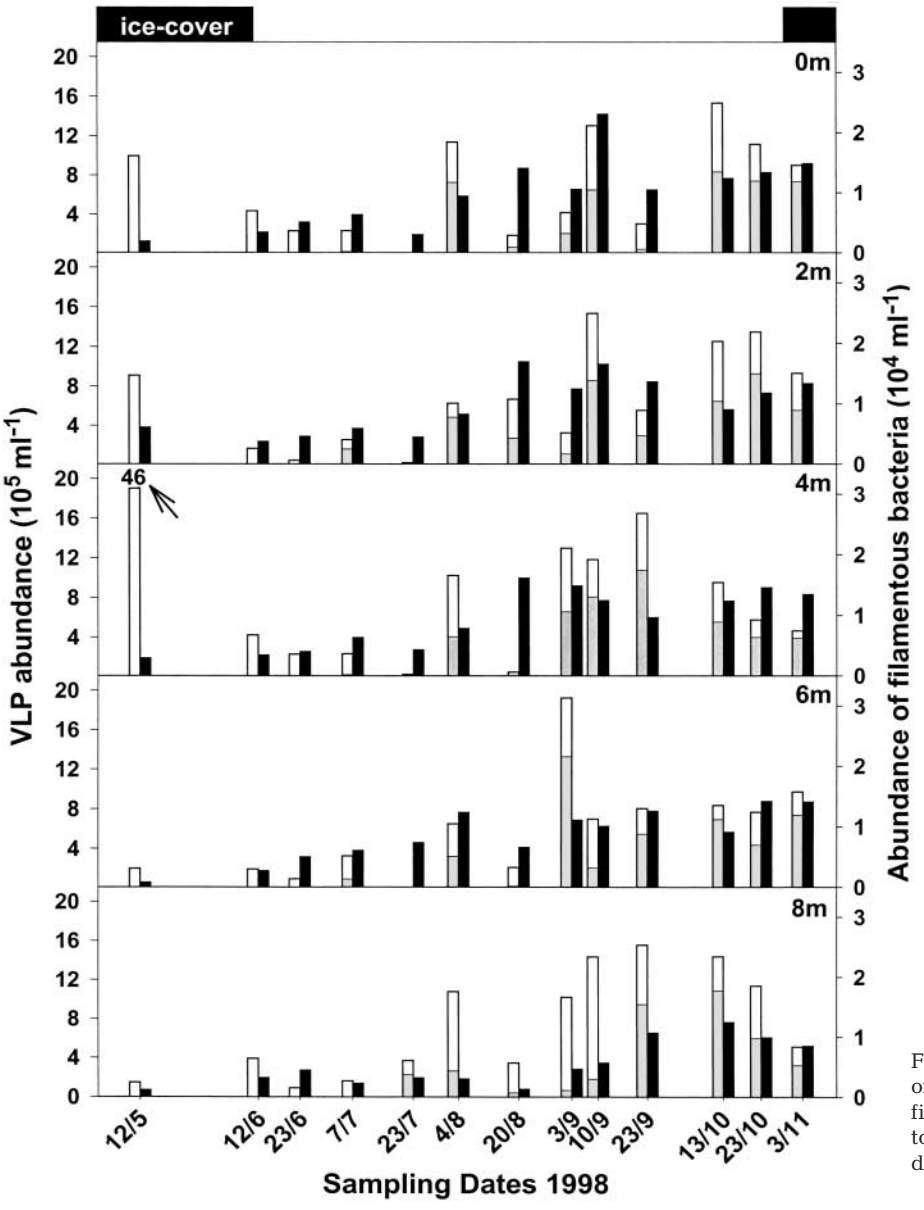

Fig. 5. Temporal and depth variation of VLP abundance separated for non-filamentous (white bars) and filamentous (gray bars) forms, and abundance of filamentous heterotrophic bacteria (black bars)

larly, bacterial activity was higher at 8 m depth (Table 2). Viral abundances ranged from 1.0 to 1.8 × 10^6 ml^{-1}, exceeding bacterial numbers by a factor of ~2 to 5 (Table 2). The frequency of VIB ranged from 0.9 to 2.3% (Table 2). The numbers of VIB did not change significantly between day and night, but more VIB were always found at 8 m than at 0.5 m (Table 2). Between 3 and 45 mature phages were observed inside VIB (Table 2), and the average burst size was higher at 8 m than at 0.5 m. However, only at 05:00 h was the difference in burst sizes between surface and bottom layers statistically significant ($p < 0.05$). Viral lysis removed between 5 and 11% of bacterial production at 0.5 m and 13 to 28% of bacterial production at 8 m (Table 2).

