

NOTE

Low consumption of virus-sized particles by heterotrophic nanoflagellates in two lakes of the French Massif Central

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ABSTRACT: Seasonal and depth-related variability in the grazing activity of heterotrophic nanoflagellates (HNF) on viruses was examined in the oligo-mesotrophic Lake Pavin and in the eutrophic Lake Aydat, French Massif Central, between May and November 2000. Ingestion rates (IR) were determined using 50 nm diameter fluorescent microspheres, as virus analogues. In both lakes, highest grazing activities on virus-sized particles were recorded in the metalimnion, at the beginning and the end of the thermal stratification period. Estimated IRs in Lake Pavin (mean = 0.4 viruses HNF⁻¹ h⁻¹, CV = 38.0%) and in Lake Aydat (mean = 0.3 viruses HNF⁻¹ h⁻¹, CV = 35.6%) were not significantly different, in contrast to clearance rates (CR), which were significantly higher in the oligomesotrophic (2.3×10^{-2} nl HNF⁻¹ h⁻¹) than in the eutrophic lake (0.7×10^{-2} nl HNF⁻¹ h⁻¹). CRs for viruses were correlated with CRs for bacteria in Lake Aydat but not in Lake Pavin, suggesting a greater abundance within the HNF assemblages of virus-sized particle feeders in the less productive lake. We estimated that 4.1 and 0.8% of viral production were grazed by HNF in Lake Pavin and Lake Aydat, respectively. Finally, although viruses seem to represent a minor food source for HNF (i.e. compared to bacteria), they may not be inconsequential in their diet, especially in oligotrophic lakes.

KEY WORDS: Lakes · Viruses · Heterotrophic nanoflagellates · Microbial food web · Predation

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INTRODUCTION

With abundances typically surpassing 10^7 particles ml⁻¹ in both fresh and marine waters, viruses are now recognized as a key component in structuring the aquatic microbial food web (Fuhrman 1999, Wommack & Colwell 2000, Sime-Ngando et al. 2003, Weinbauer 2004). Although viruses potentially infect all aquatic organisms, prokaryotes and eukaryotic phytoplankton are doubtless their major hosts, whose entire production may be occasionally controlled by these pathogens (Noble & Fuhrman 2000, Fischer & Velimirov 2002).

Furthermore, evidence is accumulating that viruses are also involved in nutrient cycling (Middelboe & Lyck 2002), influence bacterial and algal diversity (Šimek et al. 2001, Mann 2003, Weinbauer & Rassoulzadegan 2004) and contribute to the demise of phytoplankton monospecific blooms (Bratbak et al. 1993, Suttle 2000, Brussaard 2004).

With sizes commonly comprising between 20 and 200 nm (i.e. the diameter of the capsid), aquatic viruses consequently contribute to the DOM pool and may potentially represent a food source for small grazers. On the basis of this consideration, González & Suttle

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(1993) investigated grazing on viruses and virus-sized particles by natural assemblages and cultures of marine nanoflagellates using both fluorescent microspheres and labelled viruses. Despite the fact that bacteria are undoubtedly the principal constituent in the diet of heterotrophic nanoflagellates (HNF) in most aquatic systems (Sanders et al. 1992, Sherr & Sherr 1994), González & Suttle (1993) showed that viruses can also contribute significantly to the nutrition of HNF and that being consumed is one possible route for the decay of viroplankton in addition to degradation by solar radiation (Suttle & Chen 1992, Wilhelm et al. 1998), by heat-labile substances (Noble & Fuhrman 1997), or by adsorption to particulate organic matter (Kapuscinski & Mitchell 1980).

However, this novel microbial trophic link (i.e. viruses > HNF) has not been explored further as González & Suttle's (1993) work remains the only report on this topic so far. In the present study, we attempt to further examine this trophic link by investigating, for the first time in freshwater, HNF consumption of fluorescent latex virus-sized spheres. By investigating the seasonal variability in HNF 'virivory' in 2 lakes of distinct trophic status, we can confirm the ability of phagotrophic nanoflagellates to graze on virus-sized particles and discuss the significance and ecological implications of this activity.

MATERIALS AND METHODS

Samples were collected from 2 lakes of the French Massif Central differing in trophic status. Lake Pavin is a dimictic, meromictic oligo-mesotrophic lake located at 1197 m altitude and characterized by a maximum depth of 92 m and a small surface area (44 ha). Lake Aydat is also small (surface area: 60 ha; maximum depth: 15 m) and is a eutrophic, dimictic lake located at 825 m altitude. Water samples were collected monthly from April to November 2000 using a 10 l Van Dorn bottle. In both lakes, triplicate samples were collected from a central location during the period of thermal stratification at 3 depths located in the epilimnion, metalimnion, and hypolimnion: 5, 10, and 35 m in Lake Pavin and 1, 5, and 12 m in Lake Aydat. Dissolved oxygen concentrations and temperature profiles were determined *in situ* using an Oxycal-SL probe.

