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Low effect of viruses on bacteria in deep anoxic water and sediment of a productive freshwater reservoir

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ABSTRACT: The seasonal and vertical distribution of freshwater viruses and the related induced bacterial mortality were determined in the water column down to the surface sediments of the thermally stratified reservoir Lake Grangent, France, between March and November 2007 in relation to environmental parameters. Viral abundance (VA, range = 0.9 to 3.5×10^{10} l^{-1}), bacterial abundance (BA), and the frequency of infected cells were significantly higher in the oxic than in the anoxic waters, which was partly related to forcing from the thermal stratification together with vertical changes in dissolved oxygen, ammonia and phytoplankton biomass. BA was the best predictor (R^2 = 0.78) for VA in the oxygenated waters only. The virus-to-bacteria ratio (VBR) yielded less variability and did not differ significantly along the water column (mean ± SD value = 5.5 ± 1.3). In the sediments, both VA and BA were one order of magnitude higher than in the water column with a higher variable VBR ratio (10.6 ± 8.9). Paradoxically, the frequency of visibly infected bacterial cells (determined from transmission electron microscopy) in the sediments was significantly lower (on average 3- to 7-fold) compared with the water column, which is in agreement with recent benthic reports in geographically contrasting lake environments. On average, viruses destroyed 23% of bacterial production in the oxygenated surface waters, but only 9% in deep anoxic waters and 3% in the sediments. Overall, there was a remarkably low viral influence on heterotrophic bacteria in the anoxic waters and overlying sediments of Lake Grangent, where recurrent shift from summer planktonic to winter benthic phases in cyanobacterial blooms is typical.

KEY WORDS: Lakes · Seasonal dynamics · Viruses · Bacteria · Lytic infection · Microbial ecology

INTRODUCTION

With the discovery of high viral abundance in aquatic environments almost 2 decades ago (Bergh et al. 1989), it is now well established that viruses form an integral component of the microbial food web in a great variety of such environments (Suttle 2007, Middelboe et al. 2008, Wilhelm & Matteson 2008). They play an important role in the regulation of carbon and nutrient fluxes, food web dynamics and bacterial diversity (Wilhelm & Suttle 1999, Brussaard et al. 2008, Sime-Ngando & Colombet 2009). Reports have suggested that viral lysis can account for up to 90 to 100% of bacterial mortality in some freshwater systems (Weinbauer & Höfle 1998, Fischer & Velimirov 2002), which has led to the conclusion that viral lysis can be a major cause of mortality, comparable with grazing-induced mortality (Wilhelm & Matteson 2008). Attempts to predict dynamics of virioplankton in aquatic systems have revealed relationships to environmental parameters that affect the abundance and activity of the host, primarily bacterioplankton. Although estimates of viral abundance and infection rates have been made for a variety of aquatic environments, including freshwater, seawater and hypersaline ponds, such investigations pertaining to reservoirs are rare and very limited (Sommaruga et al. 1995, Šimek et al. 2001, Pradeep Ram et al. 2005). Studies in reservoirs

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are important as their artificial origin makes them useful study models, both in terms of understanding phenomena related to eutrophication and for determining the relative importance of the different environmental factors on microbial metabolism. Disturbance frequencies are much higher in reservoirs than in lakes, with rapid, often irregular and large, changes in flushing rates, water level and the stability of the water column (Wetzel 2001). Lack of opportunity to document viral ecology in such systems has resulted in a paucity of knowledge about these environments.

Lake Grangent, is a eutrophic and turbid reservoir located in the Massif Central of France on the upper reaches of the River Loire 15 km from the city of Saint-Etienne. Since 1970, high external phosphorous loading has been causing hypereutrophication of the reservoir, which has led to the recurrence of cyanobacterial blooms of *Microcystis aeruginosa* in August or sometimes in early June every year (Berthon et al. 1996), with a summer planktonic phase followed by an overwinter benthic phase (Latour et al. 2004). Thermal stratification begins in March with a weak thermocline at 30 m. Euphotic (<7 m) and aphotic zones differ significantly in terms of temperature, dissolved oxygen, ammonia concentration, organic matter and phytoplankton biomass. Therefore, the factors controlling viral abundance and phage infection can change significantly with depth, as previously inferred from Lake Pavin, France (Colombet et al. 2006).

Our knowledge of the significance of viral lysis for bacterial production in anoxic waters is sparse (Weinbauer & Höfle 1998, Gobler et al. 2008). Since grazing rates are typically low in anoxic waters (cf. Colombet et al. 2006), other mechanisms such as viral lysis must be responsible for bacterial mortality. So, we hypothesize that bacteriolysis should be much higher in the anoxic compared with the oxic zone, based on the evidence from theoretical (Pedros-Alio et al. 2000) and empirical field (Colombet et al. 2006) investigations. The results of the present study reveal the ecological importance of the depth-related fluctuations of virioplankton abundances and their infection rates linked to physicochemical variables of the water column, which has not yet been explored on a seasonal scale. The study of viral ecology in Lake Grangent is also of interest because only few data are available with regard to the seasonal trends and vertical variations of viral abundance and infectivity in eutrophic waters.

The present study reports on the seasonal and vertical variability of viruses, bacteria and phytoplankton biomass (i.e. chlorophyll *a* [chl *a*]) in the water column and water–sediment interface of Lake Grangent from March to November 2007.