DISCUSSION

Seasonal dynamics of VLP

The range of VLP abundances in Gossenköllesee (Fig. 4) was within that reported in the literature for a variety of aquatic systems (10^4 to >10^8 VLP ml^{-1}; Wommack & Colwell 2000). In comparison with other oligotrophic freshwater systems, the range of VLP abundances in Gossenköllesee was similar to the range estimated in Lake Superior (0.2 to 0.9 × 10^6 VLP ml^{-1}; Tapper & Hicks 1998) and in 5 lakes of the Vestfold Hills, Antarctica (range 1.0 to 3.3 × 10^6 VLP ml^{-1}; Laybourn-Parry et al. 2001).

Little information is available about temporal dynamics of viruses in aquatic systems, particularly in fresh-

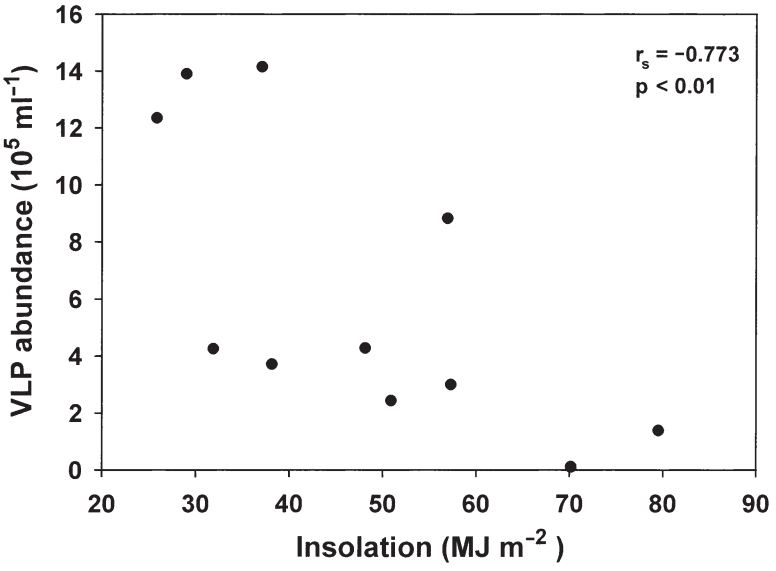

Fig. 6. Relation between the abundance of VLP for the upper 2 m of the water column and the sum of daily integrals of solar global radiation (insolation) for the 3 d before sampling

water systems and considering different depths in the water column. The temporal variation of the VLP abundance in Gossenköllesee from May (before ice-break) to November (after ice formation) was characterized by 2 maxima, 1 under ice and 1 in autumn, and a minimum after ice-break (Fig. 4). These maxima were associated with peaks in chl *a* and bacteria (Figs 1c & 4). In particular, the maximum in autumn may have been caused by the diatom *Cyclotella glomerata*, which regularly peaks in Gossenköllesee at this time. This is in agreement with several studies reporting a maximum of viral numbers in association with phytoplankton blooms (Wommack & Colwell 2000 and references therein).

A remarkable observation was that the viral abundance after ice-break decreased to very low or even undetectable values, whereas bacterial abundances did not change significantly (Fig. 4) and the chl *a* concentration increased at the lake bottom (Fig. 1c). The strong decrease of VLP abundance in Gossenköllesee after ice-break may have been caused by 2 non-exclusive factors, namely the sudden increase of solar radiation and a change in the species composition of the microbial community. The negative correlation found between viral abundances and solar radiation in the upper water layers supports the first hypothesis (Fig. 6). Because of the increase of the solar UV flux with altitude and the clear waters of alpine lakes (Sommaruga & Psenner 1997), plankton is exposed to high UV irradiance levels during certain periods. Solar radiation, especially UV-B radiation (290 to 320 nm), is an important factor in the decay of aquatic viruses (Suttle & Chen 1992, Suttle et al. 1993). Moreover, viral abundance might be indirectly affected by UV solar radiation if hosts are damaged. While the infectivity of phages can be restored inside bacteria, either through a specific host-repair machinery (= photoreactivation; Weinbauer et al. 1997, Wilhelm et al. 1998) or by a virus-encoded repair system (Shaffer et al. 1999), probably not all viruses and host bacteria have the same sensitivity to solar radiation (Wommack et al. 1996, Joux et al. 1999). Therefore, we hypothesized that UV-sensitive viral species that can reproduce successfully under the ice-cover may have been inactivated and destroyed after ice-break. The short mixing period in Gossenköllesee after ice-break might explain why the VLP abundance was affected in the whole water column. After the breakdown event, a new, more UV-tolerant viral community might have been established.

