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High Lytic Infection Rates but Low Abundances of Prokaryote Viruses in a Humic Lake (Vassivière, Massif Central, France)▽

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We explored the abundance and infection rates of viruses on a time series scale in the euphotic zone of the humic mesotrophic Lake Vassivière (Massif Central, France) and compared them to nonhumic lakes of contrasting trophy (i.e., the oligomesotrophic Lake Pavin and the eutrophic Lake Aydat) located in the same geographical region and sampled during the same period. In Lake Vassivière, the abundances of virus-like particles (range, 1.7×10^{10} to 2.6×10^{10} liter^{-1}) were significantly ($P < 0.001$) lower than in Lakes Pavin and Aydat. The percentage of virus-infected prokaryotic cells (mean, 18.0%) was significantly higher ($P < 0.001$) in Vassivière than in Pavin (mean, 11.5%) and Aydat (mean, 9.7%). In Vassivière, the abundance of prokaryotes was a good predictor ($r = 0.78$, $P < 0.001$) of the number of virus-like particles, while the potential grazing rate from heterotrophic nanoflagellates was positively correlated to the viral infection rate ($r = 0.75$, $P < 0.001$; $n = 20$), indicating the prevalence of cycling interactions among viruses, prokaryotes, and grazers, which is in agreement with past experiments. The absence of correlation between chlorophyll *a* concentrations (Chl) and viral parameters suggested that the resources for the lytic activity of viruses in Vassivière were mainly under allochthonous control, through host activity. Indeed, compilation of data obtained from several nonhumic lakes in the French Massif Central revealed that Chl was positively correlated to the abundance of virus-like particles at concentrations above 0.5 μg Chl liter^{-1} and negatively at concentrations below 0.5 μg Chl liter^{-1}, suggesting that phytoplankton-derived resources could force prokaryotic growth to attain a certain threshold level when the host availability is sufficient to boost the proliferation of viruses. Therefore, based on the high level of lytic infection rates in Lake Vassivière, we conclude that viruses are key agents for prokaryotic mortality and could influence the food web dynamics in humic lakes, which may ultimately depend on the internal cycling of resources and, perhaps, mainly on the allochthonous inputs and the associated humic substances.

Our conceptual understanding of the function and regulation of aquatic systems, from microbial to global biogeochemical processes, has changed with the discovery of the abundance and activities of viruses (7, 44, 56). Research over the past 2 decades has firmly established that viruses are the most abundant and diverse biological entities (3), thereby forming an integral component of the microbial food web in a great variety of aquatic environments (10, 29, 55). Viral lysis plays fundamental roles in cycling nutrients and organic matter (57), structuring the microbial food web dynamics (15), governing microbial diversity (49) and, to a lesser extent, by being a potential food source for protists (17). The distribution of viruses is known to be determined by factors that affect the activity and density of the host populations, mainly prokaryotes (14, 33). Reports have suggested that on average 10 to 40% of the prokaryotic production is lysed by viruses in both marine and freshwaters (48, 55) and, at times, can match grazing by bacterivorous protists as a source of prokaryotic mortality (14, 33). This is a significant departure from the traditional view that predation and resource availability are the main factors controlling prokaryotic abundance and production in pelagic systems.

Studies on the factors that may control the distribution of virioplankton on large spatial scales are limited (13), especially for freshwater lakes. As lakes are characterized by steep changes in environmental gradients over depth, most of the studies on viral ecology have focused on the vertical spatial variability (11, 16, 37) rather than trophic gradients (13). The apparent positive relation reported between viral lytic infection and the trophic state of aquatic systems is based more upon extrapolation than on direct measurements (12, 14). Few investigations conducted in Swedish lakes have revealed a very consistent relationship between lake trophic status and virus-induced prokaryotic mortality. Moreover, it has also been suggested that virus-induced mortality may be more important in oligomesotrophic than in eutrophic lakes (4, 47). Therefore, existing data on the relationship between lake trophic status and virus-induced prokaryotic mortality are inconsistent.

Besides trophic status, another important characteristic of the lakes is the humic content. Food webs in humic lakes are known to function differently than those in clear water lakes due to the so-called "reversed microbial loop" (*sensu* [22]) and have unusual microbial pathways (46). Humic lakes, which are traditionally viewed as unproductive environments, are often

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TABLE 1. Locations and morphometric characteristics of the studied lakes

Parameter	Vassivière	Pavin	Aydat
Location	45°48′51″N, 01°51′09″E	45°29′41″N, 02°53′12″E	45°39′48″N, 02°59′04″E
Altitude (m)	650	1,197	825
Origin	Human-made	Volcanic	Volcanic
Trophic status	Mesotrophic	Oligomesotrophic	Eutrophic
Humicity	Humic	Nonhumic	Nonhumic
pH	Moderately acidic (6.5)	Alkaline (9.1)	Alkaline (9.2)
Maximum depth (m)	25	92	15
Water circulation	Holomictic	Meromictic	Holomictic
Lake surface area (ha)	1,000	44	60
Lake storage capacity (10^6 m^3)	106	23	4.1
Watershed area (ha)	7,600	50	3,000
Catchment/lake area ratio	7.6	0.8	49.8

characterized by low levels of inorganic nutrients and photosynthetic activity (22). However, such systems are often supported by high levels of prokaryotic secondary production and biomass through increased inputs of high concentrations of dissolved organic matter from allochthonous inputs, which force the system to net heterotrophy (23). Several studies of humic lakes have focused on the nutrient limitation (22) and grazing loss of bacterioplankton (21). However, published reports on viral abundance and phage infection in such environments are limited (25, 47).

