RESEARCH ARTICLE

Viruses in subarctic lakes and their impact on benthic and pelagic bacteria

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Abstract

Virus–bacterium interactions were investigated in the pelagic and benthic habitats in a set of lakes along an altitudinal gradient in the subarctic northern Sweden. Viral and bacterial abundances showed a significant variation between the lakes, with the highest benthic microbial abundances recorded in a high-altitude lake [993 m above sea level (a.s.l.)], whereas the highest pelagic microbial abundances were found in a low-altitude lake (270 m a.s.l.). In the pelagic habitat, there was also a distinct difference in microbial abundances between the summer–autumn and the winter sampling occasion. A positive relationship was noted between viruses and bacteria in both the pelagic and the benthic habitats. Visibly virus-infected bacterial cells were uncommon in the pelagic habitat and undetectable in the benthos. Both lytic and lysogenic pelagic viral production rates were undetectable or low; thus, a possible explanation for the relative high viral abundances found in the water column could be an allochthonous input of viruses or release of sediment-derived viruses. Overall, our results provide novel information about the relevance of viruses in the subarctic region and indicate that viruses play only a minor role in the nutrient and carbon cycling in the microbial communities of subarctic lakes.

Introduction

Bacteria constitute an important part of basal biomass production in pelagic and benthic habitats of unproductive subarctic lakes (Vadeboncoeur *et al.*, 2002; Karlsson & Byström, 2005). High bacterial growth and respiration in relation to photosynthetic CO_2 fixation has been explained by the metabolism of allochthonous organic matter derived from the catchment (Karlsson *et al.*, 2001; Jansson *et al.*, 2008). Despite the ecological role and significance of pelagic and benthic bacteria in unproductive subarctic lakes (Karlsson & Byström, 2005; Karlsson *et al.*, 2007), information on the influence of viruses on the bacterial community in these systems is extremely scarce or completely lacking. Viral lysis of bacteria disrupts the flow of energy and organic matter from the microbial loop to higher trophic levels by forming a 'viral shunt' among bacteria, viruses and the dissolved organic matter pool (Bratbak & Heldal, 2000; Wommack & Colwell, 2000; Middelboe & Lyck, 2002; Weinbauer, 2004). It has been suggested that as much as 30% of bacterial production (BP) can be channelled through the viral shunt in aquatic systems (Bratbak & Heldal, 2000).

The interactions of bacteria and their viruses have been described for several different inland waters, ranging from tropical to polar ecosystems (Hofer & Sommaruga, 2001; Vrede *et al.*, 2003; Peduzzi & Schiemer, 2004; Weinbauer, 2004; Bettarel *et al.*, 2006; Säwström *et al.*, 2008a). Many of these studies have shown that viral abundance increases with lake productivity and that there is a close link between viruses and bacteria (Weinbauer, 2004). Säwström *et al.* (2008a) and Lymer *et al.* (2008) presented preliminary data on pelagic viral abundance and the fraction of virus-infected bacteria (FVIB) in Swedish subarctic lakes. However, no studies exist that include both pelagic and benthic viral ecology in subarctic lakes. In this study, we sampled a set of lakes in a natural climate gradient [270–1300 m above sea level (a.s.l.)] in northern Sweden to investigate the influence of viruses on pelagic and benthic bacteria in two distinct seasons (summer–autumn and winter). The lakes represented different habitats that reflected distinct differences in

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lake productivity, with decreasing productivity (i.e. bacterial activity) with increasing altitude (Karlsson *et al.*, 2001, 2005; Jansson *et al.*, 2008). We hypothesized that viruses and bacteria were closely linked in both the benthic and the pelagic habitat. As a consequence, viral abundance and production were expected to decrease in lakes with increasing altitude.

Materials and methods

Study sites and sampling

Fourteen lakes were sampled along an altitudinal gradient (270–1300 m a.s.l.) from the coniferous forest to the alpine belts (Table 1) in the Scandinavian mountains in the subarctic northern Sweden (68°N, 18°E). More detailed information of the study area was described in Karlsson *et al.* (2001). The lakes were sampled once in the summer and autumn of 2006 (July–September). In the winter of 2007 (March), five of the 14 lakes were resampled and additionally six of the 14 lakes were sampled again in winter 2008 (February and March) for measurements of lysogeny. Water samples (5–10 L) were collected, using a tube-sampler (0.5 m long, 3.4 cm diameter) or a Ruttner water sampler, from either the top layer of the water column (~1 m) or as a composite water sample (collected from every 1-m-depth interval of the entire water column) (Table 1). During the winter, water samples were collected using a Ruttner water sampler immediately under the ice. The lakes showed no indications of thermal stratification at any of the sampling occasions; thus, the whole lake volume was treated as a homogenous unit. In summer 2006, sediment samples were collected from five lakes (Erkkijaure, S2, Solbacka, Knivsjön and Suorujaure). Triplicate sediment samples were collected with a sediment corer (inner diameter = 6 cm, height = 40 cm) where the above-lying water column was between 1 and 2 m deep. The overlying water was removed carefully, and the top 3 cm of the sediment was sliced off, and transported to the laboratory in plastic containers.

Water chemistry and chlorophyll *a* (chl *a*)

Water temperature was measured in the field using a WTW multiline P4 meter. During the winter, ice thickness was also measured. Samples (~50 mL) for dissolved organic carbon (DOC) were filtered through GF/F filters (preashed for 3 h at 400 °C), acidified (10 μL of 1.2 M HCl mL^{-1} sample) and stored at 4 °C until analysis. Concentrations of DOC were determined by the high-temperature catalytic oxidation method using a Shimadzu TOC-5000 total carbon analyser equipped with an ASI-5000 auto sampler. Inorganic nutrient analyses were performed on unfiltered water [total phosphorus (T-P) and total nitrogen (T-N)] and GF/F filtered water [soluble reactive phosphorus (PO_4-P) and dissolved nitrogen (NH_4-N, NO_3-N and NO_2-N)]. Concentrations of nutrients were analysed at the Department of

Table 1. Physiochemical characteristics of the lakes studied, in summer-autumn 2006 and winter 2007, in the different vegetation belts following the altitude gradient

