SHORT COMMUNICATION

Abundance, morphology and distribution of planktonic virus-like particles in two high-mountain lakes

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Abstract. Direct counts of virus-like particles (VLP) by transmission electron microscopy revealed abundances of up to $3 \times 10^7$ ml$^{-1}$ in the plankton of two remote high-mountain lakes in the Alps and the Pyrenees. Most VLP were icosahedric without a tail, and with diameters between 40 and 50 nm, but very large ones with diameters of up to 325 nm were also observed. VLP outnumbered bacteria by a factor of 4.2-42.8 and bacterial cells were infected with large numbers (>50) of viral particles. This study constitutes the first report on aquatic viruses for alpine lakes and it suggests that they may be an important additional source of bacterial mortality in these systems.

It is now widely accepted that viruses are a dynamic and ubiquitous component of the planktonic microbial community in aquatic environments. Besides protistan grazers, viruses can be important in the control of bacterioplankton. Fuhrman and Noble (1995) presented evidence, gathered from mesocosm experiments with coastal water, that viruses were able to produce similar bacterial mortality as protists. Quantitative models showed that this may lead to a substantial decrease in the bacterial carbon exported to protistan grazers (Fuhrman, 1992). In addition, viruses, which are largely species specific, may be important in the control of bacterial community structure (Hennes et al., 1995).

The abundance of viruses in aquatic systems generally ranges from $10^5$ to $10^8$ ml$^{-1}$ (Bergh et al., 1989; Børsholm, 1993; Fuhrman and Suttle, 1993; Hennes and Suttle, 1995). Although most published data on viruses in natural waters are for sea water, Maranger and Bird (1995) indicated that the virus-to-bacteria ratio (VBR) was significantly higher in fresh water (mode = 22.5) than in marine systems (mode = 2.5). They also found that the difference in viral abundance among aquatic systems appeared to depend mainly on parameters related to bacterial production, like chlorophyll a. Within one system, the balance between loss factors and the production of viruses, mainly by lytic growth, will regulate the viral abundance (Suttle and Chen, 1992; Wilcox and Fuhrman, 1994). In addition, solar UV radiation is known to have a strong impact on the loss of infectivity of free viruses (Suttle and Chen, 1992; Noble and Fuhrman, 1997). On the other hand, damaged viruses inside bacteria may partially regain their infectivity when exposed to photoreactivating light (Weinbauer et al., 1997).

To our knowledge, information about viruses from high-mountain lakes is not
available. Most of these lakes are remote, i.e. they are not directly affected by human activities; therefore, viruses are expected to be mainly indigenous. Moreover, most high-mountain lakes are highly transparent to UV radiation (Sommaruga and Psenner, 1997) and, due to their oligotrophic condition, the microbial components play a significant role within the planktonic food web (Stockner and Porter, 1988; Felip, 1997). Here, we present for the first time data on viral abundance, morphology and VBRs for two high-mountain lakes, and discuss their potential role in controlling bacterial abundance.

The study was conducted in Gossenkölesee (GKS) located in the Tyrolean Alps (47°13'N, 11°01'E) at 2417 m a.s.l. and in Lake Redö (LR) situated in the Central Pyrenees (42°38'N, 0°46'E) at 2240 m a.s.l. Both lakes are situated above the tree line, are oligotrophic and highly transparent. LR has a surface area of 24 ha and a maximum depth of 73 m, while GKS is smaller (area = 1.7 ha) and only 9.9 m deep. Other limnological characteristics are published elsewhere (Catalan, 1992; Felip et al., 1995; Sommaruga and Psenner, 1997).

