Ecol Res (2014) 29: 271–287
DOI 10.1007/s11284-013-1121-2

ORIGINAL ARTICLE

X. Zhong · F. Rimet · S. Jacquet

Seasonal variations in PCR-DGGE fingerprinted viruses infecting phytoplankton in large and deep peri-alpine lakes

Received: 16 October 2013 / Accepted: 16 December 2013 / Published online: 10 January 2014

Abstract Double-stranded DNA viruses infecting eukaryotic algae (e.g., phycodnaviruses) and cyanobacteria (e.g., cyanophages) are now recognized as widespread and ubiquitous in aquatic environments. However, both the diversity and functional roles of these viruses in fresh waters are still poorly understood. We conducted a year-long study in 2011 of the community structure of planktonic virus groups in the upper lit layer of two important freshwater natural ecosystems in France, Lake Annecy (oligotrophic) and Lake Bourget (oligo-mesotrophic). Using PCR-DGGE to target a number of different structural and functional signature genes, i.e.,*g20*, *g23*, *psbA*, *polB*, and *mcp*, the phytoplankton viruses were shown to display temporal and spatial variability. There were marked seasonal changes in community structure for all viral groups in Lake Bourget, but only for T4-like myoviruses and *psbA*-containing cyanophages in Lake Annecy. The multivariate statistical analyses revealed that (1) various environmental factors can directly or indirectly explain the community structure observed for each phytoplankton viral group, and (2) temporal patterns of T4-like myovirus community structure were similar between the two lakes. In general, our results (1) suggest that the observed algal virus patterns were associated with significant shifts in phytoplankton biomass and/or structure, which in turn were shaped by the abiotic environment, and (2) support the Bank model proposed by Breitbart and Rohwer (Trends Microbiol 13:278–284, 2005). This study provides new evidence that freshwater lakes contain a significant diversity of algal viruses, and that the distribution of these viruses strongly mirrors that of their hosts.

Keywords Lakes · Phytoplankton · Cyanophages · Phycodnaviruses · T4-like phages · Community structure

Introduction

Phytoplankton comprises both autotrophic prokaryotes (e.g., cyanobacteria) and eukaryotic microalgae. Concentrated in the upper lit surface waters of both marine and freshwater ecosystems, they harvest solar energy and produce half of all organic matter on earth. They are responsible for fueling nearly all heterotrophic processes (Karl 2007; Boyce et al. 2010), including remineralization by bacteria, as one of the main pathways in surface waters (Kirchman et al. 2009). Phytoplankton also supports complex microbial-based food webs in aquatic environments; they are the main prey for organisms from higher trophic levels, from unicellular zooplankton all the way up to metazoans (Karl 2007).

Phytoplankton community structure is regulated by a large set of environmental factors, among which biotic interactions (such as predation and lysis) may be particularly important (Brussaard 2004; Kagami et al. 2007; Chambouvet et al. 2008). In the 1990s, it was shown that phytoplankton biomass or primary productivity can be significantly reduced due to viral infection (Suttle et al. 1990; Proctor and Fuhrman 1990). Further evidence of this effect has come from studies showing the significant role of viruses in algal bloom control or termination (Nagasaki et al. 1994; Jacquet et al. 2002; Gastrich et al. 2004). It is now widely recognized that viruses are a main cause of phytoplankton mortality and that they mediate the flow of nutrients and energy towards higher trophic levels by, for instance, diverting a significant portion of available organic matter to the dissolved pool. This process, referred to as the "viral shunt" (Wilhelm and Suttle 1999), may dissipate up to 25 % of the carbon initially fixed during photosynthesis (Suttle 2007). Although different mechanisms of resistance have been developed by phytoplankton populations (e.g., Thomas et al. 2012),

Electronic supplementary material The online version of this article (doi:10.1007/s11284-013-1121-2) contains supplementary material, which is available to authorized users.

X. Zhong · F. Rimet · S. Jacquet (✉)
INRA, UMR CARRTEL, 75 Avenue de Corzent,
74203 Thonon-les-Bains Cedex, France
E-mail: sjacquet@thonon.inra.fr
Tel.: +33-4-50267812
Fax: +33-4-50260760

algal viruses still impact, to varying degrees, the dynamics, diversity and structure of phytoplankton communities. As well, particular fluctuations in abiotic factors are likely to exert a strong control on virus–host interactions.