Vegetation belt	Lake	Date	Season	Alt (m a.s.l.)	*T* (°C)	Chl *a* ($\mu g\,L^{-1}$)	DOC ($mg\,L^{-1}$)	T-N ($\mu g\,L^{-1}$)	NH_4 ($\mu g\,L^{-1}$)	NO_3 ($\mu g\,L^{-1}$)	T-P ($\mu g\,L^{-1}$)	PO_4 ($\mu g\,L^{-1}$)
Coniferous forest	Erkkijaure*	03-07-06	S-A	270	22.5	1.7	7.1	431	15.0	5.0	15.9	1.6
	Vuoskkujaure	01-09-06	S-A	348	14.3	0.7	3.4	77.6	1.8	1.1	13.5	0.6
		(06-03-07)	W		(1.3)	(0.3)	(3.4)	(180)	(24.6)	(15.2)	(7.0)	(0.1)
Subalpine	S2*	04-07-06	S-A	376	16.3	1.7	13.1	406	4.0	2.4	16.3	2.3
	Almberga*	10-07-06	S-A	382	14.1	0.8	4.5	141	3.3	5.0	13.9	0.6
	Solbacka*	06-07-06	S-A	410	14.3	0.6	9.40	365	13.5	0	17.1	0.9
	Tjabrak*	11-07-06	S-A	508	11.5	0.7	3.2	172	1.8	0	15.5	0.9
	Kratersjön	13-09-06	S-A	547	10.5	0.6	1.4	33.7	16.4	0	13.5	0.9
		(08-03-07)	W		(0.9)	(0.2)	(3.0)	(88.5)	(14.6)	(15.2)	(8.3)	(0.2)
Low alpine	Katterjaure	16-09-06	S-A	697	9.6	0.4	1.7	12.0	7.7	0	13.1	2.3
		(09-03-07)	W		(0.4)	(0.02)	(1.3)	(16.1)	(12.6)	(40.6)	(5.7)	(0.2)
	Ruozutjaure*	12-07-06	S-A	710	12.5	1.1	3.1	195	1.1	0	16.3	0
	Vuorejaure*	13-07-06	S-A	712	12.4	1.2	3.7	141	6.9	0	14.7	0.2
Middle alpine	Knivsjön*	23-07-06	S-A	865	8.9	0.3	2.4	88.7	8.4	0	14.7	0.9
	Suorujaure*	20-07-06	S-A	993	7.6	–	1.8	–	–	–	–	–
	Njulla	29-08-06	S-A	999	11.1	0.5	1.4	51.9	9.1	9.0	15.1	0.9
		(07-03-07)	W		(1.9)	(0.4)	(4.9)	(289)	(105.2)	(21.6)	(6.8)	(0.2)
High alpine	Kuoblatjåkkajaure	10-09-06	S-A	1300	6.1	0.5	0.6	59.2	6.9	27.6	9.6	0.2
		(09-03-07)	W		(0.2)	(0.06)	(1.3)	(30.6)	(17.0)	(41.6)	(4.6)	(0.1)

Winter values are in parentheses.

*A composite water sample from the entire water column of the lake.

S-A, summer-autumn 2006; W, winter 2007; Alt, altitude; *T*, temperature; NH_4, ammonium; NO_3, nitrate.

Limnology, Uppsala University. T-P was obtained after oxidative hydrolysis with potassium peroxodisulphate (Menzel & Corwin, 1965), followed by PO_4-P analysis according to Murphy & Riley (1962). NH_4-N was determined using the indophenol method (Grasshoff *et al.*, 1983). NO_3-N and NO_2-N were determined after reduction of NO_3-N with Cd (Grasshoff *et al.*, 1983). T-N was obtained after analysing Kjeldahl-N (Jönsson, 1966).

Chl *a* was determined according to the protocol of Jespersen & Christoffersen (1987). Briefly, a known volume of water sample was filtered through a GF/F filter, which was then stored frozen at $-20\,^{\circ}C$. Filters were extracted with EtOH (95%) in the dark for 24 h and analysed on a luminescence spectrophotometer (Perkin-Elmer LS55) using a wavelength of 433 nm for excitation and 673 nm for emission measurements.

Bacterial and viral concentrations and virus–bacterium encounter rates

Duplicate 20-mL water samples for viruses and bacteria enumeration were fixed with prefiltered (0.02-m-pore-size filter) glutaraldehyde (2% final concentration). Sediment samples were homogenized by shaking, and excess water was discarded by allowing the sediment to settle in a 60-mL syringe for 1.5 h at *in situ* temperature in the dark. The sediment was transferred to a 10-mL scintillation vial, and vortexed for further homogenization. A sediment subsample of 0.5 mL was fixed with prefiltered (0.02-μm-pore-size filter) glutaraldehyde (2% final concentration). Benthic viruses and bacteria were then extracted as described by Danovaro *et al.* (2001). Bacteria and viruses were counted by epifluorescence microscopy with SYBR Green I nucleic acid stain (Molecular Probes) and a Leica DM IL microscope, according to the method of Noble & Fuhrman (1998). Samples of 0.5–1 mL were filtered on 0.02-μm-pore-size Anodisc membrane filters (Whatman), with a 0.8-μm-pore-size backing membrane filter. The filter was then laid, sample side up, on a drop of SYBR Green I working solution [25 μL of 100 × diluted SYBR Green I solution and 75 μL of filtered (0.02-μm-pore-size filter) Milli-Q water], for 15 min in the dark. After drying, the filter was mounted on a glass slide with a drop of Molecular Probe Slow Fade antifade solution. For each filter, >200 viruses and >100 bacteria were counted on 10–15 fields of view selected randomly. Contact rates (R) between viruses and bacteria were calculated using the following equation of Murray & Jackson (1992):

$$R = (Sh\,2\,\pi\omega\,D_v)VB$$

where Sh is the Sherwood number (1.06 for a bacterial community with 10% motile cells; Wilhelm *et al.*, 1998), ω is the bacterial cell diameter (calculated from the mean bacterial cell volume that was estimated for 16 lakes in the subarctic north of Sweden in 1998 and 1999; Karlsson *et al.*, 2001, and assuming that the cells are spheres), D_v is the diffusivity of viruses ($3.456 \times 10^3\ cm^2\ day^{-1}$) and V and B are the respective viral and bacterial abundances (per millilitre).