The temporal and vertical variations in the concentrations of chlorophyll *a*, as well as those of viral-related parameters (abundance, production, lytic activity, burst size) have been described in detail (cf. Bettarel et al. 2003, 2004).

HNF grazing on viruses was estimated using tracer particles and epifluorescence microscopy (González & Suttle 1993). Primarily, a stock solution of tracer parti-

cles was prepared from a concentrated suspension of 50 nm diameter Fluoresbrite plain microspheres (Polysciences) and protein-coated in 5 mg ml⁻¹ albumin solution for 24 h, to avoid clumping of the particles (Pace & Bailiff 1987). Before *in situ* grazing experiments, the tracer solution was diluted with 0.02 µm pore-size filtered lake water, and microspheres were added to 120 ml of lake water in acid-washed glass bottles (triplicates) and incubated at *in situ* depth. Microbeads were added to the samples at a final concentration of 0.5 × 10⁷ ml⁻¹ to account for approximately 10 to 20% of the viroplankton standing stock. Preliminary time-series experiments showed that 50 nm bead uptake by HNF was relatively linear during the first 15 to 20 min. Thereafter, we suspect that particles were egested. Thus, incubations at each sampling depth (epi-, meta-, and hypolimnion) were stopped after 20 min by adding ice-cold glutaraldehyde (2% final concentration). We acknowledge that egestion of a fraction of indigestible particles may happen sooner (i.e. 2 to 3 min) after ingestion (e.g. Boenigk & Arndt 2002). To determine the uptake of beads by phagotrophic HNF, triplicate 10 ml subsamples of each of the glutaraldehyde-fixed samples were filtered onto 0.8 µm pore-size Nuclepore filters, stained with primulin and examined via epifluorescence microscopy (Caron 1983). UV excitation was used to visualize 200 to 300 HNF per sample (×1.250), while blue-light excitation was used to enumerate beads present in the food vacuoles inside the flagellates. The ingestion rate (IR, virus HNF⁻¹ h⁻¹) of individual HNF was calculated by multiplying the mean number of beads ingested per protozoan per hour by the natural virus-to-bead ratio. Clearance rate (CR, 1 HNF⁻¹ h⁻¹) was calculated by dividing IR by natural viral abundance. Grazing rate (GR, virus l⁻¹ h⁻¹) was calculated as follows: GR = IR × [HNF]. Finally, the removal rate of viruses from HNF grazing (expressed in percentage of viral production, VP), was determined by dividing VP (calculated in Bettarel et al. 2004) by GR. In order to examine affiliation between HNF grazing on viruses and bacteria, we herein refer to HNF bacterivory (estimated using 500 nm fluorescent latex microbeads) reported in Bettarel et al. (2004) for the same sampling event.

RESULTS AND DISCUSSION

Methodological aspects

The use of 50 nm diameter beads as virus analogues may underestimate predation on viruses. For example, discrimination of particles in the spectrum of virus size

range has been clearly demonstrated by González & Suttle (1993), who reported that HNF can show preferences for fluorescent labelled natural viruses (FLV) concurrently with 50 nm latex beads. However, each of our attempts to use the FLV method failed because of the weak optical signal from FLVs through HNF food vacuoles in our freshwater samples. Furthermore, the standard size of the latex beads used (50 nm diameter) represents the average viral capsid size in both lakes, since about 70% of viruses in Lake Aydat and Lake Pavin were smaller than 60 nm diameter (authors' unpubl. data). Overall, because the surface properties of virus capsids and latex beads differ, we are aware that the estimated IR, GR and CR may be underestimates, and thus should be extrapolated to nature with some care.

HNF consumption of viruses

The level of HNF grazing on viruses was, on average, not significantly different in Lake Pavin (mean = 3.2×10^6 viruses $l^{-1} h^{-1}$) than in Lake Aydat (mean = 2.9×10^6 viruses $l^{-1} h^{-1}$) (Table 1). However, for both lakes, the lowest GR, along with the lowest flagellate abundances, were always recorded within the hypolimnion (Bettarel et al. 2003). In Lake Aydat, this layer was anoxic between July and October, while temperatures remained constantly low (mean = $4.00 \pm 0.05^\circ\text{C}$) within the hypolimnion of Lake Pavin (see Fig. 1 in Bettarel et al. 2004). Both anoxic conditions and low temperatures have been shown to limit flagellate proliferation (Sherr et al. 1988, Weinbauer & Höfle 1998). In contrast, viruses were grazed at higher rates in the metalimnion of the 2 lakes, where like their bacterial hosts, they are significantly more numerous (Bettarel et al. 2003). It is thus tempting to believe that virivorous organisms, like bacterivorous ones, should be able to optimize their food uptake by exploiting patches of high prey abundances.