To date, work on isolating and characterizing viruses infecting phytoplankton has focused mainly on the double-stranded-DNA viruses (Nagasaki and Bratbak 2010; Short 2012). The dsDNA viruses infecting eukaryotic phytoplankton, known as phycodnaviruses, infect a variety of eukaryotic microalgae, including Chlorophyta, Dinophyta, Haptophyta, and Heterokonta. They belong to a viral category referred to as nucleocytoplasmic large DNA viruses (NCLDVs) and have a polyhedral capsid, no tail or envelope, and large dsDNA genomes ranging from 160 to 560 kb (Wilson et al. 2009). By contrast, both ssRNA and dsRNA viruses infecting phytoplankton are generally associated with hosts from a specific group or species, such as diatoms, raphidophytes, dinoflagellates, or prymnesiophytes. These viruses have not been as well studied as their dsDNA counterparts (e.g., Brussaard 2004; Nagasaki et al. 2004; Tomaru et al. 2008), although the work of Culley and colleagues clearly suggested they are important in terms of both diversity and abundance (Culley et al. 2006, 2007; Steward et al. 2013). The viruses infecting prokaryotic phytoplankton are known as cyanophages. These dsDNA viruses belong to three families with differing tail morphology (Suttle 2000; Mann 2003): the Myoviridae are T4-like phages and have a long contractile tail, the Podoviridae are T7-like phages and sometimes have a short contractile tail, and the Siphoviridae are lambda-like phages and have a long, non-contractile tail. To date, the most studied group in aquatic ecosystems has been the Myoviridae (Breitbart et al. 2002; Clokie et al. 2010).

To study the diversity of viruses, different molecular tools (e.g., metagenomic, pulsed-field gel electrophoresis, PCR-associated approaches) have been used. Unlike prokaryotes or eukaryotes, in which the 16S rDNA or the 18S rDNA gene is conserved, there is no universal genetic marker in viruses and so the assessment of virus diversity using PCR or PCR-based approaches has to resort to group-specific gene markers (Short et al. 2010). The markers *polB* and *mcp*, which encode for DNA polymerase and the major capsid protein, respectively, have been used to examine phycodnavirus diversity in both marine and freshwater systems (Chen et al. 1996; Short and Suttle 2002, 2003; Short and Short 2008; Larsen et al. 2008; Clasen and Suttle 2009; Park et al. 2011; Gimenes et al. 2012). The T4-like portal-protein-encoding gene *g20* has been used extensively to examine cyanomyovirus communities in a variety of both marine and freshwater ecosystems (Fuller et al. 1998; Wilson et al. 1999; Zhong et al. 2002; Marston and Sallee 2003; Frederickson et al. 2003; Dorigo et al. 2004; Wang and Chen 2004; Short and Suttle 2005; Mühling et al. 2005; Wilhelm et al. 2006; Wang et al. 2010; Parvathi et al. 2012; Clasen et al. 2013; Zhong and Jacquet 2013a). By contrast, cyanopodoviruses have been studied only in marine environments, and only recently (Chen et al. 2009; Huang et al. 2010; Dekel-Bird et al. 2013), mainly using the DNA polymerase gene *polA*. Data is also lacking for cyanosiphoviruses, even though several genomes of these cyanophages are now available for the Mediterranean Sea, the Atlantic Ocean, and Chesapeake Bay (Sullivan et al. 2009; Huang et al. 2012; Mizuno et al. 2013; Ponsero et al. 2013). Other genetic markers, such as host-derived auxiliary metabolic genes (Breitbart et al. 2007; Goldsmith et al. 2011) (mainly *psbA,* which encodes the photosystem II D1 protein, but also *psbD*, which encodes the D2 protein of the photosystem II, or *phoH*, which encodes a phosphate-starvation-inducible protein), have also been used for studies in a variety of environments (Sullivan et al. 2006; Sharon et al. 2007; Sandaa et al. 2008; Chénard and Suttle 2008; Wilhelm and Matteson 2008; Wang et al. 2009; Goldsmith et al. 2011; Clasen et al. 2013; Zhong and Jacquet 2013a). Finally, the *g23* gene, which encodes the major capsid protein, can be used to identify T4-like myoviruses (including cyanomyoviruses), and several studies have been proposed for either marine or freshwater environments (Filée et al. 2005; Butina et al. 2010, 2013; López-Bueno et al. 2009; Huang et al. 2011; Jamindar et al. 2012; Bellas and Anesio 2013; Zheng et al. 2013).

