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Genotypic, size and morphological diversity of virioplankton in a deep oligomesotrophic freshwater lake (Lac Pavin, France)

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ABSTRACT

We examined changes in morphological and genomic diversities of viruses by means of transmission electronic microscopy and pulsed field gel electrophoresis (PFGE) over a nine-month period (April–December 2005) at four different depths in the oligomesotrophic Lac Pavin. We found that the majority of viruses in this lake belonged to the family of *Siphoviridae* or were untailed, with capsid sizes ranging from 30 to 60 nm, and exhibited genome sizes ranging from 15 to 45 kb. On average, 12 different genotypes dominated each of the PFGE fingerprints. The highest genomic viral richness was recorded in summer (mean = 14 bands per PFGE fingerprint) and in the epilimnion (mean = 13 bands per PFGE fingerprint). Among the physico-chemical and biological variables considered, the availability of the hosts appeared to be the main factor regulating the variations in the viral diversity.

Introduction

It is now widely accepted that viruses are the most abundant entities in aquatic ecosystems, where their abundance is about an order of magnitude greater than the number of bacteria (Weinbauer, 2004; Suttle, 2007). Viruses are ubiquitous entities in natural environments, infecting virtually every living form of life, from cellular prokaryotes to animals and plants. A given host probably has a range of different co-infectious viruses, resulting in enhanced gene exchanges between phages and hosts (Moineau et al., 1994). For example, it was suggested that bacteria and their co-infecting phages function as 'phage factories' and produce a variety of chimeric or mosaic phages that increase viral diversity (Ohnishi et al., 2001). Undoubtedly, environmental viruses are the largest reservoir of genetic diversity on the planet (Suttle, 2005, 2007). For viruses there are no universal molecular markers that are shared among all viruses, similar to molecular RNA genes for cellular organisms, that could be used to assess their diversity (Hendrix et al., 1999). In aquatic environments, only genes belonging to specific groups or families can be targeted (Culley et al., 2003). This renders the study of global viral diversity difficult by PCR-based methods. In addition to the use of transmission electronic microscopy (TEM) for estimating viral diversity based on phenotypic traits (Auguet et al., 2006; Brum and Steward, 2010; Liu et al., 2006; Weinbauer, 2004; Wommack and Colwell, 2000), metagenomic and other molecular techniques have been developed and extensively used (Angly et al., 2006; Breitbart et

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al., 2002; Hambly and Suttle, 2005; Williamson et al., 2008; Winget and Wommack, 2008; Wommack and Colwell, 2000). Among these, pulsed field gel electrophoresis (PFGE) is based on size fractionation of intact DNA and has the advantage of allowing easy and rapid estimation of the whole-genome fingerprint of natural communities of viruses (Steward and Azam, 2000; Wommack et al., 1999a, 1999b).

Results obtained with this method have showed that between 7 and 35 bands can be distinguished by applying PFGE to individual samples, as a minimum viral richness, with a dominance of <100 kb genomes in both marine (Jamindar et al., 2012; Weinbauer, 2004) and freshwater plankton (Auguet et al., 2006; Filippini and Middelboe, 2007; Tijdens et al., 2008). Some studies have shown that viral genomic diversity, determined from PFGE and the relative intensity of individual bands, can vary seasonally (Castberg et al., 2001; Jamindar et al., 2012; Larsen et al., 2001, 2004; Sandaa and Larsen, 2006; Wommack et al., 1999b; Zhong et al., 2014) and spatially (Auguet et al., 2006; Filippini and Middelboe, 2007; Jiang et al., 2004; Riemann and Middelboe, 2002; Steward et al., 2000; Tijdens et al., 2008; Wommack et al., 1999a). However, little is known about the factors controlling these variations, because only rare works have simultaneously studied the viral genomic composition (VGC) and the microbial community structure and environmental factors. A few authors have hypothesized that VGC changes with the structure of host communities (Auguet et al., 2006; Steward et al., 2000; Tijdens et al., 2008). Sandaa and Larsen (2006) have shown that seasonal changes in viral community composition can be related to changes in the abundances of cyanobacteria, autotrophic nano- and picoeukaryotes and heterotrophic bacteria, whereas Riemann and Middelboe (2002) and Tijdens et al. (2008) reported no clear link between temporal changes in VGC and bacterial community composition and/or phytoplankton abundance. With the exception of the work of Tijdens et al. (2008), the few data available were conducted in marine waters, but without taking into account phytoplanktonic species and protistan flagellates. Potential links between VGC and microbial community structure thus remain to be established, because there are many routes of interaction between viruses and microbial communities (Pradeep Ram and Sime-Ngando, 2008; Sime-Ngando and Pradeep-Ram, 2005). For example, in addition to representing a potential host reservoir for viruses (Massana et al., 2007), protistan flagellates could also graze bacteria and/or viruses (Bettarel et al., 2005), thereby influencing directly or indirectly the viral diversity (Weinbauer, 2004). Viral lytic or lysogenic activity could also affect VGC (Weinbauer, 2004). Parada et al. (2008) suggested that viral richness changes at time scales of hours to days linked with the lysing of specific bacterioplankton phylotypes. Since the genetic exchanges are probably higher among lysogenic than among lytic phages, the diversity of lysogenic phages is important (Chen and Lu, 2002), and higher viral diversity could be recorded during lysogen induction events. However, at the community level, no study to our knowledge has considered simultaneous changes in VGC together with lytic and lysogenic activities and with potential grazers as well. Finally, Jiang et al. (2004) have shown that VGC changed with oxygen conditions in the hypersaline Lake Mono (California, USA). Weinbauer (2004) suggested that certain physico-chemical parameters may define the niches for phages and thus influence viral diversity.