BP and generation times (GTs)

BP was estimated by the incorporation of [^{3}H]-leucine ($166\ Ci\ mmol^{-1}$) into the bacterial biomass using a slightly modified version (Karlsson *et al.*, 2001) of the microcentrifuge method as described by Kirchman (2001). Previous isotope addition experiments in lakes from the same region showed that [^{3}H]-leucine reached saturating concentrations at 50 nM (Karlsson *et al.*, 2001). BP samples were counted by liquid scintillation in a Beckman LS 6500 scintillation counter. Bacterial cell production was obtained by applying a conversion factor of $1.42 \times 10^{17}\ cells\ mol^{-1}$ to the incorporation rates of leucine into protein (Chin-Leo & Kirchman, 1988). Benthic BP was measured using a modified [^{3}H]-leucine incorporation method. A homogenized subsample of the sediment was collected with a 1-mL syringe, and 0.1 mL was placed in each of three Eppendorf tubes prepared with both [^{3}H]-leucine and a nonradioactive leucine. Additional experiments revealed that 10.7 μM leucine was sufficient to saturate the leucine uptake. Control triplicates were also prepared by adding 65 μL 100% trichloroacetic acid (TCA), together with the isotopes, before the addition of the sediment. The tubes were vortexed and incubated for about 45 min at *in situ* temperature, and the incubation was terminated with 65 μL 100% TCA. The following washing and analysing procedures were identical to those for the lake water, with the exception that an additional washing step was included using 1.2 mL of 80% EtOH. Bacterial GTs were estimated from bacterial abundance divided by BP.

Induction assay for lysogenic bacteria

Triplicate samples (15 mL) were either treated with Mitomycin C (a potent mutagen for prophage induction) ($1\ \mu g\ mL^{-1}$ final concentration) (Sigma, St. Louis, MO) or left untreated (controls) (Paul & Jiang, 2001). The samples were incubated in the dark at *in situ* temperature for 24 h and then fixed with 0.02 μm filtered glutaraldehyde (final concentration 2%) and stored at $4\,^{\circ}C$ (storage <24 h). Bacterial and viral abundance were then determined using SYBR Green I staining (as described earlier). The significance of each induction event was determined by comparison of Mitomycin C treatment and control levels of viruses by an independent samples *t*-test. A statistically significant increase in viral abundance in the Mitomycin C treatment relative to the control indicated the presence of lysogenic bacteria. The fraction of lysogenic bacteria (FLC) was then calculated as: $\%FLC = [(V_t - V_c)/B_z]/B_c \times 100$, where V_t is

the number of viruses enumerated in the Mitomycin C treatment at 24 h and V_c is the number of viruses enumerated in the control sample. B_c is the number of bacteria enumerated in the control sample at 24 h and B_z is the burst size, which was estimated for each study lake by transmission electron microscopy (TEM) as explained below.

Virus-infected bacteria and burst sizes

The frequency of visibly infected bacterial cells (FVIB) and burst sizes (B_z; the number of viruses released during cell lysis) were determined on water and extracted sediment samples were fixed with 0.02-μm-filtered glutaraldehyde (2% final concentration). Control samples consisting of 0.02-μm-filtered Milli-Q water were also processed and acted as blanks to ensure that there was no contamination that might be mistaken for bacterial cells. We used a modified TEM method as explained by Säwström *et al.* (2007b). In brief, approximately 30 mL of each sample was centrifuged onto replicate Formvar coated 400-mesh Cu grids in a rotor with swing-out buckets (3270 ***g*** for 164 min at 10 °C, Beckman X-12R). The grids were negative-stained for 30 s with 0.2-μm-filtered 2% uranyl acetate and then rinsed with deionized distilled water. Grids were examined for visibly infected cells with a Zeiss EM-900 transmission electron microscope at 80 kV and × 20 000 magnification. The whole electron microscope grid was examined (a 400-mesh grid has approximately 1300 fields of view) to determine FVIB and B_z. For the water samples, at least 60 bacterial cells were inspected in each sample, but for the extracted sediment samples, the number of cells inspected was lower (12–73 cells per sample) as there were only a few bacterial cells on each grid. A cell was considered infected when the phage inside could be clearly recognized on the basis of shape (round or hexagonal capsid structures), size (generally < 200 nm in diameter) and staining intensity. Similar to Säwström *et al.* (2007b), we used a lower threshold limit of two virus-like particles to score a cell as infected.

Calculations of viral proliferation and viral-induced bacterial mortality

The model of Binder (1999) was used to estimate the fraction of bacterial mortality caused by viral lysis (FMVL): $\mathrm{FMVL} = \mathrm{FVIB}/[\gamma \ln(2)\ (1 - \varepsilon - \mathrm{FVIB})]$, where $\gamma = 1$ (the ratio between the latent period and the GT) and $\varepsilon = 0.816$ (the fraction of the latent period during which viral particles are not yet visible). The value of ε was previously referenced incorrectly as 0.186 by Säwström *et al.* (2007a) and Binder (1999, see abstract). The lytic viral production (LVP; viruses produced L^{-1} day^{-1}) was calculated by multiplying the lysed BP with the estimated B_z from each lake ($\mathrm{FMVL} \times \mathrm{BP} \times B_z$). Viral turnover times (VT) were estimated as viral abundance divided by viral production. The percentage of viral–host contacts resulting in a successful infection ending with lysis (success) was calculated as follows:

$$\%\mathrm{Success} = [\mathrm{FMVL} \times \mathrm{BP}/R] \times 100$$

Statistical analyses

Statistical analyses were performed in SPSS (version 11.0.0 for Windows). Data were checked for normal distribution using the Kolmogorov–Smirnov test. Data with a non-normal distribution were ln-transformed to achieve normality. The relationships between the measured and calculated variables were determined using two different tests: Spearman rank-order correlation (r_s) for the summer–autumn data set and Pearson product–moment correlation (r) for the winter data set. Correlation coefficients with P values of < 0.1 were assumed to be statistically significant. Differences in the measured and calculated parameters between the sampling occasions (summer–autumn and winter) were analysed using the nonparametric Kruskal–Wallis test. Differences in the measured and calculated parameters between lake water and sediment were analysed using an independent samples *t*-test.