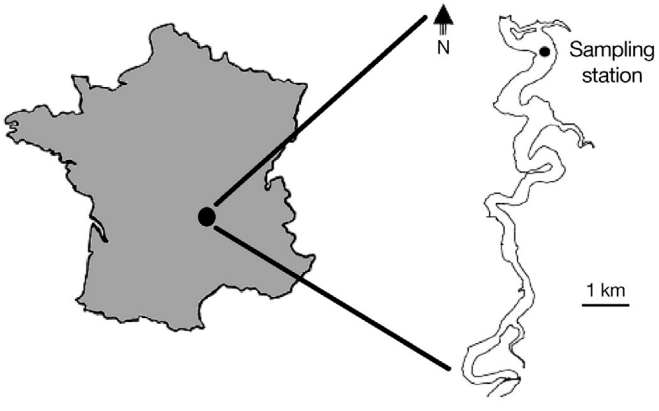

Fig. 1. Location of sampling station in Lake Grangent, France

MATERIALS AND METHODS

Sampling. For detailed hydrological and morphological characteristics of Lake Grangent see Latour et al. (2004). Water samples were collected in the downstream part of the reservoir (35° 33′ N, 75° 01′ E, Fig. 1) where blooms have been primarily occurring for the past few years (Giraudet & Berthon 1999). Samples were collected bimonthly from March to November 2007 using a horizontal 10 l Van Dorn bottle. The sampling depths were 0.5, 5, 10 and 40 m. Secchi depth (z_{SD}) measurements were used to estimate the euphotic depth (z_{eu}) according to the relationship: $z_{eu} = 2.42\ z_{SD}$, assuming that 15% of the incident light is transmitted to the z_{SD} depth (Wetzel & Likens 1995). According to the above criteria, z_{eu} did not exceed 7 m in the present study, and as a result the sampling depths of 0.5 and 5 m were considered to be located in the euphotic zone. We also collected samples in the surface sediments (i.e. 0 to 2 cm of the upper sediments) using a core drill at 42 m (i.e. z_{max}). These samples were cut out and stored in dark bottles before being brought back to the laboratory. All samples were collected in triplicate.

Physicochemical variables. Water temperature and dissolved oxygen profiles were determined *in situ* using a WTW-OXI-320 multiparameter probe and the values were interpolated through the water column by using SURFER 5.00 (Golden Software). Samples for nutrients, namely dissolved ammonium (as NH_4-N) (AFNOR 1990), nitrate (as NO_3-N) (Rodier 1996) and soluble orthophosphate (as PO_4-P) (Motomizu et al. 1982) were analyzed spectrophotometrically, whereas silica concentration was analyzed colorimetrically by means of standard methods (AFNOR 1990). Chl *a* concentrations were determined spectrophotometrically (Lorenzen 1967) from samples collected on GF/F filters

(Whatman) from a 500 ml volume of reservoir water. Pigments were extracted in 90% acetone overnight in the dark at 4°C. The supernatant was used to determine the functional chl *a* and then acidified with 0.1 N HCl to estimate phaeopigments.

Bacterial and viral abundances. For the enumeration of virus-like particles and bacteria, water samples were fixed with 0.02 µm filtered, buffered alkaline formalin (final concentration, 2% v/v from a 37% w/v solution of commercial formaldehyde). Within 1 h of sampling, 1 to 2 ml subsamples were filtered (under <15 kPa vacuum) through 0.02 µm pore size Anodisc filters (Whatman), with 1.2 µm pore size cellulose acetate backing filters. After staining with SYBR Green I fluorochrome (Molecular Probes Europe) (final dilution, 2.5×10^{-3} fold) as described by Noble & Fuhrman (1998), filters were air dried on absorbent paper, mounted on slides and covered with glass coverslips with the mountant Glycerol/PBS solution (i.e. Citifluor) amended with a special antifading solution, i.e. ca. 20% (v/v) of Vecta Shield (Vector Laboratories). This amendment significantly reduced fading of the fluorochrome and gave highly stable fluorescence (Pradeep Ram et al. 2005). When not analyzed immediately, slides were stored at –20°C until needed for counting under a model 300F epifluorescent microscope (Leica DC). Bacteria were distinguished from virus-like particles on the basis of their relative size and brightness. A blank was routinely examined to control for contamination of the equipment and reagents.

For sediment samples, 2 to 4 g of sediment (with a precision of 0.005 g) from the top layer (0 to 2 cm depth) of the core sample was transferred to a 50 ml centrifuge tube, and 4 ml of virus-free (0.02 µm filtered) bottom water and glutaraldehyde (3% final concentration) were added. Benthic viruses and bacteria were extracted within 24 h, using a modified protocol from Danovaro et al. (2001), by adding sodium pyrophosphate to a final concentration of 10 mmol, followed by 3 × 1 min of sonication on an ice bath before centrifugation for 5 min at 700 × *g*. The settled sediment samples were subsequently washed twice with 2 ml of virus-free water to enhance extractability of bacteria and viruses, and the extracted volumes were pooled with the above mentioned supernatant samples. Aliquots were then diluted 100 to 250 times, and 200 µl of extracted samples were taken for slide preparation and analyzed by means of the procedures described above for bacterial and viral abundances.