Many biological or physico-chemical factors could thus potentially affect VGC, but very few attempts have been made to study these effects, leaving open many questions of ecological interest concerning the dynamics of the biodiversity within virioplankton communities and the related environmental forcing factors. In this study, we have investigated the spatio-temporal dynamics of viral community diversity (based on genomic and morphological characteristics) and activity (lytic and lysogenic), concurrently with physico-chemical parameters (temperature, oxygen, chlorophyll *a*) and microbial community variables (bacteria, picocyanobacteria, autotrophic picoeukaryotes, autotrophic and heterotrophic nanoflagellates). We sought empirical evidence of the potential connections between the diversity of viruses and both abiotic and biotic environmental factors, at consistent seasonal and depth scales in aquatic systems. This study complements companion papers in which the standing stock of virus and microbial communities (Colombet et al., 2009) as well as grazing and viral activities (Colombet and Sime-Ngando, 2012) were examined.

1. Experimental procedure

1.1. Study site and sample collection

Samples were collected in Lake Pavin (altitude 1197 m), a meromictic and dimictic oligomesotrophic lake located in the French Massif Central, that experiences partial overturn. It is a typical crater mountain lake characterized by a maximum depth of 92 m and low surface (44 ha) and catchment (50 ha) areas. A characteristic feature of the physical structure of Lake Pavin is the existence of an oxic/anoxic interface (*i.e.*, oxycline) between 50 and 60 m depth. Samples were collected monthly (systematically between 09:00 am and 10:00 am) from April to December 2005 from a central location in the lake by using an 8-L Van Dorn bottle. Four different layers were sampled, corresponding to the epi- (5 m), meta- (12 m) and hypolimnion (30 m), and to the oxycline (57 m) (Colombet and Sime-Ngando, 2012). This was done for all biological variables, except for picocyanobacteria and autotrophic picoeukaryotes, which were determined in the epi-, meta-, and hypolimnion. All analysis was performed in triplicate, except for genomic size profiling of samples by pulsed field gel electrophoresis.

1.2. Virioplankton concentration

For the diversity study of viruses, viral concentrates are required. The strategy employed for viral concentration was based on the use of polyethylene glycol (PEG), an approach that we have recently described in detail in Colombet et al. (2007). Following successive prefiltration steps, 20 L samples were filtered with 0.2 μm pore size filters. Viruses from the filtrate were concentrated by ultrafiltration, using a high performance concentration/diafiltration system (Model DC 10LA, Amicon®, Epernon, France) equipped with a reusable hollow fiber cartridge with a 30-kDa cut-off (Amicon®, Epernon, France). Concentrated viruses in the retentate were then precipitated by 'pegylation', *i.e.*, PEG-treated. Polyethylene glycol 8000 (Catalog No. 81,268; Sigma, Saint-Louis, USA)