Results

Physiochemical lake environment

There were large variations in the measured parameters between the lakes and also considerable variations between the two sampling occasions (Table 1). Lake water temperature ranged between 6.1 and 22.5 °C in the summer–autumn, but significantly lower temperatures were recorded in the winter (0.2–1.9 °C) (Kruskal–Wallis, $P < 0.01$). There was a trend of decreasing water temperature ($r_s = -0.858$, $P < 0.01$, $N = 14$), T-N ($r_s = -0.539$, $P < 0.1$, $N = 13$), DOC ($r_s = -0.776$, $P < 0.01$, $N = 14$) and chl *a* ($r_s = -0.604$, $P < 0.05$, $N = 13$) with increasing altitude in the summer–autumn. Both NH_4 and NO_3 concentrations (Kruskal–Wallis, $P < 0.05$, $N = 5$) were significantly lower in summer–autumn than in the winter when the lakes were ice covered (ice thickness: 65–121 cm). On the other hand, the concentrations of PO_4 ($P < 0.01$, $N = 5$), T-P ($P < 0.01$, $N = 5$) and chl *a* ($P < 0.01$, $N = 5$) were significantly higher in the summer–autumn.

Variations in pelagic and benthic viral and bacterial abundance

The abundance of viruses in the water column (pelagic viruses) ranged from 0.67 to 28.89×10^9 viruses L^{-1} in the summer–autumn and from 0.72 to 3.07×10^9 viruses L^{-1} in the winter (Table 2). Viral abundances were positively associated with lake water temperature (summer–autumn, $r_s = 0.482$, $P < 0.1$, $N = 14$; winter, $r = 0.944$, $P < 0.05$, $N = 5$) and concentrations of T-N (Fig. 1c; summer–autumn,

Table 2. Mean viral and microbial parameters for the pelagic habitats of the lakes studied in summer-autumn 2006 and winter 2007

Lake	Season	Viruses ($\times 10^9$ L^{-1})	Bacteria ($\times 10^9$ L^{-1})	VBR	R ($\times 10^9$ L^{-1} day^{-1})	BP ($\times 10^6$ cells L^{-1} day^{-1})	GT (days)	FVIB (%)	B_z	FMVL (%)	LVP ($\times 10^6$ L^{-1} day^{-1})	VT (days)	Success (%)
Erkkijaure	S-A	28.9 ± 1.3	3.8 ± 0.2	7.6 ± 0.4	103.3 ± 7.9	39.8 ± 2.4	96	0	0	–	–	–	–
Vuoskkujaure	S-A	6.9 ± 0.2	1.0 ± 0.3	6.7 ± 0.6	6.71 ± 0.3	112.1 ± 12.9	9	0.29	17	2.31	44.0	157	0.04
	W	(2.8 ± 0.3)	(0.7 ± 0.2)	(3.9 ± 0.2)	(7.4 ± 0.5)	(10.9 ± 3.5)	(66)	(0)	(0)	(–)	(–)	(–)	(–)
S2	S-A	5.5 ± 0.5	1.4 ± 0.1	3.8 ± 0.4	7.3 ± 1.0	433.4 ± 16.0	3	0	0	–	–	–	–
Almberga	S-A	3.0 ± 0.4	0.7 ± 0.1	4.4 ± 0.3	1.9 ± 0.4	223.1 ± 7.5	3	0.23	33	1.83	134.5	22	0.21
Solbacka	S-A	19.6 ± 1.4	2.7 ± 0.5	7.3 ± 1.0	49.3 ± 10.6	117.4 ± 6.1	23	0	0	–	–	–	–
Tjabrak	S-A	2.2 ± 0.1	0.6 ± 0.04	3.9 ± 0.3	1.1 ± 0.1	–	–	0	0	–	–	–	–
Kratersjön	S-A	2.2 ± 0.01	0.4 ± 0.03	5.6 ± 0.5	0.8 ± 0.08	35.8 ± 3.6	11	0	0	–	–	–	–
	W	(1.8 ± 0.2)	(0.5 ± 0.02)	(3.7 ± 0.6)	(3.2 ± 0.07)	(10.6 ± 2.9)	(46)	(0)	(0)	(–)	(–)	(–)	(–)
Katterjaure	S-A	1.4 ± 0.01	0.2 ± 0.3	6.0 ± 1.5	0.3 ± 0.04	36.9 ± 1.4	6	0.56	21	4.53	35.1	40	0.56
	W	(1.6 ± 0.1)	(0.2 ± 0.04)	(9.1 ± 0.4)	(1.1 ± 0.06)	(10.3 ± 7.9)	(17)	(0)	(0)	(–)	(–)	(–)	(–)
Ruozut	S-A	3.4 ± 0.3	0.6 ± 0.1	5.9 ± 0.5	1.8 ± 0.3	–	–	0	0	–	–	–	–
Vuore	S-A	2.8 ± 0.2	1.2 ± 0.5	2.2 ± 0.4	3.2 ± 1.4	–	–	0	0	–	–	–	–
Knivsjön	S-A	5.9 ± 0.2	0.9 ± 0.04	6.3 ± 0.4	5.2 ± 0.3	202.8 ± 24.3	3	0	0	–	–	–	–
Suorujaure	S-A	7.2 ± 0.2	0.9 ± 0.1	8.2 ± 0.5	5.9 ± 0.4	122.1 ± 5.3	6	0	0	–	–	–	–
Njulla	S-A	5.9 ± 0.1	0.6 ± 0.04	10.3 ± 1.8	3.1 ± 0.2	112.7 ± 8.0	5	0	0	–	–	–	–
	W	(3.1 ± 0.2)	(0.8 ± 0.2)	(3.7 ± 0.5)	(2.4 ± 0.6)	(70.4 ± 6.9)	(12)	(0.40)	(5)	(3.21)	(11.3)	(274)	(0.09)
Kuoblatjåkkajaure	S-A	0.7 ± 0.1	0.1 ± 0.4	7.0 ± 2.9	0.06 ± 0.01	37.4 ± 8.9	3	2.34	8	21.03	62.9	11	13.11
	W	(0.7 ± 0.1)	(0.2 ± 0.1)	(3.5 ± 1.3)	(0.1 ± 0.02)	(5.0 ± 0.5)	(41)	(1.97)	(15)	(17.30)	(13.0)	(54)	(0.87)

Values are given as mean ± SE. Winter values are in parentheses. The bacterial production value for Almberga was taken from July 2005 (J. Karlsson *et al*., unpublished data). FVIB values are presented as the mean of two analysed grids. Total number of bacterial cells analysed with TEM, ranged between 64 and 1102 cells per sample.
S-A, summer-autumn; W, winter; R, virus–bacterium encounter rate; success, percentage virus–bacterium encounters ending in cell lysis.