Although a body of data is now available on the significance of viruses to prokaryotic mortality in aquatic systems, it is largely unclear as to which factors will determine their importance, specifically, in humic lakes. In the present study, a comparison of a mesotrophic humic lake (Vassivière) with an oligomesotrophic lake (Pavin) and a eutrophic lake (Aydat), all located in the French Massif Central, was carried out on a time series scale to determine the interactions of viruses with other microbial components, together with physicochemical parameters. In addition to the above parameters, depth-related variability in viral and prokaryotic parameters was also examined, but only in Lake Vassivière during the stratified summer period. The main aim of the present investigation was to evaluate the dynamics of bacterioplankton, virioplankton, and phage-infected bacterioplankton in a humic lake on a time series scale and to discuss these findings in light of those found in nonhumic lakes of the same geographical region. As humic lakes are classified as nutrient-poor environments (dystrophic), we hypothesized that viral abundance and infection rates would be lower than in productive nonhumic lakes (4, 37). The present study sought to uncover the environmental factor(s) responsible for the variations in viral abundance, infection rates, and burst size in humic lakes and bring out the relative importance of virus-induced versus the potential grazer-induced prokaryotic mortality.

MATERIALS AND METHODS

Study sites. Samples were collected from three freshwater lakes, namely, the mesotrophic humic Lake Vassivière, the oligomesotrophic Lake Pavin, and the eutrophic Lake Aydat, which differed in watershed characteristics but were located in the same regional area, the French Massif Central. The characteristics of the studied lakes are presented in Table 1. Unlike Lake Pavin, both Lakes Vassivière and Aydat receive a high input of organic matter from terrestrial sources. While the humic Lake Vassivière is surrounded by peat bog, wetland, heath lands, and forests of pine, oak, and beech, Lake Aydat is largely surrounded by intensive agricultural lands. There is no riverine inflow in Lake Pavin, and the watershed essentially consists of beech forests.

Sampling. During all sampling occasions, integrated water samples, representative of the whole euphotic zones, were obtained from Lakes Vassivière, Pavin, and Aydat. Water samples from the lakes were collected every month from April to December 2007. All samples were collected manually at the deepest central point of the lakes, by using a flexible plastic tube (diameter, 4 cm) provided by a rope connecting the weighted bottom of the tube with a surface manipulator. Analytic samples were thus considered integrated samples representative of the euphotic layers of the lakes. In addition, water samples from Lake Vassivière were collected with a 10-liter Van Dorn bottle at different depths (including aphotic depths) of the water column (i.e., at 1, 3, 8, 10, and 19 m) during the stratified period (June, July, and August 2007) to determine the depth-related variability in the abundance of prokaryotes and viruses and the percentages of infected prokaryotic cells. All samples were collected in triplicate, i.e., from three independent sampling operations. Collected samples were immediately prefiltered through a 150-μm-pore-size nylon filter (to eliminate the predatory metazoan zooplankton) when poured into clean recipients previously washed with the lake water.

Physicochemical analyses. Water temperature and dissolved oxygen concentration were measured *in situ* with a WTW OXI 197 multiparametric probe. Secchi depth (Zs) measurements were used to estimate the euphotic depths (Zeu) in the sampled lakes, based on the general limnological assumption that Zs corresponds to the depth of approximately 10% of surface light. This assumption has been shown to be approximately correct in a variety of inland water bodies, especially during the ice-free period (34). Samples for nutrients, namely, total nitrogen and total phosphorus, were analyzed spectrophotometrically (1, 45). Total organic carbon (TOC) concentrations were determined by high-temperature combustion in an Apollo 9000 TOC analyzer set at 700°C and calibrated with standard additions of potassium hydrogen phthalate, with a precision of 0.2 μM (1). Chlorophyll *a* concentrations (Chl) were determined spectrophotometrically from samples (500 ml) collected on Whatman GF/F filters. Pigments were extracted in 90% acetone overnight in the dark at 4°C, and concentrations were calculated from SCOR UNESCO (40) equations. Nutrients and Chl concentrations were analyzed in the triplicate samples.

Abundances of prokaryotes and virus-like particles. For the measurements of virus-like particles (VLP) and prokaryotic abundances (PA), 50-ml water samples were fixed with 0.02-μm filtered buffered alkaline formalin (final concentration, 2% [vol/vol], from a 37% [wt/vol] solution of commercial formaldehyde). Subsamples (1 to 2 ml) were then filtered (<15-kPa vacuum) through 0.02-μm-pore-size Anodisc filters (Whatman, Maidstone, England), with 1.2-μm-pore-size cellulose acetate backing filters. After they were stained with SYBR green I fluorochrome (final dilution, 2.5×10^{-3}-fold; Molecular Probes Europe, Leiden, Netherlands) as described by Noble and Fuhrman (32), filters were air dried on absorbent paper and mounted between slides, and glass coverslips with the mountant glycerol–phosphate-buffered saline solution (Citifluor, London, United Kingdom) amended with a special antifading agent, i.e., ca. 20% (vol/vol) VectaShield (Vector Laboratories Inc., Burlingame, CA). This amendment significantly reduced fading of the fluorochrome and gave highly stable fluorescence (36). Slides were stored at −20°C and counted within a week using a model 300F epifluorescence microscope (Leica DC). Prokaryotes were distinguished from VLP on the basis of their relative size and brightness. A blank was routinely examined to control for contamination of the equipment and reagents.

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Lytic phage infection. Viral lytic infection was inferred from the percentage of visibly infected cells (VIC) according to the methods described by Pradeep Ram and Sime-Ngando (36). Prokaryotic cells contained in 8-ml formalin-fixed water samples were collected on copper electron microscope grids (400-mesh, carbon-coated Formvar film) by ultracentrifugation. Each grid was stained at room temperature (ca. 20°C) for 30 s with uranyl acetate (2% [wt/wt]), rinsed twice with 0.02-μm-filtered distilled water, and dried on a filter paper. Grids were examined using a JEOL 1200Ex transmission electron microscope 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 five viruses, identified by their shape and size, were clearly visible inside the host cell (51). At least 600 prokaryotic cells were inspected per grid to determine the percent VIC. Burst size (BS, in number of viruses prokaryote^{-1}) was estimated for every infected cell as the average number of viral particles in a minimum of 15 visibly infected prokaryotes. Because mature phages are visible only late in the infection cycle, the VIC counts were converted to the percentage of infected cells (IC) by using the following equation: IC = (9.524 × VIC) − 3.256 (53). Assuming that in steady state, infected and uninfected cells were grazed at the same rate and that the latent period equalled the prokaryotic generation time, the percent IC was converted to prokaryotic mortality (VIBM, as the percentage of prokaryotic production) using the equation VIBM = [IC + (0.6% IC^2)]/[1 − (1.2% IC)] (5).