The microbial and viral ecology of western European peri-alpine lakes (and fresh waters in general) have been poorly investigated. For viruses, studies have largely focused on phages, and algal viruses (mainly cyanophages) have only been investigated on a few occasions in these ecosystems (Dorigo et al. 2004; Duhamel et al. 2006; Personnic et al. 2009; Parvathi et al. 2012). Recently, we showed the prevalence of a variety of algal virus signature genes in two peri-alpine lakes, Annecy and Bourget (France), and attempted to assess algal virus diversity (e.g., Zhong and Jacquet 2013a, b). However, the community structure dynamic of various algal virus groups has not been provided yet. We therefore decided to use PCR-DGGE with five different primer-sets targeting different groups of phytoplankton viruses, the objective being to more fully describe algal virus community dynamics. We also quantified a number of biotic (e.g., host) and abiotic factors in order to study their influence on algal virus community structure. Our aims were to (1) uncover the community dynamics of different viral groups infecting phytoplankton, (2) compare the observed patterns between two ecosystems located in the same eco-region but characterized by different trophic states and phytoplankton communities, and (3) relate viral community structure dynamics to host diversity or abundance and abiotic variables.

Materials and methods

Sample collection and processing

Water samples were collected once or twice each month between January and November 2011 from Lake Annecy and Lake Bourget. For each lake, the samples were

taken at a single reference station (corresponding to the place where the limnological monitoring survey is performed) located at the deepest part of the lake (e.g., 45.8727°N, 6.1645°E for Lake Annecy and 45.94167°N, 6.0305°E for Lake Bourget). We obtained 14 samples for Lake Annecy, and 18 for Lake Bourget. We collected >20 l, integrating the water column from the surface to a depth of 20 m using an electric pump and tubing. The water was stored in a polycarbonate flask in the dark at 4 °C until filtration. A few hours after sampling, we filtered the 20-l samples through a 60-µm mesh to remove large particles, and then through 1-µm filters (Millipore, Bedford, MA, USA). The filtrate (<1-µm fraction) was concentrated to a volume of 200–250 ml using several Millipore spiral-wound ultrafiltration cartridges along the year (up to 4 for each lake and always used for the same lake in order to prevent cross-contamination, and the cartridge was cleaned before and after each application using hot 0.1 N NaCl solution and sterile Milli-Q water) with a molecular weight cutoff of 30,000 Da (regenerated cellulose, PLTK Prep/scale TFF, 1 ft2; Millipore). Finally, we filtered the <1-µm concentrated fraction through 0.45-µm filters (Millipore) twice. We then stored 10-ml aliquots of this <0.45-µm viral concentrate (VC) at −20 °C until further processing.

Physicochemical and biological variables

Classical relevant limnological parameters for assessing lake characteristics and better understanding plankton dynamics were measured (Wetzel 2001; Jacquet et al. 2012b). Nutrient concentrations (total nitrogen, N-NH_4, N-NO_3, SiO_2, P-PO_4, and total phosphorus) were measured at each station following standard AFNOR protocols (details are available at http://www.afnor.org). We used a conductivity, temperature, and depth measuring device (Sea-Bird SEB 19 SEACAT Profiler) and a chlorophyll (Chl) fluorescence fluoroprobe (BBE Moldenke, Germany) to obtain vertical profiles for water temperature, pH, conductivity, and dissolved oxygen and chlorophyll *a* concentrations.

Phytoplankton cells were enumerated using optical microscopy as described in Rimet et al. (2009). The cyanomyoviruses were enumerated using qPCR carried out on the viral concentrate (VC) using the non-degenerate primer set CPS4/5 (Wilson et al. 1999). The 20-µl qPCR reaction contained 1× QuantiTect SYBR Green PCR Master Mix (Qiagen), 0.5 µM of each primer, and 2 µl of the viral concentrate. The thermal cycling was conducted in a Rotor-Gene 3000 (Corbett Research) with the following program: 5 min denaturing at 95 °C, followed by 45 cycles of denaturing at 95 °C for 30 s, annealing at 48 °C for 30 s, and extension at 72 °C for 30 s. Nine tenfold serial diluted standards (ranging from 9×10^{-1} to 9×10^{7} molecules) were run in duplicate along with two no-template control reactions containing 2 µl of nuclease-free water. The plasmid standards contained a cloned *g20* gene amplified from environmental samples, which was linearized by a restriction digest of EcoR I, purified by the illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Waukesha, WI, USA), and quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The (correct) size of the amplicon (i.e.,165 bp) was checked using gel-electrophoresis, and the melting curves also confirmed that the fluorescence signal corresponded to a single-sized DNA fragment. The qPCR amplification efficiency was 1.003 for the cloned amplicons (with $r > 0.99$, $n = 9$). The calculation of concentrations was based on the assumption that cyanomyoviruses contained only one *g20* gene copy per particle, following Sandaa and Larsen (2006).