As stated above, another explanation for the breakdown of VLP abundance after ice-break could be a change in the microbial community and consequently in the availability of viral hosts. The maintenance of stable virus populations requires a specific density of

Table 2. Background parameters, frequency of visibly infected bacteria (VIB), burst size, virus to bacteria ratio (VBR), bacterial production and bacterial mortality due to viral lysis measured on September 10, 1998, at 05:00 h (night) and at 16:00 h (day). Values of viral abundance are given as mean of 2 replicates ± 1 SD. Values of burst size and viral lysis are expressed as means, and ranges are given in parentheses. The range of bacterial production is based on estimations using the theoretical and the empirical conversion factor. Other abbreviations as in Table 1

Time (h)	Depth (m)	Temp. (°C)	Chl *a* (µg l^{-1})	BAC (10^5 ml^{-1})	VIB (% bacterial abundance)	VLP (10^6 ml^{-1})	VBR (VLP cell^{-1})	Burst size	Bacterial production (10^5 cells produced l^{-1} h^{-1})	Viral lysis (% of bacterial production)
05:00	0.5	9.9	1.2	3.4	1.0	1.8 ± 0.6	5.2	7 (4 – 12)	2 ± 0.3 – 8 ± 1	8 (6 – 11)
	8	6.8	5.4	4.9	2.3	1.0 ± 0.04	2.1	18 (7 – 38)	9 ± 0.6 – 34 ± 3	21 (13 – 28)
16:00	0.5	12.3	0.8	5.5	0.9	1.3 ± 0.07	2.4	12 (3 – 45)	3 ± 0.2 – 11 ± 1	7 (5 – 10)
	8	7.4	5.2	7.5	2.2	1.4 ± 1.2	1.9	17 (4 – 44)	6 ± 0.3 – 24 ± 1	20 (13 – 27)

host cells (Wiggins & Alexander 1985), so that viruses can come in contact with a host before they are destroyed. Pernthaler et al. (1998) observed in Gossenköllesee that specific bacterial populations show different dynamics and that the composition of the bacterial assemblage (Bacteria and Archaea) changes noticeably after ice-break. In our study, no correlation was found between VLP and bacterial abundance (Table 1), but this does not rule out a possible relation between specific virus-bacteria host systems. Compositional change of the bacterial and the viral communities after the ice-break were indicated by the appearance of FVLP.

FVLP

The virioplankton consisted of VLP commonly found in other aquatic systems (Bergh et al. 1989, Klut & Stockner 1990, Sommaruga et al. 1995, Wommack & Colwell 2000) but also of FVLP 450 to 730 nm long and 30 to 40 nm wide (Fig. 2). Variability in the length of these filamentous forms (Fig. 3) may be related to breakage during sample preparation either of the long tail-like structure (ca 200 nm long; Fig. 2a,b) or at the second electron-dense part in the middle of the particle.