Contact rate. The rate of contact (R) between viruses and prokaryotes was calculated by using the following formulae (30): (i) $R = (Sh2wDv)VP$, where Sh is the Sherwood number (1.06 for a prokaryotic community with 10% motile cells [57]), w is the cell diameter (calculated from the mean prokaryotic cell volume assuming that the cells are spheres), V and P are the abundances of viruses and prokaryotes, respectively, and Dv is the diffusivity of viruses; (ii) $Dv = kT/3dv$, where k is the Boltzmann constant (1.38×10^{-23} J K^{-1}), T is the *in situ* temperature (in degrees Kelvin), μ is the viscosity of water (in pascals per second, calculated from values given by Schwörbel [39] for temperatures in the range from 4 to 15°C), and dv is the mean (± standard deviation [SD]) diameter of the viral capsid, estimated at 55 ± 10 nm for Lake Pavin (11) and at 65 ± 15 nm for Lakes Vassivière and Aydat (A. S. Pradeep Ram, unpublished data). The contact rate was corrected for prokaryotic abundance to estimate the number of contacts per cell on a daily basis (50).

Heterotrophic nanoflagellate abundance and grazing potential in Lake Vassivière. Since time series data on heterotrophic nanoflagellates (HNF) and potential flagellate grazing rates (FG) are already available from previously published reports in Lakes Pavin and Aydat (4), the above-mentioned variables were determined only for Lake Vassivière in this study. Samples for the measurements of HNF abundance were fixed immediately after sampling with alkaline Lugol solution (final concentration, 0.5%) and decolorized with borate-buffered formalin (final concentration, 2%). Primulin-stained HNF were then collected on 0.8-μm polycarbonate black filters (25-mm diameter) and counted under UV excitation in a LEICA epifluorescence microscope (9). At least 20 microscopic fields and 200 HNF cells were counted per slide. To estimate the rate of potential bacterivory by HNF in Lake Vassivière, an approach based on the average flagellate clearance rate (1.9 nl individual^{-1} h^{-1}) obtained from published reports for freshwater lakes (25), was used for calculations. Potential HNF grazing rates (in cells ml^{-1} h^{-1}) were calculated as follows: *in situ* prokaryotic abundance × *in situ* HNF abundance × mean flagellate clearance rate (1.9 nl individual^{-1} h^{-1}).

Statistical analyses. Differences in physicochemical and biological variables between lakes and seasons (spring, April to June; summer, June to September; autumn, September to December) were tested by one-way analysis of variance (ANOVA). Interactions between sampled depths and months were tested in Lake Vassivière by two-way ANOVA. Linear regression analysis was used to test the relationship between heterotrophic nanoflagellate grazing and viral infection of prokaryotes and between chlorophyll concentrations and viral abundance. 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 Minitab software for Windows (release 12).

RESULTS

Water chemistry. The mean physicochemical and microbiological characteristics of the sampled euphotic zones of the lakes under study are listed in Table 2. Water temperature showed strong changes ($P < 0.01$) with sampled months in the euphotic zone of the three lakes, which were typical of temperate systems. The euphotic depth in Lake Vassivière ranged from 1.5 to 4.5 m with a mean Secchi value of 2 m and was generally well oxygenated (mean ± SD, 9.3 ± 2.4 mg liter^{-1}) during the entire study period. The three lakes differed significantly ($P < 0.01$) in terms of total nitrogen, organic carbon, and chlorophyll concentrations. The lowest concentration of total phosphorus (0.02 ± 0.009 mg liter^{-1}) and the highest concentration of total organic carbon (7.6 ± 2.1 mg liter^{-1}) were recorded in Lake Vassivière, where total organic carbon was significantly higher and varied significantly ($P < 0.001$) with time, compared to the other lakes (Table 2). No clear trend in Chl with sampled months was observed in Lake Vassivière, with the values being significantly lower ($P < 0.01$) than in Lake Aydat but higher ($P < 0.01$) than in Lake Pavin (Table 2).

TABLE 2. Physicochemical characteristics, chlorophyll *a* concentrations, and prokaryotic and viral parameters of Lakes Vassivière, Pavin, and Aydat for the euphotic depth integrated samples during the study period

Parameter	Mean (CV) finding		
	Vassivière	Pavin	Aydat
Temp (°C)	13.2 (44)	11.6 (43)	13.7 (39)
Dissolved oxygen (mg liter^{-1})	9.1 (25)	9.6 (20)	8.9 (18)
Total nitrogen (mg liter^{-1})	0.6 (53)	0.2 (25)	0.9 (51)
Total phosphorous (mg liter^{-1})	0.02 (54)	0.03 (35)	0.03 (45)
Total organic carbon (mg liter^{-1})	7.6 (35)	2.8 (28)	5.1 (41)
Chlorophyll *a* (μg liter^{-1})	10.8 (33)	3.9 (71)	17.9 (59)
Virus-like particle abundance (10^{10} liter^{-1})	1.9 (26)	2.3 (26)	5.0 (32)
Prokaryote abundance (10^9 cells liter^{-1})	5.7 (11)	3.0 (23)	5.9 (28)
Virus-to-prokaryote ratio	3.3 (19)	7.6 (13)	8.5 (37)
Percentage of infected cells	17.6 (27)	11.2 (49)	8.3 (42)
Virus-induced prokaryotic mortality (%)	25.6 (23)	15.3 (70)	12.2 (61)
Burst size (no. of viruses prokaryote^{-1})	17.0 (10)	34.5 (27)	37.2 (38)
Specific contact rate (contacts cell^{-1} day^{-1})	174 (29)	183 (26)	482 (37)
Heterotrophic nanoflagellates (10^6 cells liter^{-1})	1.5 (81)	ND[a]	ND
Flagellate grazing (10^6 prokaryotes liter^{-1} h^{-1})	16.8 (77)	ND	ND

[a] ND, not determined.