We counted the virus-like particles, heterotrophic prokaryotes (mainly bacteria), picocyanobacteria [represented by the phycoerythrin-rich (PE-rich) *Synechococcus* spp. (Personnic et al. 2009)], using a FACSCalibur flow cytometer (Becton-Dickinson) equipped with an air-cooled laser set at 15 mW and 488 nm. Prior to the FCM analysis, the virus-like particles (VLPs) were fixed with a 0.5 % glutaraldehyde solution (grade I, Merck) for 30 min in the dark, then diluted in 0.02-µm filtered TE buffer (0.1 mM Tris–HCl and 1 mM EDTA, pH 8), and incubated with SYBR Green I (at a final 5×10^{-5} dilution of the commercial stock solution; Molecular Probes) for 5 min at ambient temperature, followed by 10 min at 75 °C, and then another 5 min at room temperature (procedure based on Brussaard (2004) and modified by Personnic et al. (2009)). We also obtain heterotrophic bacterial counts on fixed samples that were diluted in 0.02-µm filtered lake water sample and then incubated with SYBR Green I (10^{-4} dilution of the commercial stock solution) for 15 min. For photosynthetic cells (i.e.,the picocyanobacteria), we used no fixative or fluorochrome. Instead, we used fresh samples to which we added a suspension of 1-µm beads (Molecular Probes). We transferred the FCM listmode files to a PC and analyzed them using the custom software CYTOWIN (Vaulot 1989). More details about the FCM analysis and data treatment can be obtained in Marie et al. (1999, 2000).

PCR amplification and DGGE

In order to detect a majority of the sequences present in the environment and to avoid any possible interference of the GC clamp on natural samples, we conducted the PCR in two stages as previously described (Short and Suttle 2002; Sandaa et al. 2010). Briefly, the first PCR stage using the (treated) VC as template was conducted with the primer set in the absence of the GC clamp. The second PCR was then performed on the product of the first stage PCR, using the GC-clamp-containing primer set (i.e., with a 40-nt GC clamp attached to the 5′ of the forward primer). The PCRs were performed using a TProfessional Thermocycler (Biometra, Göttingen,

Table 1 List of primers and optimized conditions used in this study

Gene markers	Targeted viruses	Encoded proteins	Primers (5′–3′)	Annealing temperature (°C)	DGGE gradient (%)	References
g20	Cyanomyovirus	T4-like portal protein	CPS 1.1: GTAGWATWTTYTAYATTGAYGTWGG CPS 8.1: ARTAYTTDCCDAYRWAWGGWTC	46	25–50	Sullivan et al. (2008)
g23	T4-like myovirus	Major capsid protein	MZIAlbis: GATATTTGIGGIGTTCAGCCIATGA MZIA6: CGCGGTTGATTTCCAGCATGATTTC	50	45–70	Filée et al. (2005)
psbA	Cyanophage	Photosystem II protein D1	Pro-psbA-lF: AACATCATYTCWGGTGCWGT Pro-psbA-lR: TCGTGCATTACTTCCATACC	50	40–60	Sullivan et al. (2006)
polB	Phycodnavirus	Family B DNA polymerase	AVS 1: GARGGIGCIACIGTIYTIGAYGC AVS 2: GCIGCRTAICKYTTYTTISWRTA	51	40–70	Chen et al. (1996)
mcp	Phycodnavirus	Major capsid protein	mcp Fwd: GGYGGYCARCGYATT mcp Rev: TGIARYTGYTCRAYIAGGTA	45	45–70	Larsen et al. (2008)

Germany) with 25 µl of reaction mix containing 1× PCR buffer, 4 mM $MgCl_2$, 200 µM of each dNTP, 0.4 µM of each primer (Table 1), 0.5 U of Platinum® Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) and 1 µl of (treated) VC (containing approximately 10^6 VLPs). Prior to the PCR with the *psbA* gene, the VC was treated with DNase (0.002 unit DNase I for 1 µl VC, Sigma, St. Louis, MO, USA) to remove environmental free DNAs and thus avoid the potential amplification of *psbA* sequences from free DNAs released from cellular hosts. The removal of host DNAs from PCR templates was checked by verifying negative amplification of 16S rRNA gene in a polymerase chain reaction using universal primers (i.e.,Eubacteria-specific primer set 358F/907rM; Berdjeb et al. 2011). For PCR conducted with the *polB* and *mcp* genes, VCs were treated using the freeze–thaw method described by Short and Short (2008), which consists of three repetitions of heating for 3 min at 95 °C followed by freezing at −20 °C until the liquid becomes solid (see the Supporting Information and Fig. S1).