Samples were collected at different depths in the water column from the central part of the lakes during the ice-free period in 1995 (30 July, and 2, 6 and 10 August in GKS, and 18 and 26 October in LR). On all occasions, sampling was carried out close to midday. In GKS, the weather was partially cloudy during the first two sampling dates and sunny during the rest, while in LR it was partially cloudy on 26 October. Subsamples for bacteria and heterotrophic nanoflagellates (only considered in GKS) were fixed with 37% formaldehyde at a final concentration of 2% v/v and stored at 4°C until further processing. Bacteria and heterotrophic nanoflagellates were enumerated by epifluorescence microscopy after staining with the fluorochrome 4'6'-diamidino-2-phenylindole (DAPI) on black polycarbonate membrane filters (0.2 μm pore size) according to Porter and Feig (1980). Samples for chlorophyll a analysis were filtered through Whatman GF/F filters and extracted with acetone (90%). The formulae of Jeffrey and Humphrey (1975) were used to calculate the pigment concentration. Virus-like particles (VLP) were enumerated by transmission electron microscopy according to Bergh et al. (1989). Briefly, 10 ml of lake sample fixed with formaldehyde (final concentration 2%) were harvested directly on electron microscope grids (400-mesh Ni grids) supported with carbon-coated Formvar film, using a Beckman L8-60M ultracentrifuge (197 500 g for 90 min) with an SW41 swing-out rotor. Afterwards, the supernatant was withdrawn, the grids air-dried and the sample stained with 3% uranyl acetate for 20 s. VLP were counted in view fields selected randomly until total numbers exceeded 200, or in the case of low density, until 200 fields were examined, using a Hitachi-600AB transmission electron microscope at 75 kV and ×80 000 magnification. Taper corrections were implemented in final calculations (Suttle, 1993). The size of the VLP was estimated directly on micrographs obtained at different magnifications.

The most frequently observed VLP in both lakes were icosahedric without a tail, and with a diameter between 21 and 290 nm, although most were between 40 and 90 nm (Figure 1e). Viruses with a different tail length, either rigid or retractile (Figure 1a–d), were also observed. Maranger and Bird (1995) reported that in 22 lakes >80% of the VLP were <70 nm. The authors did not find a
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statistically significant relationship between size and trophic degree of the lakes. Large VLP of 300 nm diameter, like those found in the present study (Figure 2c and d), are usually considered to originate from eukaryotic hosts (Van Etten et al., 1991), and their presence in fresh water was reported for the first time in a eutrophic Spanish reservoir (Sommaruga et al., 1995). Occasionally, we found particles of unusual morphologies, such as rod shaped (740 nm long; Figure 2a), granulated (Figure 2b) or star shaped (70 nm diameter; Figure 2e) in GKS and

Fig. 1. Different morphologies of virus-like particles found in Lake Redó (a–d) and Gossenköllesee (e) observed with the transmission electron microscope. Bar = 100 nm.

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LR. Recently, Oren et al. (1997) described star-shaped particles in samples from the Dead Sea; however, this morphology is unknown for viruses.

In GKS, the abundance of VLP at the surface changed within 3 days by one order of magnitude from $10^6$ to $10^7$ ml$^{-1}$. No clear pattern was found in the vertical distribution of VLP for the four sampling dates, with maxima present at different depths (Figure 3). There was a clear relationship between the vertical

Fig. 2. Unusual morphologies of virus-like particles found in Lake Redó (a) and Gossenköllesee (b–e) observed with the transmission electron microscope. Bar = 100 nm.
distribution of bacterial and viral abundance only on 10 August (Figure 3d). In addition, there was no significant correlation between the viral abundance and chlorophyll $a$ (range 0.8-3.4 µg l$^{-1}$) or the abundance of heterotrophic nanoflagellates (range 590-6680 cells ml$^{-1}$) in GKS (data not shown). In LR, we found a higher abundance of VLP than in GKS. For example, on 18 October, the abundance of VLP was $3.2 \times 10^7$ ml$^{-1}$ at 1 m depth and $2.2 \times 10^7$ ml$^{-1}$ at 32 m depth. On 26 October, a more detailed profile showed the minimum abundance of VLP ($3.4 \times 10^6$ ml$^{-1}$) at 50 m depth and the maximum ($2.1 \times 10^7$ ml$^{-1}$) at 30 m depth (Figure 4). This maximum was coincident with that of bacterial abundance ($6.7 \times 10^5$ ml$^{-1}$) and that of chlorophyll $a$ (1.3 µg l$^{-1}$; data not shown); however, no significant correlations were detected. The abundance of VLP observed in these two high-mountain lakes was in the lower range of values reported for other