Standing stocks of prokaryotes and virus-like particles. We looked for evidence of time series variabilities in VLP and PA and the differences between the lakes under study. In Lake Vassivière, VLP and PA ranges were at 1.7×10^{10} to 2.6×10^{10} liter^{-1} and 4.3×10^9 to 6.5×10^9 cells liter^{-1}, with the highest values noted in May and June, respectively. Similar peaks were also noted in Lake Aydat, but in June and July, respectively, while in Lake Pavin both maxima were observed later in August (Fig. 1A and B). For the three lakes, the time series variabilities in VLP and PA were thus generally weak and nonsignificant, after excluding the two spring peaks noted in May and June for VLP in Lake Aydat (Fig. 1A). In spite of the significantly ($P < 0.001$) lower VLP in Lake Vassivière than in Lakes Pavin and Aydat, prokaryotic standing stock in Vassivière equaled that in Aydat and was even significantly

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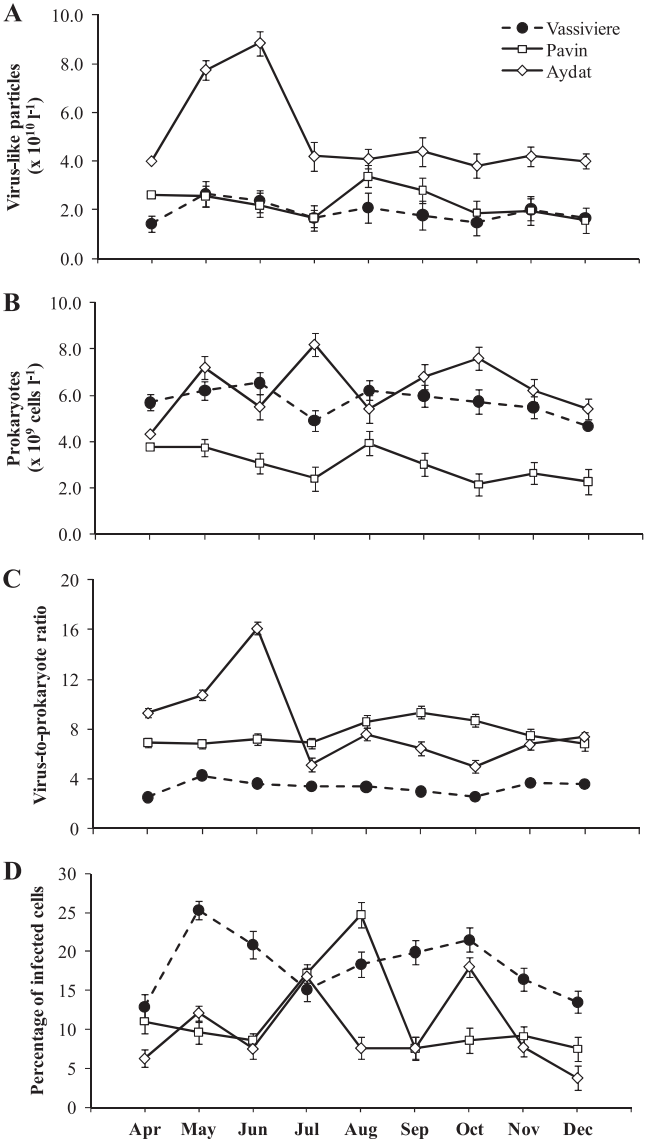

FIG. 1. Time series variations in the abundances of viruses (A) and prokaryotes (B), the virus-to-prokaryote ratio (C), and the percentage of infected prokaryotic cells (D) in the euphotic zones of Lakes Vassivière, Pavin, and Aydat. Error bars indicate standard errors of the means ($n = 3$).

higher ($P < 0.001$) than in Pavin (Table 2). Virus-to-prokaryote ratios in Lake Vassivière ranged from 2.6 to 4.3, with the average being significantly lower ($P < 0.003$) than in Lakes Pavin and Aydat (Table 2). VLP was significantly correlated to PA in Vassivière ($P < 0.001$) and in Pavin ($P < 0.001$), and it was significantly correlated to Chl in Pavin (Table 3).

Phage infection and burst size. The time series variability in the percentage of IC in Lake Vassivière varied over a range of 9.0% to 25.3%, with a mean value (18.0 ± 4.7%) that corresponded to 25.6 ± 8.9% of virus-induced prokaryotic mortality (i.e., VIBM). The maximum value of IC was observed in May, which coincided with the peak in VLP (Fig. 1A and D) and corresponded to a VIBM level of 41.9%. In contrast to VLP, the IC in Lake Vassivière was significantly ($P < 0.007$) higher

TABLE 3. Pearson's correlation coefficients for different variables in the euphotic zones of the studied lakes