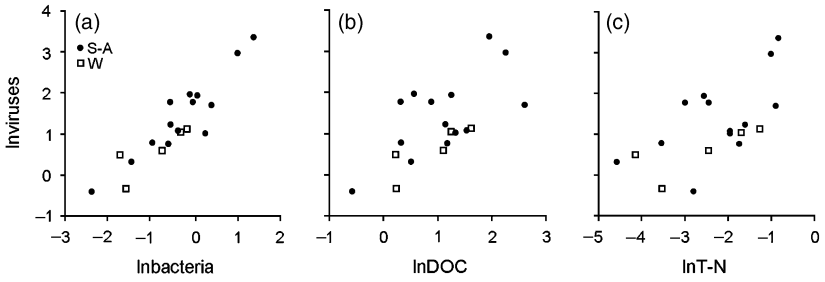

Fig. 1. Relations between viral abundance and (a) bacterial abundance (summer–autumn, $r_s = 0.802$, $P < 0.01$, $N = 14$; winter, $r = 0.922$, $P < 0.05$, $N = 5$), (b) DOC (summer–autumn, $r_s = 0.477$, $P < 0.1$, $N = 14$; winter, $r = 0.891$, $P < 0.05$, $N = 5$) and (c) T-N (summer–autumn, $r_s = 0.525$, $P < 0.1$, $N = 13$; winter, $r = 0.898$, $P < 0.05$, $N = 5$) in the pelagic habitat in the summer–autumn (S-A) and winter (W) in a set of subarctic lakes in northern Sweden.

Table 3. Mean viral and microbial parameters for the benthic habitats of the five lakes studied in summer-autumn 2006

Lake	Season	Viruses ($\times 10^{11}$ L^{-1})	Bacteria ($\times 10^{11}$ L^{-1})	VBR	R ($\times 10^{14}$ L^{-1} day^{-1})	BP ($\times 10^{9}$ cells L^{-1} day^{-1})	GT (days)	Benthic viruses : pelagic viruses
Erkkijaure	S-A	2.1 ± 0.1	1.1 ± 0.1	1.9 ± 0.6	0.2 ± 0.02	1.7 ± 0.2	65	7.2
S2	S-A	5.1 ± 0.6	3.0 ± 0.3	1.7 ± 0.1	1.4 ± 0.3	0.7 ± 0.1	405	93.9
Solbacka	S-A	3.2 ± 0.3	1.6 ± 0.2	2.0 ± 0.4	0.5 ± 0.1	8.3 ± 0.6	19	16.2
Knivsjön	S-A	7.5 ± 1.4	3.0 ± 0.7	2.5 ± 0.3	2.1 ± 1.1	0.6 ± 0.2	508	126.2
Suorujaure	S-A	14.0 ± 2.1	8.0 ± 2.0	1.8 ± 0.4	10.5 ± 4.3	1.9 ± 0.2	424	195.4

Values are given as mean ± SE.
R, virus–bacterium encounter rate.

$r_s = 0.522$, $P < 0.1$, $N = 13$; winter, $r = 0.899$, $P < 0.05$, $N = 5$) and DOC (Fig. 1b; summer–autumn, $r_s = 0.477$, $P < 0.1$, $N = 14$; winter, $r = 0.891$, $P < 0.05$, $N = 5$). Pelagic viral numbers were the highest in lake Erkkijaure (270 m a.s.l.) in the summer–autumn whereas in winter the highest value was recorded in lake Njulla (999 m a.s.l.) (Table 2). Benthic viruses ranged from 2.07 to 14.00 $\times 10^{11}$ viruses L^{-1}, with the highest value recorded in Suorujaure (993 m a.s.l.) (Table 3). The ratio of benthic viral abundance to pelagic viral abundance ranged from 7.2 to 195.4 (Table 3). Bacterial abundance in the water column ranged from 0.10 to 3.82 $\times 10^{9}$ cells L^{-1} in the summer–autumn and from 0.18 to 0.83 $\times 10^{9}$ cells L^{-1} in the winter (Table 2). As with pelagic viral numbers, the highest pelagic bacterial numbers were recorded in lake Erkkijaure in the summer–autumn and in the winter the highest value was recorded in lake Njulla (Table 2). Bacterial abundances were positively associated with lake water temperature (summer–autumn, $r_s = 0.730$, $P < 0.01$, $N = 14$; winter, $r = 0.973$, $P < 0.01$, $N = 5$) and concentrations of T-N (summer–autumn, $r_s = 0.768$, $P < 0.01$, $N = 13$; winter, $r = 0.963$, $P < 0.01$, $N = 5$), DOC (summer–autumn, $r_s = 0.837$, $P < 0.01$, $N = 14$; winter, $r = 0.973$, $P < 0.01$, $N = 5$) and chl *a* (summer–autumn, $r_s = 0.604$, $P < 0.05$, $N = 13$; winter, $r = 0.988$, $P < 0.01$, $N = 5$). Furthermore, in the summer–autumn bacterial abundance decreased with increasing altitude ($r_s = -0.552$, $P < 0.05$, $N = 14$).

The highest bacterial abundance was recorded in the sediment (8.01 $\times 10^{11}$ cells L^{-1}) of lake Suorujaure (Table 3). There was a positive relationship between viruses and bacteria in the water column (Fig. 1a) and in the sediments ($r = 0.981$, $P < 0.01$, $N = 5$). Virus-to-bacterium ratios (VBR) in the water column ranged between 2.24 and 10.32 in the summer–autumn and between 3.51 and 9.14 in the winter (Table 2). VBR values were significantly lower (*t*-test, $P < 0.01$, $N = 5$) in the sediments (mean 1.97 ± 0.14, $N = 5$) than in the above water column (mean 6.62 ± 0.76, $N = 5$). Pelagic and benthic virus–bacterium contact rates (R) showed considerable variation between the lakes (Table 3). In the summer–autumn, pelagic virus-bacterium *R*s varied from 0.06 to 103.27 $\times 10^{9}$ contacts L^{-1} day^{-1}. In the winter, pelagic *R*s varied from 0.14 to 2.39 $\times 10^{9}$ contacts L^{-1} day^{-1}. The highest pelagic R was found in lake Erkkijaure, which also had the highest viral abundance (Table 2). Benthic *R*s were significantly higher than pelagic R (*t*-test, $P < 0.01$, $N = 5$) and varied from 0.2 to 10.5 $\times 10^{14}$ contacts L^{-1} day^{-1}.