Phage infection. In glutaraldehyde-fixed experimental samples, bacterial cells contained in 8 ml water samples were collected on copper electron microscope (EM) grids (400 mesh, carbon-coated Formvar film) by centrifugation according to Sime-Ngando et al. (1996). Each grid was stained at room temperature (ca. 20°C) for 30 s with uranyl acetate (2% w/w), rinsed twice with 0.02 µm filtered distilled water and dried on a filter paper. Grids were examined in a JEM 1200 EX transmission electron microscope (TEM) (JEOL) operated at 80 kV at a magnification of 20 000 to 60 000× to distinguish between prokaryotic cells with and without intracellular viruses. A prokaryote was considered infected when at least 5 viruses, identified by their shape and size, were clearly visible inside the host cell. At least 500 prokaryote cells were inspected per grid to determine the frequency of visibly infected cells (FVIC). Because mature phages are visible only late in the infection cycle, FVIC counts were converted to the frequency of infected cells (FIC) using the equation FIC = 9.524 × FVIC – 3.256 (Weinbauer et al. 2002). Assuming a steady state existed, that is, the infected and uninfected cells were grazed at the same rate, and that the latent period equalled the prokaryotic generation time, the FIC were converted to bacterial mortality (VIBM, as percentage of prokaryotic production) using the equation: $VIBM = (FIC + 0.6 \times FIC^2)/(1 - 1.2 \times FIC)$ (Binder 1999). For determination of FVIC in sediment samples, the aliquots of water samples extracted from sediments were filtered through 3 µm filters and diluted 2 to 8 times before bacteria were harvested on EM grids (Middelboe et al. 2003). For sediment samples, a minimum of 800 cells were inspected per grid to determine FVIC. The mean viral burst size (no. viruses bacterial $cell^{-1}$) was estimated for each sample by counting the number of viral particles in a minimum of 15 and 5 visibly infected bacteria in water and sediment samples, respectively.

Statistical analysis. Differences in physicochemical and biological variables between sampled depths and seasons (Spring: March to June, Summer: June to September, Autumn: September to November) were tested by 2-way ANOVA. Potential relationships among variables were tested by linear pairwise correlations (i.e. Pearson correlation analysis) and stepwise multiple regressions. Data were log transformed to satisfy the requirements of normality and homogeneity of variance necessary for parametric statistics. All statistical analyses were performed with Release 12 software for Windows (Minitab).

RESULTS

Temperature and dissolved oxygen

Water temperature showed strong seasonal changes ($p < 0.001$); the values increased from 7.9°C (April) to 22.3°C (August) in the surface waters (0.5 m depth) and then gradually decreased to 15.6°C in October. A

similar trend was also observed at 5 and 10 m depths, but with lower values (Table 1). Temperature at 40 m depth was low and less variable (4.9 to 7.2°C) with an average ± SD of 6.4 ± 0.7°C. Thermal stratification started in late April with a weak thermocline at 30 m (Fig. 2A). The water column above 10 m depth was generally well oxygenated (>6 mg O_2 l^{-1}) during the entire study period, contrasting with the deep anoxic zone (i.e. 40 m) and the sediments as well. The anoxic layer increased with seasons, from about 38 m depth in spring to <30 m from late August onwards (Fig. 2B).

Nutrients

In most cases, nutrients did not vary significantly with seasons or with depths. Concentrations of potentially limiting nutrients such as dissolved reactive phosphate and ammonium were well above the threshold concentration to induce any kind of limitation for the growth of planktonic organisms (Table 1). The ammonia concentration in the anoxic zone was significantly higher ($p < 0.001$) than in the oxic zone. Concentrations of other nutrients were generally higher in the anoxic waters and sediments than in the upper water column, but differences were not significant.

Chl *a* and phaeopigments

Chl *a* concentration showed large variability with seasons (coefficient of variation [CV] = 199%) and depths (CV = 171%). At the 0.5 m depth, there were 2 peaks of chl *a*, one in April (15.5 µg l^{-1}) and a larger one in June (56.1 µg l^{-1}) coinciding with the spring phytoplankton bloom. Chl *a* in the oxic depths (mean ± SD = 6.6 ± 12.2 µg l^{-1}) differed significantly ($p < 0.02$) from those in the anoxic zone (0.6 ± 1.7 µg l^{-1}) (Table 1). Phaeopigment concentrations showed large variability (CV = 143%) in the anoxic zone (40 m), and the values were significantly higher ($p < 0.01$) than in the upper oxic waters. The average phaeopigment concentrations in the oxic and deep anoxic waters were 15.2 ± 15.7 and 69.9 ± 93.7 µg l^{-1}, respectively.

Standing stocks

In the water column, VA and BA peaked on 6 August in the surface waters at 0.5 m (VA = 3.5×10^{10} l^{-1}; BA = 7.4×10^9 cells l^{-1}) and 5 m (VA = 3.0×10^{10} l^{-1}; BA = 6.3×10^9 cells l^{-1}), which was 2- and 4-fold higher than the lowest mean value obtained in autumn for all sampling depths (VA = 1.3×10^{10} l^{-1}; BA = 1.9×10^9 cells l^{-1}) (Fig. 3A,B). Two-way ANOVA indicated that both VA and BA exhibited strong variability ($p < 0.001$) with seasons and depths, both of which interacted significantly (for all microbial variables) because of the spatial differences that occurred during the period of thermal stratification (Table 2, Fig. 3). Among abiotic variables, temperature indeed exerted a significant influence on BA ($p < 0.001$) and VA ($p < 0.01$) in the surface oxic waters (Table 3). BA and VA were strongly correlated in these waters (Table 3). VA and BA in the euphotic zone (i.e. at 0.5 and 5 m) were significantly ($p < 0.001$) higher than in the aphotic zone (10 and 40 m). In the sediments, both VA and BA were one order of magnitude higher than in the water column (Table 1). Unlike the water

Table 1. Mean (% coefficient of variation) physicochemical characteristics, chl *a*, phaeopigment concentrations and bacterial and viral variables in the water column and sediments of Lake Grangent, France, from March to November 2007; na: no data available