Variable	Correlation coefficient with the second variable (r)[a]						
	Temp	Chlorophyll *a*	Prokaryote abundance	Virus-like particle abundance	% of infected cells	Flagellate grazing	Specific contact rate
Chlorophyll *a*	**0.71*****, *NS/NS*	**NS** 0.69***/NS					
Prokaryote abundance	**0.71*****, *0.74****/*0.52**	**NS** 0.74***/NS	**0.78*****, *0.87****/*NS*				
Virus-like particle abundance	**0.66*****, *0.61***/*NS*	**NS** NS/NS	**0.49***, *0.49*/*NS*	**0.71*****, *0.53*/*NS*			
Percentage of infected cells	**0.80*****, *0.49*/*0.48**	**NS**	**NS**	**NS**	**0.80*****		
Flagellate grazing	**0.57****	**NS**	**0.83*****, *0.88****/*NS*	NA	**0.72*****, *0.60***/*0.78****	**NS**	
Specific contact rate	**0.71*****, *0.66****/*NS*	**NS** *0.51*/*NS*	**NS**, *NS/NS*	**0.69*****, *NS/NS*	**0.69*****, *NS/0.61***	**NS**	**0.50***, *NS/0.83****
Burst size	**0.57****, *NS/NS*	**NS**, *0.59***/*NS*					

[a] Values for Lake Vassivière are indicated in boldface and in italics for Lakes Pavin/Aydat. Flagellate grazing was compared with other variables in Lake Vassivière only. Levels of significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant. NA, not applicable (autocorrelation).

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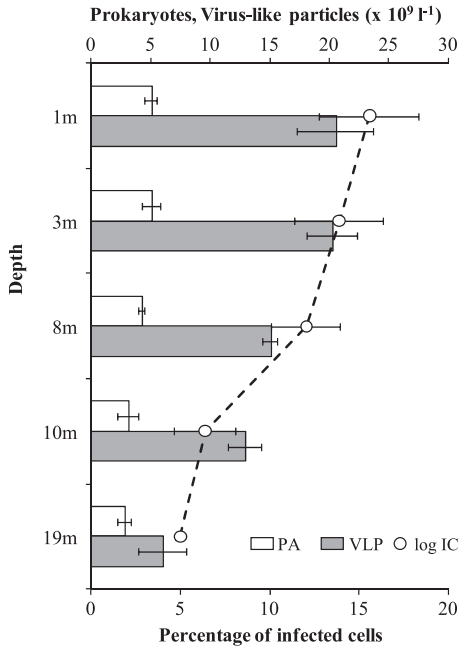

FIG. 2. Depth-related variability in the abundances of viruses (dark bars), prokaryotes (light bars), and the percentages of infected cells (dotted lines) during the stratified period in Lake Vassivière. Values correspond to means ± standard errors ($n = 3$), for samples collected in June, July, and August 2007.

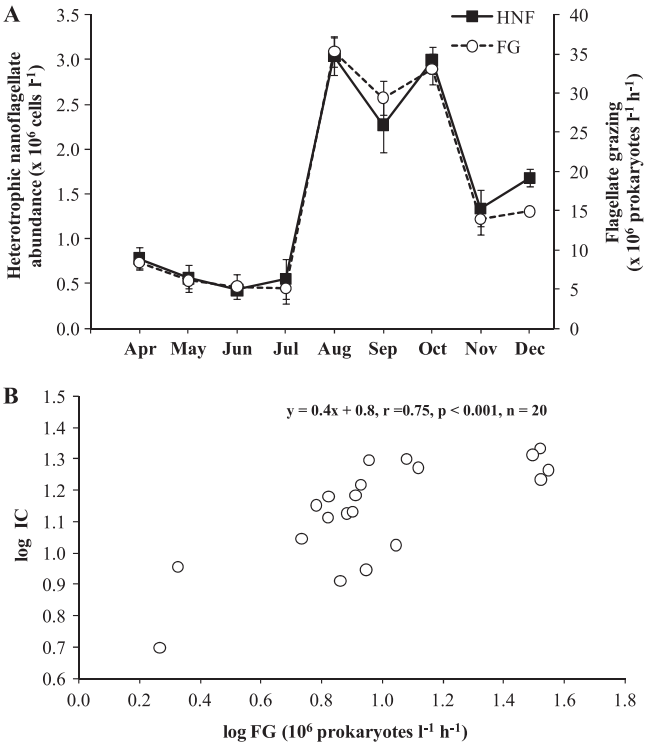

FIG. 3. Time series variability in the abundance (HNF) and grazing rate (FG) of heterotrophic nanoflagellates (A) and the relationship between FG and the percentage of prokaryotic cells infected with viruses (IC) (B) in Lake Vassivière.

than in Lakes Pavin (range, 6.3 to 24.7%; mean ± SD, 11.5 ± 5.4%) and Aydat (range, 3.8 to 18.0%; mean ± SD, 9.7 ± 4.8%) (Table 2; Fig. 1D). The IC was significantly correlated to VLP and PA in Lakes Vassivière and Pavin and to the water temperature in the three lakes (Table 3).

The mean number of intracellular viruses observed per infected cell in Lake Vassivière varied from 6 to 42 and averaged 17 ± 4 viruses prokaryote^{-1}, which was significantly lower ($P < 0.001$) than in the other sampled lakes. In Lakes Vassivière and Aydat, the variation observed for the IC was reflected in the BS, and both were significantly correlated ($P < 0.001$) to each other (Table 3).

In Lake Vassivière, data on VLP, PA, and IC during the stratification period (i.e., June, July, and August 2007) were pooled and are presented in Fig. 2. VLP, PA, and IC values were significantly higher ($P < 0.001$) in the euphotic (<5 m) than in the aphotic depths (5 to 20 m). During the above period, 2-way ANOVA indicated that both VLP [$F_{(2,4)} = 17.4$; $P < 0.001$] and IC [$F_{(2,4)} = 17.7$; $P < 0.001$] varied significantly with sampling month and decreased with depth. This contrasts with PA, for which the temporal-related (i.e., for the three summer months) and depth-related variability were low and not significant [$F_{(2,4)} = 0.12$; $P > 0.05$].