BP and GTs

BP in the pelagic habitat ranged between 35.8 and 433.4 $\times 10^{6}$ cells L^{-1} day^{-1} in the summer–autumn and between 5.0 and 70.4 $\times 10^{6}$ cells L^{-1} day^{-1} in the winter. The highest pelagic BP rate was recorded in lake S2 in the summer–autumn whereas in winter the highest rate was recorded in lake Njulla (Table 2). BP rates in the summer–autumn showed a positive correlation with the concentrations of T-P ($r_s = 0.571$, $P < 0.1$, $N = 10$), T-N ($r_s = 0.648$, $P < 0.05$, $N = 10$) and DOC ($r_s = 0.600$, $P < 0.1$, $N = 11$). In the winter, BP was positively correlated with concentrations of inorganic nitrogen (NH_4 and T-N) ($r = 0.991$, $P < 0.01$,

$N = 5$; $r = 0.851$, $P < 0.1$, $N = 5$, respectively) and temperature ($r = 0.818$, $P < 0.1$, $N = 5$). The BP rates in the sediments ranged between 0.59 and 8.29×10^9 cells L^{-1} day^{-1} (Table 3) and were significantly higher than the rates in the water column (*t*-test, $P < 0.01$, $N = 5$). In the summer–autumn, pelagic bacterial GTs ranged between 2.7 and 96.0 days and were positively correlated with viral abundance ($r_s = 0.555$, $P < 0.1$, $N = 11$). In the winter, bacterial GTs ranged between 11.8 and 66.4 days; however, no significant relationship was found between GT and viral abundance. The pelagic bacterial GTs were shorter in the summer–autumn (mean 6.79, $N = 5$) than in the winter (mean 36.4, $N = 5$) (*t*-test, $P < 0.01$, $N = 5$). Furthermore, benthic bacterial GTs were significantly longer (19–508 days, mean 285, $N = 5$) than pelagic bacterial GTs (*t*-test, $P < 0.01$, $N = 5$).

Lytic infection

Based on electron microscopy, we could estimate the percentage of bacteria being visibly infected with viruses (FVIB). In the water column, FVIB varied from undetectable to 2.34% in the summer–autumn and from undetectable to 1.97% in the winter. The maximum values were obtained for Kuoblatjåkkajaure, a high-altitude lake (1300 m a.s.l.) (Table 2). Pelagic FVIB values were negatively correlated with the concentrations of T-P and T-N in the summer–autumn ($r_s = -0.756$, $P < 0.01$, $N = 13$; $r_s = -0.480$, $P < 0.1$, $N = 13$). In the winter there was a positive relationship between pelagic FVIB values and altitude ($r = 0.927$, $P < 0.05$, $N = 5$). We found no virus-infected bacterial cells in any of the sediment samples. In total, we inspected 161 bacterial cells from the extracted sediment samples but none of these cells contained viruses. Because of the low number of cells inspected, it is hard to make any meaningful interpretations of these results and subsequently we could not calculate the fraction of bacterial mortality caused by viral lysis (FMVL) or virus production in the sediments. The presence of virus-infected bacteria in the water column was rare, with only four out of 14 lakes containing virus-infected bacteria in the summer–autumn. In the winter, a slightly higher proportion of the lakes (two out of five) contained virus-infected bacteria. FMVL in the water column was always $< 5\%$ of the BP, except in Kuoblatjåkkajaure, where up to 21% of the bacterial mortality was caused by viruses (Table 2).

The burst size (B_z) in the pelagic samples from summer–autumn ranged from 8 (lake Kuoblatjåkkajure) to 33 (lake Almberga), whereas in the winter a B_z of 5 was recorded in lake Njulla and a B_z of 15 in lake Kuoblatjåkkajaure (Table 2). In the summer–autumn, B_z was negatively correlated with the concentrations of T-P ($r_s = -0.685$, $P < 0.01$, $N = 13$). In the winter, there was a positive correlation between B_z and altitude ($r = 0.912$, $P < 0.05$, $N = 5$).

Pelagic LVP rates were low ($11.3–134.5 \times 10^6$ viruses produced L^{-1} day^{-1}), which resulted in long VT (11–275 days) (Table 2). The pelagic BP rates were generally higher than the pelagic LVP rates in the lakes, with the exception of Kuoblatjåkkajaure (Table 2). Virus–bacterium contact rates indicate how often a virus particle bumps into a bacterial cell; however, every virus–bacterium contact does not result in a successful infection. The percentages of pelagic virus–bacterium contacts resulting in a successful infection ending with lysis (success) were low and showed considerable variation between the lakes (Table 2). In lake Kuoblatjåkkajaure, one out of eight contacts resulted in a successful viral infection in the summer–autumn; however, the success rate was over 14 times lower in the winter (0.865%). The success rate was extremely low in lake Vuoskkujaure, where only one of 2500 contacts resulted in a successful viral infection in the summer–autumn.

Lysogenic infection

The presence of lysogenic bacteria in the water column was investigated in six of the 14 lakes in the winter of 2008. Lysogenic bacteria were only found in lake Kuoblatjåkkajaure, with a low calculated FLC (2.3%) (Table 4). Thus, lysogenic viral production seemed to be of minor importance in the pelagic habitat of the investigated lakes.

Table 4. Estimation of percentages of lysogenic bacteria in the total bacterial community in the pelagic habitats of the six lakes studied in winter 2008

Lake	Date	Viruses (control $\times 10^9$ L^{-1})	Mitomycin C (% of the control)	% FLC
Vuoskkujaure	20-02-08	3.3 ± 0.2	105.0 NS	NA
Almberga	27-02-08	12.2 ± 2.3	80.1	NA
Kratersjön	11-03-08	3.7 ± 0.3	58.9	NA
Katterjaure	29-02-08	0.9 ± 0.1	85.3	NA
Njulla	03-03-08	4.5 ± 0.3	107.6 NS	NA
Kuoblatjåkkajaure	11-03-08	0.7 ± 0.02	110.1*	2.3 ($B_z = 15$)

Values are given as mean $\pm$ SE.