Variable	Oxic				Anoxic	
	0.5 m	5 m	10 m	Average	40 m	Sediments
Temperature (°C)	17.5 (26)	16.9 (25)	16.5 (26)	16.9 (25)	6.4 (10)	na
Dissolved oxygen (mg l^{-1})	8.9 (22)	8.0 (22)	7.6 (25)	8.2 (23)	1.1 (197)	na
Oxygen saturation (%)	98.5 (22)	86.7 (17)	81.3 (17)	88.8 (19)	9.4 (191)	na
NH_4-N (mg l^{-1})	0.07 (51)	0.07 (58)	0.08 (65)	0.07 (58)	0.8 (65)	1.5 (40)
NO_3-N (mg l^{-1})	2.6 (37)	2.6 (31)	2.7 (38)	2.6 (35)	3.1 (47)	3.1 (46)
PO_4-P (mg l^{-1})	0.05 (117)	0.1 (60)	0.11 (59)	0.09 (79)	0.03 (55)	0.1 (40)
SiO_2 (mg l^{-1})	7.1 (32)	7.4 (33)	7.8 (33)	7.4 (33)	8.3 (10)	8.3 (17)
Chl *a* (µg l^{-1})	9.7 (200)	8.5 (180)	1.5 (133)	6.6 (171)	0.6 (119)	na
Phaeopigments (µg l^{-1})	19.6 (143)	14.4 (69)	11.4 (79)	15.1 (97)	70 (134)	na
Viral abundance (10^{10} l^{-1})	2.2 (24)	2.1 (17)	1.6 (22)	2.0 (21)	1.2 (19)	48.2 (21)
Bacterial abundance (10^9 cells l^{-1})	4.3 (32)	4.1 (24)	3.0 (27)	3.8 (28)	2.5 (22)	45.5 (25)
Virus-to-bacteria ratio	5.3 (18)	5.2 (19)	5.5 (23)	5.3 (20)	5.8 (27)	10.6 (85)
Frequency of infected cells (%)	18.8 (42)	16.9 (50)	14.3 (27)	16.7 (34)	7.9 (32)	2.8 (32)
Viral induced bacterial mortality (%)	27.0 (60)	23.3 (70)	18.7 (36)	23.0 (55)	9.1 (37)	3.0 (73)
Burst size (no. viruses bacterial $cell^{-1}$)	29.0 (27)	27.1 (34)	23.1 (49)	26.4 (37)	23.2 (41)	16.2 (20)

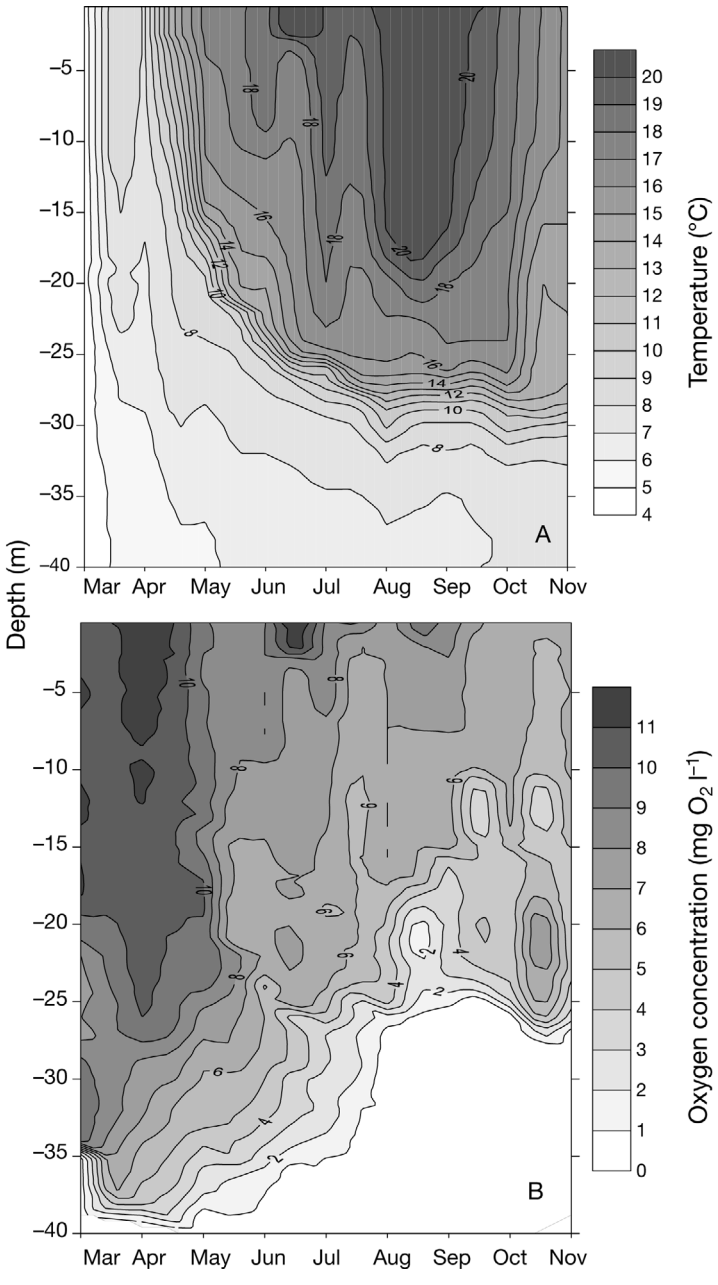

Fig. 2. Spatio-temporal variations of (A) temperature and (B) dissolved oxygen concentration in the water column of Lake Grangent from March to November 2007

column, the highest values for VA (66.5×10^{10} l^{-1}) and BA (67.0×10^{9} cells l^{-1}) in the sediments did not occur at the same time (Fig. 3A,B). Despite large variability, VBR did not differ significantly between the sampling depths, averaging 5.3 and 5.8 for oxic and anoxic waters, respectively. In the sediments, VBR (mean = 10.6) was significantly ($p < 0.001$) higher compared with the water column values (Table 1).