Contact rates. Theoretical contact rates between viruses and their potential prokaryotic hosts, which are necessary to quantify the rate of successful infection, were calculated according to the model of Murray and Jackson (30). In Lake Vassivière, the specific contact rate (i.e., the number of viruses encountering a single prokaryote per specified time) ranged between 84 and 253 and averaged 174 contacts cell^{-1} day^{-1} (Table 2), with a peak in June. Specific contact rates in Lake Vassivière were similar to those calculated for Lake Pavin (mean, 183 contacts cell^{-1} day^{-1}) but were significantly lower ($P < 0.001$) than in Lake Aydat (mean, 482 contacts cell^{-1} day^{-1}). Despite this, the IC was significantly higher ($P < 0.002$) in Lake Vassivière than in Lake Aydat (Table 2).

Heterotrophic nanoflagellates in Lake Vassivière. The abundance of HNF in Lake Vassivière was marked by two similar peaks of about 3.0×10^6 cells liter^{-1} in August and October (Fig. 3A) and varied significantly with season ($P < 0.003$). Flagellate grazing potential on prokaryotes, which was measured only in Lake Vassivière in the present study, showed large (coefficient of variation [CV], 77%) and significant ($P < 0.03$) variability by sample month, ranging from 4.3×10^6 to 35.3×10^6 prokaryote liter^{-1} h^{-1} (mean, 16.8×10^6 prokaryote liter^{-1} h^{-1}), with a maximum in August (Fig. 3A). Flagellate grazing potential was significantly correlated to IC, which was best described by a linear function ($\log y = 0.4x + 0.8$; $r = 0.75$, $P < 0.001$) (Fig. 3B). Flagellate grazing potential was also significantly correlated with the water temperature (Table 3).

Regression analyses. Forward stepwise multiple regression analysis using all the environmental variables, as provided in Table 2, was conducted using the time series data obtained in order to select the variables that significantly accounted for the variability in VLP and IC in the three lakes. Results indicated that PA was the lone significant predictor of VLP in Lake Vassivière (i.e., VLP = $0.50x^2 - 5.16x + 14.8$; $r^2 = 0.61$; $n = 18$). In Lake Pavin, Chl and PA were strong predictors for VLP [VLP = $-0.212 + 0.0965$(Chl) + 0.96(PA); $r^2 = 0.90$; $n = 18$].

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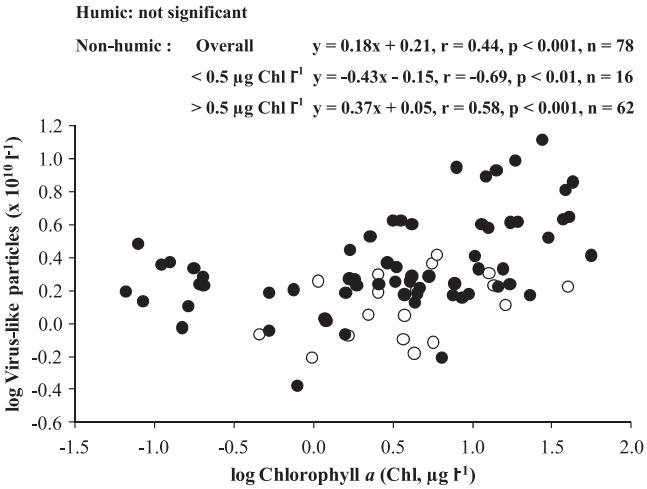

FIG. 4. Scatter plot of chlorophyll *a* concentration versus the abundance of virus-like particles in the humic Lake Vassivière (white circles) and in several other nonhumic lakes (dark circles) located in the same geographical areas. Data for the nonhumic lakes were from this study for Lakes Pavin and Aydat and our recent previous studies of Lakes Sep and Grangent (41).

In Lake Aydat, none of the measured variables accounted significantly in the variability in VLP. For IC, VLP along with temperature, BS, and PA were selected as significant ($P < 0.05$) predictor variables in Lake Vassivière [IC = −9.7 + 0.122(temperature) + 2.50(VLP) + 2.53(PA) + 0.417(BS); $r^2 = 0.68$; $n = 18$]. Similar variables was also found to predict IC in Lake Aydat, with the exception of temperature [IC = −21.9 + 2.96(PA) + 0.644(BS); $r^2 = 0.95$; $n = 18$). In Lake Pavin, the measured variables failed to significantly predict IC.

Relationship of VA with Chl, as an indicator of trophic status of an ecosystem, was determined for both humic and nonhumic lakes using the data from the present and previously published studies of lakes in the same geographical region. Scatter plots suggested that VLP was strongly positively correlated ($P < 0.001$) to Chl in nonhumic lakes only, with an upper threshold Chl concentration of 0.5 μg Chl liter^{-1} (Fig. 4). The inverse was observed when Chl concentration was <0.5 μg Chl liter^{-1}. No significant correlation was observed between Chl and VLP in Lake Vassivière.

DISCUSSION

Time series abundances. Among freshwater systems, our knowledge on viral activities in humic lakes is limited, and the present investigation is one of the few that documents the time series standing stock of viruses and phage infection in relation to environmental parameters in the pelagic realm. Our time series on viral and prokaryotic variables collected in the upper euphotic zone of Lake Vassivière revealed known and new features regarding the potential links between viruses and other microbial components, i.e., in the general context of aquatic viral ecology. The virioplankton abundances in Lake Vassivière were within the previously reported ranges of values for temperate lakes (48, 55), including those from humic lakes in Sweden (2, 47). A prominent feature was the significant correlation between PA and VLP, which so far has not been established in humic lakes (25, 47). PA alone explained 78% of the variance in VLP, which suggested that PA is a good predictor of VLP in Lake Vassivière, as prokaryotes are known to be the main hosts for pelagic viruses (32, 38). With the exception of temperature, none of the measured abiotic variables was able to explain the variations in viral parameters in Lake Vassivière (Table 3). Although the abundances of both viruses and prokaryotes in Lake Vassivière were rather homeostatic, as they did not vary more than 2-fold (Fig. 1A and B), similar to those reported by Mathias et al. (27) for the backwater systems of the River Danube, by Hennes and Simon (19) for the mesotrophic Lake Constance (Germany), by DeBruyn et al. (13) for Lake Erie (United States and Canada), and by ourselves in the eutrophic Lake Grangent in France (37). Unlike in Lakes Pavin and Aydat, the virus-to-prokaryote ratio in Lake Vassivière was relatively constant and was at the lower end of the range (i.e., 3 to 10 viruses prokaryote^{-1}) reported for other pelagic environments (48). This corroborates similar findings by Vrede et al. (47), who conducted a comparative study of Swedish humic lakes and clear water lakes.