Seasonally, peaks in GR occurred at the beginning and at the end of the period of thermal stratification in both lakes (Fig. 1), along with the maxima in bacterial, viral, and protistan abundances and activities (Bettarel et al. 2004). Interestingly, although HNF IR were similar in both lakes, individual CR were significantly higher in Lake Pavin (mean = 2.3×10^{-2} nl HNF $^{-1} h^{-1}$) than in Lake Aydat (mean = 0.7×10^{-2} nl HNF $^{-1} h^{-1}$). In the productive Lake Aydat, where bacteria and viruses are abundant, viruses may not represent an important component of HNF diet and may have been ingested passively along with the prokaryotic prey. Conversely, in Lake Pavin, the relatively low bacterial abundances may drive HNF to actively include viruses in their daily diet.

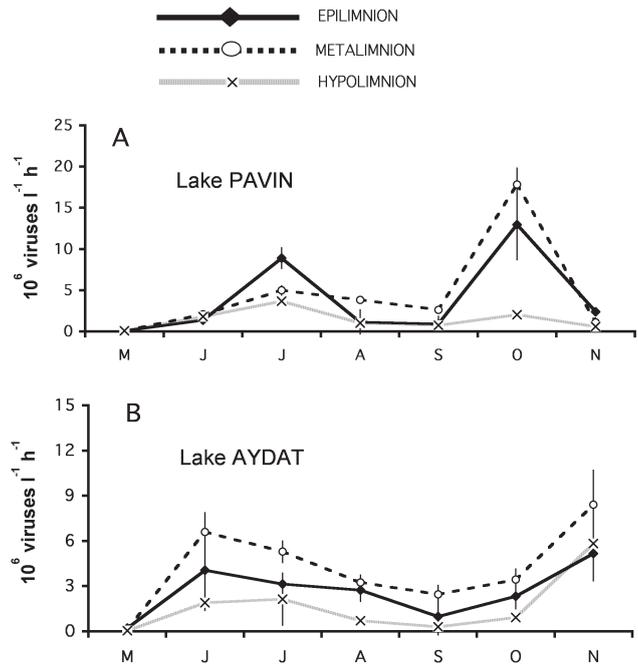


Fig. 1. Temporal and vertical variations of heterotrophic nanoflagellates (HNF) grazing rates on viruses in (A) Lake Pavin and (B) Lake Aydat

HNF grazing: virus vs bacteria

The scenario described above does not seem unreasonable since seasonal CR estimated from ingestion of virus analogues (50 nm beads) were more highly correlated with those of bacterial analogues (500 nm beads) in Lake Pavin ($r_{\text{epi}} = 0.13$, $r_{\text{meta}} = 0.06$, $r_{\text{hypo}} = 0.14$, $n = 7$) than in Lake Aydat ($r_{\text{epi}} = 0.36$, $r_{\text{meta}} = 0.81$, $r_{\text{hypo}} = 0.95$, $n = 7$). This might indicate that viruses and bacteria were grazed by the same nanoflagellate community in Lake Aydat, while in Lake Pavin this community may include more specific grazers on virus-sized particles, as already hypothesized by González & Suttle (1993). However, our observations clearly contrast with those of González & Suttle (1993), who reported a strong positive relationship between IR on virus-sized particles and their concentration, thus suggesting that the contribution of viruses to the nutrition of nanoflagellates is proportionally much greater at high viral densities. Although their conclusions are valuable for a given community, such may be not the case for all flagellate assemblages. The present study suggests that HNF grazers on virus-sized particles may occur preferentially in oligotrophic systems in which viruses may potentially represent a substantial food source. Further experiments are needed to confirm whether enough nutrition can be gained from viruses to maintain a population of virivory specialists under oligotrophic conditions.