Phage infection and burst size

The FIC in the oxic waters varied in the range of 5.8 to 34.5%, with a mean value of 16.7% that corresponded to 23% of the bacterial mortality caused by viruses (i.e. VIBM). Maxima in FIC at 0.5 m were observed on 6 August, which coincided with peaks in VA and BA (Fig. 3C) and corresponded to a VIBM level of 71%. FIC in the oxic waters was about 2-fold and significantly ($p < 0.001$) higher than in the anoxic water at 40 m depth (range: 5.8 to 12.8%, mean = 7.9%) (Fig. 3C, Table 1). In the oxic waters, FIC was strongly correlated with VA and BA (Table 3). The significantly higher levels of VA and BA in the sediments (i.e. compared with the water column) did not result in higher viral infection. FIC values in the sediments (range: 0.6 to 6.3%, mean = 2.8%) were, on average, 3- to 7-fold lower ($p < 0.001$) than in the water column (Table 1). Burst size (BS) varied from 6 to 160 viruses bacterial cell^{-1} with an average value of 26 and 23 viruses cell^{-1} in the oxic and anoxic waters, respectively. The difference between the 2 zones was not significant. In the sediments, BS averaged 16.2 viruses cell^{-1} (Table 1) and the values were not significantly different compared with the water column.

A multiple regression analysis was carried out to determine the relative importance of the main correlates for VA and FIC. For the oxic waters, the models for which the highest R^2 values were obtained clearly highlight the importance of BA for VA (i.e. VA = 0.62 – 0.004 × Temp + 0.37 × BA, $R^2 = 0.78$, n = 49), and of the combination of BA and VA for FIC (FIC = 3.73 + 4.51 × BA – 2.8 × VA, $R^2 = 0.35$, n = 49). For the anoxic waters and sediments, the measured environmental variables

Table 2. Two-way ANOVA results for the effects of seasons and depths on viral and bacterial abundances, frequency of infected cells and burst size in Lake Grangent. Degrees of freedom are 15, 3, 45 and 128 for A, B, A × B and error, respectively

Source	Viruses		Bacteria		Frequency of infected cells		Burst size	
	F	p	*F*	p	*F*	p	*F*	p
Seasons (A)	78.82	0.0001	444.38	0.0001	632.72	0.0001	13.56	0.0001
Depths (B)	4.98	0.0001	86.72	0.0001	114.81	0.0001	15.15	0.0001
Interactions (A × B)	5.98	0.0001	20.28	0.0001	37.65	0.0001	10.36	0.0001

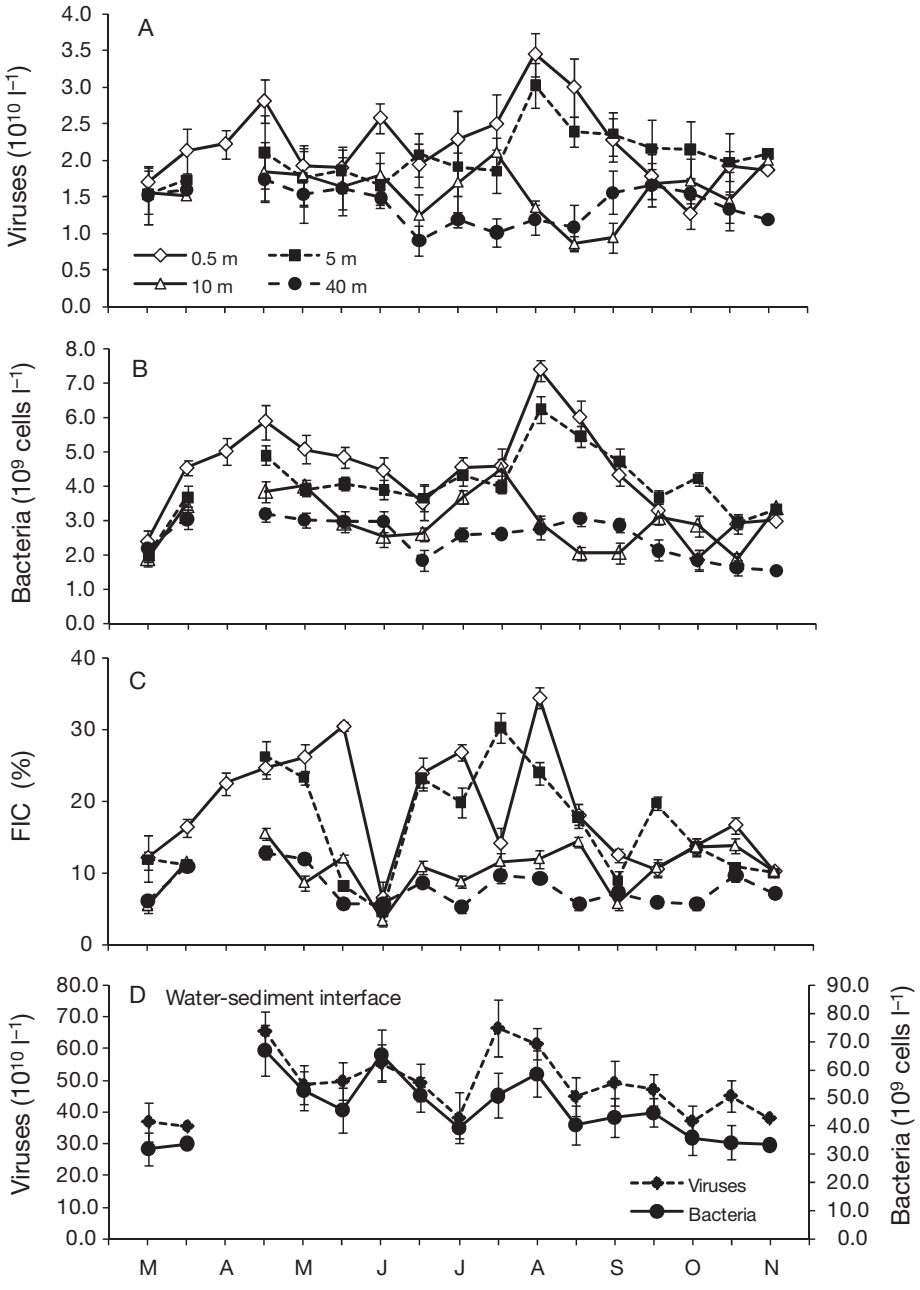

Fig. 3. Viral and bacterial parameters of Lake Grangent, March to November 2007. Spatio-temporal variability in (A) viral abundance and (B) bacteria abundance, and (C) the frequency of infected bacterial cells (FIC) in the water column. (D) Viral and bacterial abundances in the surface sediments (0 to 2 cm). Error bars are SE

only explained part of the variance in VA and FIC, but did not yield significant correlates.