The brown water color of Lake Vassiviére indicated its dissolved organic matter was mainly composed of terrestrial-derived humics, which are intrinsically refractory and therefore less prone to rapid prokaryotic incorporation. Such substances have a high capacity to absorb the light energy required for photosynthesis (42). This explains the general low primary production in Lake Vassivière (<15 mg m^{-3} h^{-1}), where an incident light energy in the surface waters as low as <20 μmol s^{-1} m^{-2} has been recorded (28). Under such conditions, prokaryotes have a competitive advantage over light-limited phytoplankton to harness inorganic nutrients, which could thus help to explain the high prokaryotic abundance in Lake Vassivière. Moreover, the inflow of labile organic carbon from terrestrial inputs could also equally help to sustain high prokaryotic activity (43). Although studies pertaining to the effect of dissolved humic substances on prokaryotic activity and its impact on prokaryotic community structure have been carried out in freshwater systems (8, 20), the influences of humic substances on viral infection and production have received less attention. Earlier reports suggested that viruses can be negatively influenced by binding to humic substances (2). We believe such inactivation could likely affect more the numerical abundance of viruses rather than their lytic activity in Lake Vassivière, which is one of the original findings of the present study.

Phage infection and burst size. Recent studies have suggested that viral lytic infection contributes significantly to the bulk of prokaryotic mortality in aquatic ecosystems, and the VIC (expressed as the percentage of total prokaryotic cells) is a measure of the magnitude of this process (16, 38). We used the transmission electron microscopy (TEM) method (i.e., whole-cell approach) for the determination of the VIC, which provided direct evidence of phage infection. As most of the literature to date on VIC and burst size are derived from TEM-based estimates (18, 48), comparisons among aquatic systems are relatively easy. In our study, a minimum of 500 to 800 cells were examined for 1.3 to 3.0% visibly infected cells in Lake Vassivière, which was comparable to the typical range of VIC (i.e., <5%) reported for limnetic systems (48, 55), including humic environments (30, 52). However, the VIC in Lake

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Vassivière was significantly higher than in Lakes Pavin and Aydat, in contrast with the virus-like particle abundance (Table 2). We consider that this was not an artifact due to methodological problems, because the same approaches were applied in the different lakes tested. The comparatively high infection rate in Lake Vassivière agreed well with the high prokaryotic standing stock, similar to those observed in Swedish humic lakes (47). Lymer et al. (25) also emphasized the relatively higher importance of viruses as agents of prokaryotic mortality in humic than in clear water lakes in an investigation of a set of 21 Swedish lakes with differing trophic statuses. Such trends occurring in humic lakes suggest that when the labile substrates are in short supply for prokaryotic production, viral lysis might represent an important source of dissolved organic substrates and inorganic nutrients. This is supported by the coupling between prokaryotes and both virus-like particle abundance and infection rate in Lake Vassivière (Table 3). It is also likely that lytic infection is prevalent over lysogeny in Lake Vassivière, based on the finding that the two viral lifestyles often are mutually exclusive (i.e., from negative correlations) in pelagic systems, where high host abundances generally favor lytic infection (33).

The BS level reported across lakes in this study was relatively stable, irrespective of trophic status or humic content. This contrasts with other studies where BS estimates were found to be higher in productive systems where both cell size and growth were generally greater than in oligotrophic environments (35). TEM observations revealed that, in Lake Vassivière, BS estimates were indeed lower than the values (mean around 34 viruses prokaryote^{-1}) reported for freshwater systems (48), which could be explained when lytic phages have short latent period due to the short generation time of hosts.

Infection paradox. The high viral lytic production in Lake Vassivière was not reflected in the low VLP or the related contact rates (Table 3), which is a paradox. One of the possible reasons for the low ambient viruses is the consistently low viral burst size in Lake Vassivière, as discussed above. In addition, viral particles are good candidates for absorption to humic substances (24), which are well known as complex natural heterogenous substances with acidic functional groups (COOH) that are reaction sites on the molecule (42). The trend of low VLP and virus:prokaryote ratio arising due to high prokaryotic standing stock can also be the result of the growth of phage-resistant populations within prokaryotic communities (31, 58). It is important to note that even if the specific contact rate (SCR) in Lake Vassivière (mean, 174 contacts cell^{-1} day^{-1}) was lower than in Lake Aydat (mean, 482 contacts cell^{-1} day^{-1}), the rates still were considerably high to represent a major factor for prokaryotic mortality.

Depth-related variability in Lake Vassivière. The significant decreases in both VLP and IC with depth during the stratified summer period in Lake Vassivière are similar to a recent finding in the eutrophic Lake Grangent, located in the same regional area (37). This contrasts with deep stratified lakes, such as the meromictic Lake Pavin, France (11), and the moderately hypersaline Mono Lake, CA (6), where VLP abundance and activity are higher in deeper than in surface waters, due to dramatic differences in environmental gradients, with persistent anoxic bottom waters. VLP abundance and infection rates were clearly higher in the photic than in the aphotic zones of Lake Vassivière, contrasting with the vertical distribution in PA, which also decreased with depth, but this was low and nonsignificant. However, these variables were tightly coupled to each other on a vertical basis ($r > 0.70$; $P < 0.05$), suggesting that the viral attack in the two regimens could be rather dependent on the density of the susceptible host populations, rather than to the density of the whole host community.