*$P < 0.05$. The significance of each induction event was determined by comparison of treatment and control levels of viruses by an independent samples *t*-test.

NA, not applicable; NS, not significant.

Discussion

Pelagic virus–bacterium interactions

Overall, the range of pelagic viral abundance observed in the subarctic lakes (0.67×10^9–28.89×10^9 viruses L^{-1}) falls within previously reported values for temperate and polar inland waters (Wommack & Colwell, 2000; Weinbauer, 2004; Säwström *et al.*, 2008a). There was no indication of decreasing viral abundance with increasing altitude. Nevertheless, the highest viral abundance was noted in the low-altitude lake Erkkijaure (28.89×10^9 viruses L^{-1}) and the lowest value was recorded in the high-altitude lake Kuoblatjåkkajaure (0.67×10^9 viruses L^{-1}), which was in the same range as the values reported from ultraoligotrophic Antarctic lakes (Säwström *et al.*, 2007a). Even though not significant, the mean viral abundance appeared to be higher in summer–autumn than in winter.

Viral abundance exceeded bacterial abundance in all the lakes on both sampling occasions. The VBR values were fairly stable, with low variability between the two sampling occasions (summer–autumn, 2.2–10.3; mean, 6.1; winter, 3.5–9.1; mean, 4.8). The rather low values of VBR (< 10) indicated that viral-induced bacterial mortality was low in the lakes in both the summer–autumn and the winter period. The percentages of visibly virus-infected bacteria in the lakes on the two sampling occasions were indeed exceptionally low (range, undetectable to 2.3%; mean 0.3%), compared with the *c.* 2% usually found in temperate freshwaters (Parada *et al.*, 2006; Säwström *et al.*, 2007b). In contrast, the calculated contact rates indicated that there was a high probability of contact between viruses and bacteria, particularly in two of the lakes below the tree line (< 600 m a.s.l.) on the summer–autumn sampling occasion (49 and 103×10^9 contacts L^{-1} day^{-1}). In a set of transplantation experiments conducted in the glacial freshwater environment of the high Arctic (Svalbard), *R* ranged from 0.06 to 11×10^9 contacts L^{-1} day^{-1} whereas in the deep-water masses of the North Atlantic, *R* ranged from 1 to 4×10^9 contacts L^{-1} day^{-1} (Anesio *et al.*, 2007; Parada *et al.*, 2007). Our *R*s, in the winter period (0.1–2.4×10^9 contacts L^{-1} day^{-1}; mean 1×10^9 contacts L^{-1} day^{-1}), ranged between the values reported by Anesio *et al.* (2007) and Parada *et al.* (2007), but in summer–autumn, *R*s often exceeded this range (0.06–103×10^9 contacts L^{-1} day^{-1}; mean 14×10^9 contacts L^{-1} day^{-1}).

It should be noted that only a fraction of the contacts will result in viral infection; thus, contact rates are not necessarily proportional to infection rates. In fact, a small percentage of contacts resulted in a viral infection ending with cell lysis (< 14% success). LVP was low (11.3–134.5×10^6 viruses produced L^{-1} day^{-1}), with previously reported values being over one order of magnitude higher (Weinbauer, 2004; Bongiorni *et al.*, 2005). There was no indication of decreasing LVP with increasing altitude. The low viral production rates resulted in long VT, suggesting that the rates of destruction and inactivation of viruses were low in these lakes.

In all the sampled lakes, except Kuoblatjåkkajaure, viral-induced bacterial mortality was < 5% of the BP. Kuoblatjåkkajaure had a viral-induced bacterial mortality of 21% in the summer–autumn, which is similar to previously reported values from freshwater environments (Wommack & Colwell, 2000; Weinbauer, 2004). With the exception of Kuoblatjåkkajaure, the values of viral lysis are among the lowest reported for marine and freshwater systems (Weinbauer, 2004). This implies that other bacterial loss factors such as protist or metazoan grazing of bacteria were more important than viral-induced bacterial mortality in our lakes. Indeed, Lymer *et al.* (2008) found that flagellate bacterivory was the main source of bacterial mortality in a set of 21 Swedish lakes (six were subarctic lakes situated in the coniferous forest and subalpine belt).

Burst size estimates were variable in the lakes and ranged from undetectable to 33 viruses. In Fig. 2, we plotted the reported mean B_z values for freshwaters in relationship with the mean FVIB values. We found that B_z decreased with a higher percentage of infected cells, which corroborates the observations of Säwström *et al.* (2007b), but is contrary to the findings of Parada *et al.* (2006). Interestingly, the data summarized in Fig. 2 support the hypothesis that a high frequency of visibly infected cells may offer a novel way for the viral population to survive when only a few viruses are released per infected cell (Säwström *et al.*, 2007b).

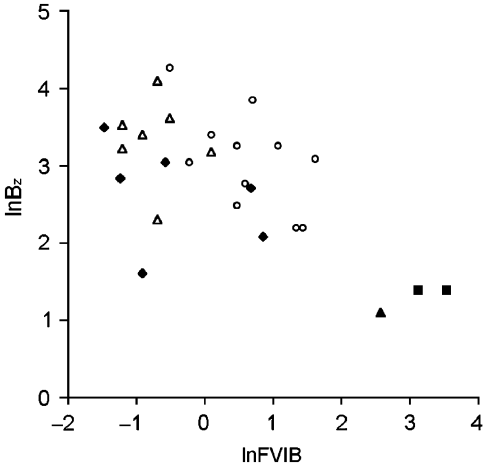

Fig. 2. Relationship between burst sizes (B_z) and frequencies of visibly infected bacteria (FVIB) in a variety of inland waters ($r = -0.637$, $P < 0.01$, $N = 28$). Antarctic waters (filled squares) (Säwström *et al.*, 2007b); arctic waters (filled triangle) (Säwström *et al.*, 2007b); subarctic waters, this study (filled diamond); temperate waters (open circle) (Hennes & Simon, 1995; Mathias *et al.*, 1995; Weinbauer & Höfle, 1998; Wilhelm & Smith, 2000; Hofer & Sommaruga, 2001; Fischer & Velimirov, 2002; Vrede *et al.*, 2003; Bettarel *et al.*, 2004; Jacquet *et al.*, 2005; Lymer *et al.*, 2008); tropical waters (open triangle) (Bettarel *et al.*, 2006).