DISCUSSION

Pelagic interactions

The present study is one of the few of its kind conducted in freshwater reservoirs (Sommaruga et al. 1995, Šimek et al. 2001, Pradeep Ram et al. 2005) and the first study of the eutrophic Lake Grangent that documents the seasonal standing stock of viruses and phage infection in relation to environmental variables in the water column down to the surface sediments. The virioplankton abundances were within the range reported in other lakes (Weinbauer 2004) and similar to those from temperate eutrophic lakes such as Lake Aydat, France (Bettarel et al. 2003), Lake Plußsee, Germany (Weinbauer & Höfle 1998), and Lake Loosdrecht,

Table 3. Pearson product moment correlation (r) between different variables in the oxic (n = 49) or anoxic (n = 16) waters during March to November 2007 in Lake Grangent. Levels of significance: *p < 0.05, **p < 0.01, ***p < 0.001; ns: not significant

Variable	Temperature	Bacterial abundance	Viral abundance	Frequency of infected cells
Temperature				
Bacterial abundance	0.48***/ns			
Viral abundance	0.41**/ns	0.89***/ns		
Frequency of infected cells	ns/ns	0.68***/0.59**	0.58***/ns	
Burst size	ns	ns/0.47*	ns/ns	0.30*/ns

The Netherlands (Tijdens et al. 2008). Concentrations of both viruses and bacteria in the oxygenated waters differed significantly from those at the anoxic depth (i.e. 40 m), a situation that also corroborates findings in eutrophic waters (Jiang et al. 2004). In Lake Grangent, the spatial differences were partly forced by thermal stratification together with dramatic differences between oxic and anoxic waters in terms of dissolved oxygen, ammonia concentration and phytoplankton biomass. Indeed, viral and bacterial abundances were significantly correlated to water temperature and to each other as well, but the latter correlation was clearly due to the patterns recorded in oxygenated depths (R^2 = 0.78 versus 0.11 in anoxic waters). In the oxic waters, bacteria thus appeared to be the major hosts for viruses, as is generally known from temperate lakes (Bettarel et al. 2003, Pradeep Ram et al. 2005). Overall, the seasonal abundances of both viruses and bacteria in Lake Grangent were rather homeostatic as they did not vary by more than 5-fold for all sampled depths (Fig. 3A,B), which is similar to those reported by Mathias et al. (1995) in a backwater system of River Danube and Hennes & Simon (1995) in the mesotrophic Lake Constance, Germany. The VBR also did not fluctuate greatly (range: 4 to 8) over the study period, suggesting a tight coupling between bacteria and viruses and a relatively constant level of viral production and loss (Pradeep Ram et al. 2005).

Recent studies have suggested that viral infection may contribute significantly to bacterial mortality in aquatic systems, and FVIC is a measure of the magnitude of this process (Pradeep Ram et al. 2005, Gobler et al. 2008). We used TEM methods (i.e. whole cell examination) to determine FVIC (expressed as percentage of total prokaryotic cells), which provides direct evidence of phage infection. As most of the data to date on FVIC and burst sizes are derived from TEM-based estimates (Weinbauer 2004), comparison among aquatic systems are relatively easy. Discussions about interpreting the estimates of bacterial mortality due to viral lysis based on the whole cell FVIC approach are detailed in Weinbauer & Höfle (1998), Guixa-Boixereu et al. (1999), and Bettarel et al. (2004), and thus, will not be reiterated here. However, because a minimum of 500 to 800 cells were examined for 0.6 to 3.9% target-infected cells, our FVIC values in the lower range would be error prone due to the general rarity of infected cells, especially in the sediment samples (Sime-Ngando & Colombet 2009).

In the plankton of Lake Grangent, FVIC values were comparable and within the typical range (i.e. < 5%) reported for limnetic systems (Sime-Ngando et al. 2003, Weinbauer 2004). Similar to viral and bacterial abundances, significant difference in viral infection was observed between oxic and anoxic waters, probably because of dramatic changes in the availability of hosts and the factors that control their abundance and activity in oxic versus anoxic environments (DeBruyn et al. 2004, Gobler et al. 2008). Such depth-related differences have been poorly resolved in stratified productive lakes such as Lake Aydat, France, (Bettarel et al. 2003) and Mono Lake, USA (Brum et al. 2005). On average the viral infection rate was 2-fold higher in the oxic than in anoxic waters. This vertical pattern that we found in Lake Grangent differs from previous results obtained in other stratified water bodies, where studies have found that viral lysis is more prominent in the anoxic than in the oxic waters (Weinbauer & Höfle 1998, Colombet et al. 2006, Gobler et al. 2008). Viral production might be suppressed in the deeper layer of Lake Grangent because of low bacterial activity (as indicated by low bacterial abundance) and depletion of dissolved oxygen (average concentration at 40 m depth = 1.1 mg O_2 l^{-1}). In addition to the anoxia, studies on bacterial community composition could also help explain the variability in viral infection rates. Although viruses apparently contributed little to bacterial mortality in anoxic waters and sediments in our study, they can exert a strong influence on clonal diversity of bacteria in such environments by selecting for virus resistant strains.