![VLP and Bacteria Distribution](image)

**Fig. 3.** Vertical distribution of virus-like particles (VLP) (○) and bacterial (●) abundance in Gossenköllesee: (a) 30 July; (b) 2 August; (c) 6 August; (d) 10 August 1995.
freshwater lakes, although most data in the literature are for single-depth measurements. In mesotrophic Lake Constance (Germany), the abundance of VLP ranged between $1$ and $4 \times 10^7$ ml$^{-1}$ (Hennes and Simon, 1995), while in the eutrophic Plussee (Germany) a single measurement indicated an abundance of $2.54 \times 10^8$ ml$^{-1}$ (Bergh et al., 1989). In 22 lakes from Quebec (Canada), with a chlorophyll $a$ range between 1.5 and 32.8 µg l$^{-1}$, the VLP abundance varied from 4.1 to $25 \times 10^7$ ml$^{-1}$ (Maranger and Bird, 1995).

The minimum and maximum VBR (4.2 and 31.1, respectively) in GKS were found at the surface on 30 July and 2 August, respectively (Figure 3). The mean VBR ± 1 SD during the study period for the different depths was $15.3 \pm 11.6$ at the surface, $22.3 \pm 8.4$ at 2.5 m, $19.6 \pm 15.8$ at 5 m and $13.3 \pm 6.0$ at 9 m depth. In LR, the minimum and maximum VBRs on 26 October (9.1 at 20 m depth and 42.8 at 10 m depth, respectively) were higher than those found in GKS. Our data support the suggestion of Maranger and Bird (1995) that the VBR is higher in fresh water than in marine systems.

Elevated values of the VBR may indicate either a high infection rate or a long persistence of viruses in the plankton. The loss of VLP from surface waters is thought to be mainly affected by sedimentation after adsorption to microaggregates, grazing by protists and digestion by proteolytic enzymes (Suttle and Chen, 1992). Among these factors, sedimentation was estimated to be the most important in coastal waters.

Fig. 4. Vertical distribution of virus-like particles (VLP) (O) and bacterial (●) abundance in Lake Redó on 26 October 1995.

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Another important physical variable affecting viruses as well as their hosts is solar UV radiation. For example, the infectivity of viruses has been found to be negatively affected by wavelengths in the UV range and particularly by UVB radiation (280–320 nm) (Suttle and Chen, 1992; Noble and Fuhrman, 1997). Considering the high solar UV radiation at the ground in GKS and LR, and its low attenuation in the water column (10% of surface UVB irradiance found at 9.6 and 6.6 m depth in GKS and LR, respectively; Sommaruga et al., 1996; Sommaruga and Psenner, 1997), we can expect that the infectivity of free viruses

Fig. 5. Transmission electron micrographs showing virus-like particles attached to bacteria or infected bacteria in Gossenköllesee (a–c) and Lake Redó (d). Bar = 0.5 μm.
may be reduced in the upper layers during sunny days. Consequently, in surface layers, direct counts may overestimate the abundance of potentially infective viruses (Wommack et al., 1996) and thus the significance of the VBR as an indicator of the potential importance of viruses to control bacterial abundance is uncertain. However, a recent study carried out with marine viral isolates suggested that despite high UVB doses, a substantial fraction of the viruses (>50%) at the surface were still infective (Wilhelm et al., 1998). Repair of damaged viral DNA was accomplished by the host-cell photoreactivation (light-mediated) mechanism. Although in GKS and LR, bacteria with viral particles (>50) inside (Figure 5c and d) or attached to the cell surface were observed at different depths (Figure 5a), we have no information about infectivity rates. Further studies should address the importance of solar UV radiation for viral infectivity as well as the temporal and spatial dynamics of this process in high-mountain lakes. In addition, the contribution of viruses as another source of bacterial loss needs to be investigated. Considering the low bacterial activity and bacterivory rates measured in these lakes (Felip, 1997; Sommaruga et al., 1997), even a small mortality rate caused by viruses may have a considerable impact.

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References

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