FVLP of the same morphology and length as those observed free in the plankton were found occasionally inside filamentous bacteria (Fig. 2c,d) and indeed their respective abundances were positively correlated (Table 1). Filamentous heterotrophic bacteria in Gossenköllesee are a small percentage of the total abundance (Fig. 5) but up to 70% of the bacterial biomass (Sommaruga et al. 1999). The FVLP seem to be a constant component of the viral assemblage in this lake because the same particles were observed in summer 1995 (S. Pina pers. comm.). Moreover, they are probably common to other alpine lakes since they were also observed in one alpine lake of the Pyrenees (Pina et al. 1998). With the exception of these observations in alpine lakes, filamentous viruses have, as far as we are aware, never been reported in natural aquatic ecosystems. Filamentous viruses, however, are known to infect bacteria growing in cultures. Examples of well-studied filamentous viruses are the phages M13, fd and f1, which infect *Escherichia coli* and have lengths of ca 760 to 1950 nm but diameters of only 6 to 8 nm (Murphy et al. 1995). All of these viruses are assembled during passage through the membrane of the host without killing them, i.e., they adopt, in contrast to most aquatic viruses, a strategy known as a chronic cycle (Fuhrman 1999, Marciano et al. 1999). This strategy, however, does not seem to apply to the FVLP found in our study because they were observed as complete particles in the bacterial cytoplasm (Fig. 2c,d). Moreover, they were ~5 times wider than filamentous *E. coli* phages; thus, passage through the membrane without killing the bacteria seems improbable (see Marciano et al. 1999 and references therein).

The FVLP appeared for the first time ca 3 wk after ice-break (Fig. 5), when the whole viral assemblage recovered in number. The absence of FVLP before ice-break and their large numbers found afterwards, despite low host numbers, suggest that these filamentous forms have an efficient strategy of replication. One such strategy could be pseudolysogeny or carrier state. In this phage-host interaction, the viral nucleic acid resides inert in the host cytoplasm until the lytic response is triggered, for example, by better host growth conditions or higher host density (Ripp & Miller 1998). In other cases, such as in the archaebacterial phage Hs1 infecting *Halobacterium salinarium*, the carrier state takes place under favorable growth conditions, e.g., at high salinities, whereas the lytic phase is triggered by low salinity that leads to host death (Reiter et al. 1988).

Besides the fact that FVLP were observed inside filamentous bacteria and their respective abundances were correlated, we cannot totally exclude the possibility that these particles are other types of biological agents such as, for example, bacteriocins. Although no bacteriocin of this dimension or morphology is known (Bradley 1967), a final answer can only be given by the isolation and cultivation of the bacteria producing these filamentous forms.

VIB and bacterial mortality by viral lysis

Because of the different ways of calculating viral mortality of bacteria from the frequency of VIB, it seemed more accurate to compare our data based on the percentage of VIB. The frequency of VIB has been measured in a variety of aquatic environments and ranges from 0.9 to 4.6% in marine and <0.1 to 9.0% in freshwater systems (reviewed in Wommack & Colwell 2000). In Gossenköllesee, the fraction of VIB ranged from 0.9 to 2.3% (Table 2). To our knowledge, there are no VIB data available for other oligotrophic freshwater systems. The range of Gossenköllesee was somewhat higher than the range found in the mesotrophic Lake Constance, i.e., <0.1 to 1.8% (Hennes & Simon 1995), and similar to the range for the epilimnion and metalimnion of the eutrophic Plußsee, i.e., 0.5 to 1.8 and 1.4 to 3.4%, respectively (Weinbauer & Höfle 1998). The VIB data, however, should be compared with caution because most estimates correspond to a single date.

Similarly to the burst size, the percentage of VIB was higher at 8 m than at the lake surface, both at 05:00

and 16:00 h (Table 2). This can be attributed to the more favorable growth conditions for bacteria as indicated by the higher bacterial activity at the deep chl *a* maximum. In addition, the higher abundance of bacteria at 8 m than at the surface probably favors a higher contact rate with phages. Solar radiation may also have affected the number of VIB as well as VLP abundance at the lake surface by inactivation of phages and host bacteria. The frequency of VIB did not significantly change between night and day (Table 2) probably because of the long generation times of bacteria in this lake (Sommaruga et al. 1999).

CONCLUSIONS

This study shows for the first time that viruses are a very dynamic component of the plankton in alpine lakes. The disappearance of the ice-cover and the sudden onset of high fluxes of solar UV radiation seem to play a major role in the dynamics and composition of the viral assemblage in this type of lake. Unlike other studies on viruses in different aquatic ecosystems, the virioplankton of Gossenköllesee was characterized by the presence of filamentous forms that reached high abundances. The absence of these conspicuous forms in other aquatic ecosystems suggests that FVLP are well adapted to the harsh environmental conditions or are specific to bacterial hosts found in alpine lakes. Although our data correspond to only one day, they suggest that in this lake viral lysis is a modest cause of bacterial mortality.

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