The increase in viral infection with increasing VA and BA would suggest that the lytic mode of infection is important in Lake Grangent, primarily in the oxygenated waters where viral proliferation leads to high encounter rates between viruses and their heterotrophic bacterial hosts. This implies that the bacterioplankton community could be dominated by only a few species, favoring specific adsorption of viruses to their

co-occurring hosts, and that the maintenance of a high number of viruses is dependent on active bacterioplankton populations (Weinbauer 2004, Pradeep Ram et al. 2005). Multiple regression analysis suggested that the predictability of FIC from VA and BA was weak (R^2 = 0.35) and substantially lower than that of VA from BA (R^2 = 0.78). One explanation assumes that a portion of the viral community in Lake Grangent infects cyanobacterial hosts (A. S. Pradeep Ram unpubl.). Cyanobacteria are highly abundant in Lake Grangent (Latour et al. 2004), and they can also represent major viral hosts in pelagic environments (cf. Sime-Ngando & Colombet 2009). So, the chance for viral proliferation is linearly dependent on the density of abundant and susceptible hosts, i.e. both auto- and heterotrophic prokaryotes (Murray & Jackson 1992). An alternative explanation would be that viral infection is not as host specific as previously assumed, or it may depend on the environmental conditions that influence receptor expression on host cell surfaces.

Calculations based on the model by Weinbauer et al. (2002) and Binder (1999) suggest that, on average, 17 and 8% (i.e. FIC) of bacteria in oxic and anoxic waters, respectively, were infected by viruses in Lake Grangent. This corresponds to about 23 and 9%, respectively, (i.e. VIMB) of bacterial production being destroyed by viruses. Depth-related differences in viral lysis could be linked to bacterial diversity, thus tempting us to assume that host genetic diversity might be important in controlling viral infection rates. High ammonia concentration in the anoxic bottom waters is the consequence of ammonia build-up in the water–sediment interface, primarily due to degradation of phytoplankton biomass as evidenced by high phaeopigment concentrations. These zones could be dominated by ammonia oxidizing bacteria capable of anaerobic oxidation of ammonium (Freitag & Prosser 2003), a process that has been less studied in deep anoxic freshwaters. Such zones could also harbor many novel non-extreme environmental *Archaea*, which have been previously reported in eutrophic lakes (Jurgens et al. 2000, Eller et al. 2005).

In Lake Grangent, BS (derived from TEM observation of intracellular phages) varied from 6 to 160 viruses cell^{-1} with an average value of 26 and 23 viruses cell^{-1} for oxic and anoxic waters, respectively. Unlike VA and phage infection, there was no significant difference in BS between the 2 zones, which is consistent with observations in other stratified aquatic environments (Choi et al. 2003, Bettarel et al. 2004, Gobler et al. 2008). However, these results were in contrast to other studies in which BS were found to be significantly larger in anoxic bottom waters relative to the overlying oxic waters (Weinbauer & Höfle 1998, Weinbauer et al. 2003). Similar to observations reported for Lake Erie, USA (Gobler et al. 2008), no consistent pattern seemed to emerge in our study concerning the vertical variability in BS, and the few data available prevent us from making any simple generalization. The transmission electron micrographs of viral infected cells (Fig. 4) reveal that the BS observed in Lake Grangent samples were indeed much lower than the global average of 34 and 57 viruses cell^{-1} reported for freshwaters and eutrophic waters, respectively (Prada et al. 2006). Low BS could be explained when lytic phages have a short latent period due to short host generation time, or when host abundances are high (Prada et al. 2006). Nevertheless, BS could also be influenced by a number of factors, such as the size of viruses or the size and metabolic activity of hosts.

Benthic interactions

The present understanding that viruses are more abundant in aquatic sediments than in the overlying water column (Fischer et al. 2003, Mei & Danovaro 2004, Bettarel et al. 2006) was confirmed in our study. The origin of the high abundances of viruses in aquatic sediments is still an uncertainty, particularly when we take into account the low occurrence of visibly infected cells in TEMs and the related low bacteriolysis reported in geographically contrasting environments, which include tropical (Bettarel et al. 2006), temperate (Filippini et al. 2006, the present study) and subarctic (C. Säwström et al. unpubl.) lake sediments. If benthic bacteria are not susceptible to viral infection, then the high concentrations of viruses in the sediments may be due to sedimentation, accumulation and persistence of viruses that originated from the surface waters (Bettarel et al. 2006). In our sediment samples, the low levels of FVIC (i.e. <0.6%) could not be entirely attributed to methodological bias, given that higher levels were detected in pelagic bacteria. In all sediment samples analyzed, bacteria were clearly identifiable on the basis of staining quality, cell transparency and membrane refringence. However, uncertainties remain because filtration (through 3 µm membranes) or the sonication procedure used to extract bacteria from sediments may result in loss or disruption of cells, and this effect may be more pronounced with large-sized fragile infected cells (Bettarel et al. 2006).

Bacteria are known to be very active in sediments (Kirschner & Velimirov 1999, Haglund et al. 2003), but the reasons why they are less susceptible to viral infection are unclear and need to be elucidated. Our low FVIC-derived viral lysis in sediment bacteria together with those reported in other lake sediments using the same methodological approach (i.e. Bettarel et al. 2006, Filippini et al. 2006, C. Säwström et al.