Table 1. Mean values (CV) for samples taken from Lake Pavin and Lake Aydat from May to November 2000, in the epilimnion (E), the metalimnion (M), and the hypolimnion (H). FB⁵⁰/FB⁵⁰⁰: ratio between clearance rates obtained from 50 and 500 nm fluorescent beads. HNF: heterotrophic nanoflagellates. Pavin/Aydat: ratio between mean values obtained in Pavin with those obtained in Aydat

Depth	Grazing rates (10 ⁶ viruses l ⁻¹ h ⁻¹)	Ingestion rates (cell HNF ⁻¹ h ⁻¹)	Clearance rates		Removal rates (% viral production)
			(×10 ⁻² nl HNF ⁻¹ h ⁻¹)	FB ⁵⁰ /FB ⁵⁰⁰ (%)	
Lake Pavin					
E	4.0 (115.8)	0.5 (92.2)	2.1 (129.2)	11.9	4.8
M	4.6 (120.3)	0.6 (100.6)	2.2 (100.2)	11.5	3.6
H	1.0 (67.2)	0.2 (45.0)	2.7 (157.6)	10.3	3.8
Mean	3.2 (50.1)	0.4 (38.0)	2.3 (10.6)	11.2 (6.2)	4.1 (12.3)
Lake Aydat					
E	2.7 (59.3)	0.3 (75.7)	0.6 (65.4)	3.1	1.0
M	4.2 (60.5)	0.5 (109.1)	0.9 (90.6)	4.1	1.2
H	1.7 (108.6)	0.2 (133.4)	0.5 (118.3)	2.5	0.3
Mean	2.9 (36.6)	0.3 (35.6)	0.7 (23.3)	3.2 (20.4)	0.8 (46.3)
Pavin/Aydat	1.1	1.4	3.3	3.5	5.1
ANOVA:					
<i>F</i>	0.09	1.39	5.28		
df	41	41	41		
<i>p</i>	0.767	0.24	0.027		

Our calculated CR in Lake Aydat, determined using 50 nm diameter beads, represented only 3.2% of those with 500 nm beads, versus 11.2% in Lake Pavin (Table 1). For comparison, González & Suttle (1993) reported that CRs of natural HNF assemblages feeding on viruses and virus-sized particles were only about 4% of those from HNF bacterivory.

Impact of grazing on viral losses

This study provides the first estimates of viral losses from HNF predation, with removal rates from HNF grazers averaging 4.1 and 0.8% of viral production in Lake Pavin and Lake Aydat, respectively. We suggest that viruses are probably of higher nutritional significance for HNF in oligotrophic systems than in productive ones. Nonetheless, despite the plausible underestimation of grazing activity on viruses from the latex bead method, phagotrophy by nanoflagellates is of minor importance as a loss process for natural viroplankton communities.

Higher contributions of flagellate grazing to bacterial mortality were also recorded in Lake Pavin (mean = 37.7% of bacteria production) than in Lake Aydat (mean = 18.5%) (Bettarel et al. 2004). These results agree with the assumption that the control of bacterial production by protistan predation is higher in pelagic environments with low productivity (Strom 2000). However, more studies are unquestionably needed to confirm the higher contribution of HNF to viral mortality in low-productivity microbial loop dominated systems. With HNF responsible for less than 5% of viral

mortality, the largest fraction of viral production in both lakes was surely controlled by factors other than flagellate predation. Besides lysogenic conversion, solar radiation (mostly UV-B wavelengths) has been identified as a prominent virucidal agent in aquatic systems (Noble & Fuhrman 1997, Wilhelm et al. 1998, Fuhrman 1999), but temperature and heat-labile particulate material may also cause viral decay (Moebus 1992, Suttle & Chen 1992).

Nutritional significance of viruses: ecological implications

According to González & Suttle's (1993) calculations of the amount of carbon (C), nitrogen (N) and phosphorus (P) contained per single virus and bacterium, we estimated that ingested viruses would constitute only 0.56, 0.71 and 1.42% of C, N and P, respectively, in the total diet of HNF in Lake Pavin, and only 0.13, 0.20, and 0.39% in Lake Aydat. Given mean virus:bacteria ratios of 7.1 and 9.0 reported in Lake Pavin and Lake Aydat, respectively (Bettarel et al. 2003), this clearly suggests that viruses are not as energetically valuable as bacteria in the HNF diet. González & Suttle (1993) hypothesized that a virus:bacteria ratio >50 would render viruses a significant source of nutrients to nanoflagellates. However, one should keep in mind that, as with bacterioplankton, 'patches' of viruses likely occur with burst events.

Finally, our study supports González & Suttle's (1993) conclusions that being grazed by protists is one possible fate for viruses in aquatic systems. In addition,

our results suggest that although viruses would represent a diminutive food source for HNF (i.e. compared to bacteria), they still may not be inconsequential for the HNF diet, primarily in systems with low productivity. These results also extend the current concept of the viral loop, where the fraction of the viroplankton that is grazed by nanoflagellates does not participate in bacteria infection and is also paradoxically confiscated from the pool of DOM available for bacteria. This study strengthens the ecological key role of flagellates in aquatic systems and confirms the existence of a presumed new link between HNF and viruses in the functioning of aquatic microbial food webs. A question that now emerges is to what extent does ingestion of viruses by HNF represent a source of infection for these protists?

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