Heterotrophic nanoflagellates and potential grazing estimates in Lake Vassivière. The potential F observed in Lake Vassivière (mean, 16.8×10^6 prokaryotes liter^{-1} h^{-1}) was higher than previous reports for oligomesotrophic Lake Pavin (mean, 3.8×10^6 prokaryotes liter^{-1} h^{-1}) and eutrophic Lake Aydat (mean, 10.4×10^6 prokaryotes liter^{-1} h^{-1}) (5). Among the sampled lakes, data from Lake Vassivière indicated a strong correlation between IC and FG, suggestive of synergistic interactions between lysis and grazing activity relative to their prokaryotic resources, which agrees with our recent experiments in nutrient-limited freshwater microcosms from the same geographical area (36). In addition, consistent with our results, the IC tended to be high in the Rimov Reservoir (South Bohemia, Czech Republic) when FG rates were high (41). These data might suggest that viral infectivity increases with increasing grazing activity of HNF, because grazers provide substrates to uninfected prokaryotes (36). Such trophic cascading cycling of substrates and nutrients was suggested to be of major importance under oligotrophic conditions (36), likely including light-limited humic lakes. Under high grazing pressure, grazing-resistant forms of prokaryotes (e.g., filamentous and floc-forming prokaryotes) become abundant in freshwater lake communities (41). Some studies have reported that these grazing-resistant forms of prokaryotes are more susceptible to viral infection, presumably because there is a trade-off between grazing resistance and viral resistance (36, 52). This hypothesis needs to be tested in future studies. Comparison of prokaryotic losses resulting from protistan predation and viral lysis in aquatic systems is often calculated and expressed in terms of the percentage of prokaryotic production (4). Since prokaryotic production was not measured in our study, we could not directly estimate and compare prokaryotic mortality between the two sources. Given the fact that VIBM and FG were high, especially during the summer period, both viruses and predators could have contributed to the bulk of prokaryotic mortality rates. Lymer et al. (25) suggested that flagellate grazing appeared to be more important for prokaryotic mortality than viral contribution, as inferred from a survey conducted in a set of 21 boreal lakes in Sweden along the trophic gradient, including humic lakes similar to Lake Vassivière.

Humic content versus trophic status. Studies examining a larger data set (e.g., studies based on regression analysis of reported values) have revealed that viral and prokaryotic abundances are significantly correlated to each other, which is in turn ultimately dependent on the levels of primary production (4). Investigation of this possibility is also important for elucidating the link between trophic conditions, humic content, and viral parameters in humic lakes. It has been proposed that trophic status is a possible driving force in controlling the spatial distribution of viruses, the rationale being that eutrophic environments support a higher standing stock of prokaryotes and consequently of viruses, compared to oligotrophic systems (54). In this study, the investigated lakes displayed

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clear gradients in chemical and biological parameters. In order to draw comparisons between humic versus nonhumic lakes with respect to the trophic state control hypothesis (12), data on VLP and infection rates from the present study and from the previous published reports in regional lakes located in the French Massif Central were plotted against Chl, considered an index of trophy. Scatter plots indicated that both VLP and infection were not correlated to Chl in the humic Lake Vassivière, which suggests that prokaryote-virus interactions could largely be forced by exogenous supplies of organic carbon. In nonhumic systems, VLP was positively correlated to Chl. This followed an apparent trend with a clear increase in VLP along the trophic gradient only from 0.5 μg Chl liter^{-1}. Below this value, the relationship was negative (Fig. 4), suggesting that phytoplankton-derived resources could force prokaryotic growth to attain a certain threshold level at which the host availability is sufficient to boost the lytic proliferation of viruses. A similar positive virus versus trophy pattern has been previously observed in the northern Adriatic Sea (52) and in nonhumic Swedish lakes (25). However, contrasting reports exist in wchih VLP does not seem to be related to the trophic status, such as in Quebec lakes in Canada (26) and in the Adriatic Basin (Mediterranean) (12). The VIC levels in Lake Vassivière were within the range which covers almost the entire span of previously published data obtained from a number of systems of different trophic status. Although studies have stressed the importance of viral infection in oligo- versus eutrophic systems (4, 47), such an apparent trend in viral infection from oligo- to eutrophic systems, derived from estimates from different temperate lakes in the same regional location (Table 3), was not observed in our comparative analysis for nonhumic lakes. We therefore conclude that in our study systems, humic content prevailed over the ecosystem productivity which, alone, appeared to be a poor predictor of the level of viral infection in freshwater lake ecosystems.

Conclusions. This study provides original data on viruses, with a focus on one site (i.e., the humic Lake Vassivière) that offers unique peculiarities, with few available data in the literature. The methodological approaches used were those commonly applied in aquatic viral ecology, providing a good basis for comparative ecology. Although VLP abundance and the virus-to-prokaryote ratio in Lake Vassivière were lower than in the productive Lake Aydat, data from visibly infected cells provided concrete evidence that viruses were important agents for prokaryotic mortality. This indicated that the impact of viruses on the food web dynamics of humic lakes might be substantial, which may ultimately depend on internal cycling of resources and/or on allochthonous inputs. The paradox between the low occurrence of viruses and high infection rates in Lake Vassivière was mainly related to low burst size estimates, which were characteristic of this ecosystem, in which humic content apparently prevailed over the trophic status in constraining microbial communities. Based on the substantially high level of viral infection rates in Lake Vassivière, we reject our initial hypothesis that viral infection is of minor importance in humic lakes, which are traditionally viewed as unproductive environments often characterized by low levels of inorganic nutrients and photosynthetic activities. Clearly, because Lake Vassivière may not be fully representative of the humic lakes in the world, additional data on viral ecology are needed for humic lakes where, for example, the nature of the association between viruses and humic substances indeed deserves further investigation to precisely determine the interactions between viruses and prokaryotic diversity.

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