Low LVP rates imply that there might be high proportions of lysogenic bacteria in the lakes as these cells could act as sinks for viruses. Surprisingly, lysogenic bacteria were only detected in the high-altitude lake Kuoblatjåkkajaure, which had an extremely low incidence of lysogeny (2.3% lysogeny). There are a limited number of reports on lysogeny in inland waters, and the majority of them have been conducted in Arctic and Antarctic waters (Tapper & Hicks, 1998; Anesio *et al.*, 2004, 2007; Lisle & Priscu, 2004; Bettarel *et al.*, 2006; Laybourn-Parry *et al.*, 2007; Säwström *et al.*, 2007a, c, 2008a, b). These studies show a considerable variation in the incidence of lysogeny, from undetectable to 73%. A high incidence of lysogeny (up to 73%) has been reported from Antarctic inland waters (Lisle & Priscu, 2004; Laybourn-Parry *et al.*, 2007; Säwström *et al.*, 2007a), whereas a low incidence of lysogeny (0.1–7.4%) has been reported from temperate and tropical inland waters (Tapper & Hicks, 1998; Bettarel *et al.*, 2006). Lysogeny may be a strategy for viruses to survive periods of low host availability and productivity in harsh environments. Indeed, the low bacterial activity in the high-altitude lake Kuoblatjåkkajaure during the winter period may have stimulated the development of lysogenic cells.

Benthic virus–bacterium interactions

Benthic viral abundance was up to three orders of magnitude higher than pelagic viral abundance and corresponded to observations from other inland waters (Ricciardi-Rigault *et al.*, 2000; Mei & Danovaro, 2004; Bettarel *et al.*, 2006; Fischer *et al.*, 2006; Filippini & Middelboe, 2007). The sediment environments in our study lakes harboured a large amount of viruses. Previous studies have speculated that the high concentrations of benthic viruses may be due to sedimentation, accumulation and persistence of viruses that originated from the overlaying waters (Mei & Danovaro, 2004; Bettarel *et al.*, 2006). An alternative explanation to the high concentrations of benthic viruses is that some of the fluorescent particles that are counted as viruses under the microscope are free nucleic acids. Dell'Anno *et al.* (1999) found high concentrations of free nucleic acids in marine sediments; thus, it is possible that free nucleic acids may interfere with the quantification of benthic viruses when using fluorescent nucleic acid dyes and epifluorescent microscopy.

BP rates were extremely high in the sediments, but this did not coincide with high benthic viral production rates. On the contrary, despite the high benthic-to-pelagic viral abundance ratios (7.2–195.4) and high theoretical contact rates ($0.2–10.5 \times 10^{14}$ contacts L^{-1} day^{-1}) in the benthic samples, our TEM analyses showed that there was a complete absence of virus-infected cells in the benthic samples. Such a virtual absence of virus-infected cells in the benthos has also been reported from other freshwater habitats (Bettarel *et al.*, 2006; Filippini *et al.*, 2006). In this single-time study, the failure to detect infected cells in the benthos along with low VBR values (< 3) implies that there was a low impact of freshwater benthic viruses on bacteria in our study lakes, which agrees with previous findings from temperate and tropical sediments (Fischer *et al.*, 2003; Bettarel *et al.*, 2006; Filippini *et al.*, 2006).

The exact reason behind the lack of viral infection, even though high viral numbers were present, in the benthos of our lakes and previous studies (Bettarel *et al.*, 2006; Filippini *et al.*, 2006) remains largely unknown. One possible explanation for the lack of virus-infected cells could be that there is a prevalence of temperate benthic viruses. However, because we have no information about the incidence of lysogeny in our sediment samples, we can neither accept nor reject this explanation. In the only study of lysogeny conducted with freshwater lake sediments (Senegal freshwaters), the authors reported a small FLC ranging from 0.3% to 4.2% (Bettarel *et al.*, 2006). Furthermore, a recent review of viriobenthos indicated that the lysogenic life cycle contributed little to benthic virus production in marine and freshwater environments (Danovaro *et al.*, 2008).

Conclusions

The large range in virus abundances in the samples from 0.67 to 28.89×10^9 viruses L^{-1} in the pelagic habitat and 2.1 to 14.0×10^{11} viruses L^{-1} in the benthic habitat indicated that the selected lakes represented distinct environments providing very different habitat conditions for viral proliferation. The environment where viruses are residing may be more or less good to support viral proliferation. Favourable conditions for pelagic viral proliferation were found in the lakes below the treeline, where high concentrations of DOC and bacteria occurred. In spite of this, there was no general trend of decreasing viral abundance and activity with increasing altitude.

Even though high benthic-to-pelagic viral abundance ratios suggested that the sediments were more favourable habitats for viral proliferation than the overlaying waters we found no indications of this. In benthic habitats, adsorption of viruses and bacteria to sediment particles may prevent the contact between viruses and bacteria. Furthermore, humic substances and complex organic matrices rich in exoenzymes have been shown to have a negative effect on viral abundance and VBR (Danovaro *et al.*, 2002a, b; Anesio *et al.*, 2004). In pelagic habitats, there is less organic matter and also lower viscosity than in the sediments, allowing a good dispersal of virus particles.

Lytic or/and lysogenic viral production could not explain the relatively high pelagic and benthic viral abundances in the investigated lakes. This may indicate that alternative life cycles may contribute to the observed viral abundances, such as pseudolysogeny (Weinbauer, 2004; Danovaro *et al.*,

2008). Another possible explanation for the rather high pelagic viral abundance might be a large allochthonous input of viruses and bacteria and/or the release of viruses and bacteria from the sediments into the overlying waters. Allochthonous input of viruses and bacteria can significantly influence both virio- and bacterioplankton assemblages in lakes (Lindström & Bergström, 2004; Sano *et al.*, 2004). The release of organic substances from lake sediments can increase the concentrations of dissolved organic matter (this fraction would also include viruses) in the overlying waters (Jansson, 1979, 1980). Our preliminary data set indicates a low impact of viruses on benthic and pelagic bacteria in subarctic lakes even though paradoxically, viruses are relatively abundant in both the benthos and the overlying waters. However, both temporal and spatial lake surveys are needed before we can firmly establish the relevance of viruses in the subarctic.

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