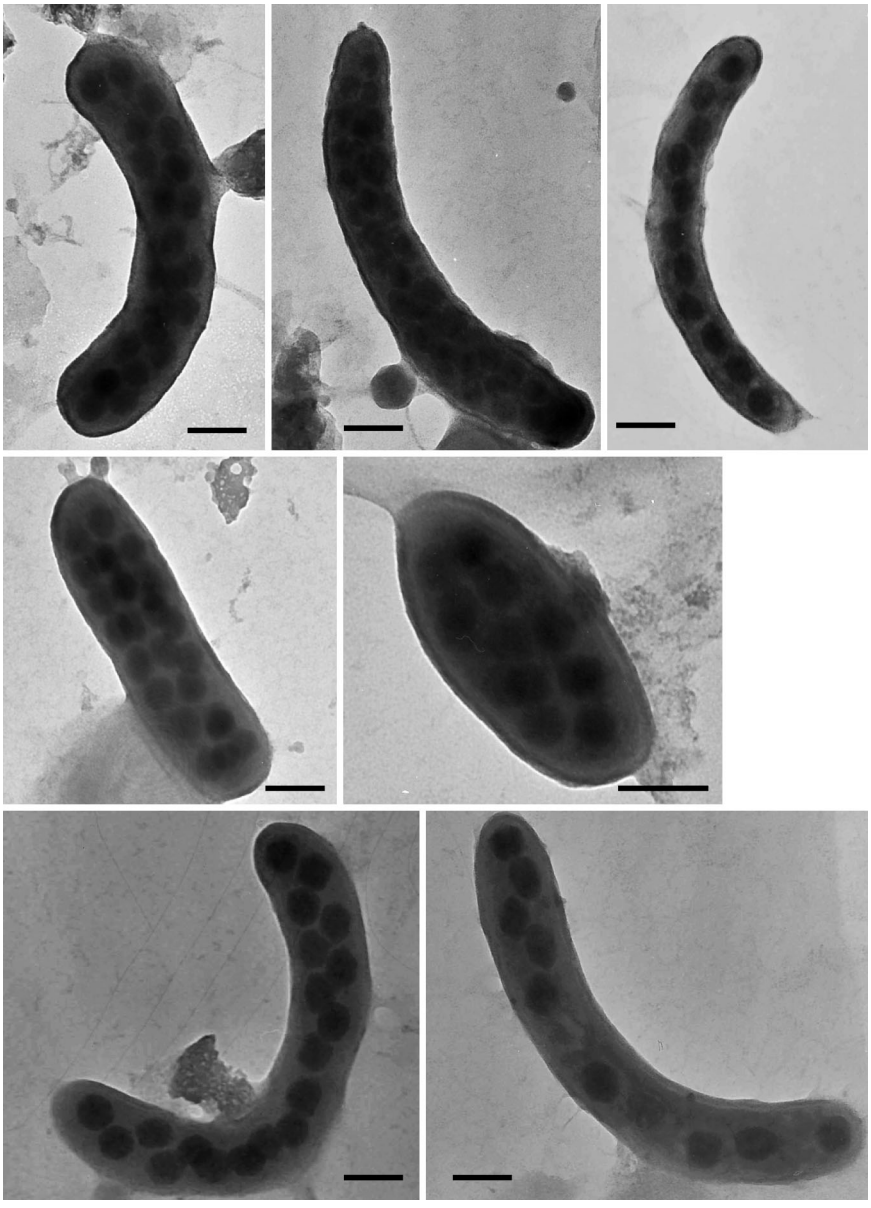

Fig. 4. Transmission electron micrographs of typical infected bacterial cells from the productive Lake Grangent with low and varying burst sizes, i.e. the number of intracellular viruses per infected cell. Each scale bar denotes 100 nm

unpubl.) are indications that strengthen the nation that aquatic sediments provide good conditions for viral preservation, but paradoxically, are poor environments for viral proliferation. In such environments, lysogeny could be a predominant mode of infection, but this still needs to be demonstrated. A prevalence of temperate viruses in aquatic sediments may indeed immune-suppress lytic viral attacks, resulting in low FVIC levels. Viral proliferation in sediments may also be limited by physical or biochemical conditions that prevent high encounter rates (Fischer et al. 2004, Filippini et al. 2006). Massive adsorption of viruses on sediment particles may also weaken their infectivity (Hewson & Fuhrman 2003), alter the physiology of bacteria and, to an unknown extent, render host cells more resistant to viral attack (Fischer et al. 2003). Alternatively, diverse prokaryotic communities within aquatic sediments (Torsvik et al. 2002) may represent a barrier for viral proliferation. Further studies on the changes in microbial community composition with time and space would be useful in the context of the viral ecology of Lake Grangent.

CONCLUSIONS

Overall, our study presents the first data on seasonal abundance and lytic activity of viruses in the water column down to the surface sediments of the eutrophic Lake Grangent, France. The abundances of viruses and bacteria were one order of magnitude higher in the sediments than in the water column. Astonishingly, the viral-to-bacterial abundance was rather stable in the water column, independently of the oxygen concentration, but was twice as high and more variable in the sediments. However, few visibly infected bacteria, and the related bacteriolysis (<10% of bacterial production), occurred in the deep anoxic waters and sediments, compared with the surface oxic waters. In these waters, about one quarter of bacterial production ended up in viral synthesis, indicating that a significant portion of organic matter could be recycled via the viral shunt into regenerated nutrients in the lake. In such a eutrophic turbid reservoir where cyanobacterial blooms are recurrent, viral-derived cell lysis products could also act like glue for the formation of organic aggregates and facilitate sinking of aggregates (Proctor & Fuhrman 1991), thereby resulting in the accumulation of pelagic organic particles into the sediments. Together with other factors such as changes in host diversity, this may account, at least partly, for the low effect of viruses on bacteria in the deep anoxic waters and sediments of the Lake Grangent reservoir.

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