The typical program for the first-stage PCR was a 15-min virion lysing and denaturation at 95 °C followed by 34 cycles of denaturation at 95 °C for 30 s, annealing for 30 s, extension at 72 °C for 45 s, and a final extension at 72 °C for 5 min. The program for the second-stage PCR was a 5-min denaturation at 95 °C, followed by 24 cycles of denaturation at 95 °C for 30 s, annealing for 30 s, extension at 72 °C for 45 s, and a final extension at 72 °C for 5 min. The annealing temperatures for each primer set were tested and optimized as summarized in Table 1.

The DGGE was conducted in a 6 % polyacrylamide gel with an optimized linear denaturing gradient (100 % denaturant is defined as 7 M urea and 40 % deionized formamide). The linear denaturing gradient was optimized and determined for each set of amplicons (Table 1). Approximately 200 nanograms of amplicons (estimated using gel densitometry) from the second-stage PCR (for samples of all marker genes) were loaded into wells. Electrophoresis was carried out for 16 h in 1× TAE buffer (pH 7.4) (40 mM Tris-base, 20 mM sodium acetate, 1 mM EDTA) at 120 V and a constant temperature of 60 °C using the CBS-DGGE 2000 system (C.B.S. Scientific Co., San Diego, CA, USA). The gels were stained in a 30-ml TAE solution (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.4) containing 2X SYBR Green I (10,000×, Molecular Probes, Invitrogen) for 45 min before visualizing on a UV transilluminator (Tex-35 M, Bioblock Scientific) and photographing with GelDoc (Bio-Rad, Hercules, CA, USA). Due to the limited number of wells in our DGGE device, we ran samples in two gels (one for Lake Annecy and the other for Lake Bourget for each marker gene). To standardize the banding pattern in each gel, a pooled sample (corresponding to equal volumes of all obtained samples from the two lakes mixed together) was loaded in the middle and each side of the gels. To identify the DGGE bands shared by the two lakes, in addition to the pooled sample, the samples of the other lake (those possessing typical distinct banding pattern) were also loaded at the same time in the DGGE migration to serve as a reference. It is noteworthy here that, for each marker gene, the reproducibility of the observed patterns was checked through the repetition of DGGE runs giving the same results (i.e., band number, position, and relative intensity). At last, it is noteworthy that we confirmed that the bands did indeed correspond to expected viral DNAs by isolating, cloning, and sequencing a representative of each band type (Zhong and Jacquet 2013a, b, 2014).

DGGE banding pattern analysis and statistical analysis

The DGGE banding patterns were analyzed using the GelCompare II software package (Applied Maths, Kortrijk, Belgium) after digitalization of the DGGE

gels. We selected the bands manually, one by one, and not by using the automatic function of the software, since we noticed that the automatic selection of bands could lead to underestimation. Two operators repeated twice this operation. Similarities between samples were estimated by Jaccard's similarity index based on the absence or presence of bands for which positions were discriminated unambiguously with a 1 % tolerance. The UPGMA method was used to construct the dendrogram from a binary matrix of similarity values.

We used the Mantel test applying Spearman's rank correlation coefficient as the test statistic and 999 permutations to test the null hypothesis of "no relationship between matrices". We could thus analyze whether the Jaccard's similarity matrix of the viral community of Lake Annecy was related to that of Lake Bourget for each gene examined.

We ran a Pearson's correlation analysis to identify relationships between the number of DGGE bands and either phytoplankton taxa number or cell abundance, and between the relative abundance of specific bands and specific phytoplankton taxa.

To investigate the relationships between each virus community structure and measured environmental variables, a canonical correlation analysis (CCA) was performed using the software package XLSTAT-ADA. CCA generates an ordination plot that shows the main pattern of variation in community structure as accounted for by measured environmental variables. Different variables were submitted to the forward selection procedure, in which the statistical significance of the term was tested by the unrestricted Monte Carlo permutation test (999 permutations). Explanatory variables with p values greater than 0.05 were excluded from further analyses. The obtained ordination axes (based on community structure data) are linear combinations of environmental variables that best explain community structure data. Our interpretation refers to canonical variation only.

Results

Environmental and biological characteristics

Table 2 reports the minimum, maximum, and mean values for the different variables monitored during the study. For both lakes, water temperature (Temp) in surface waters began to increase in late February, reached its maximum in September, and then decreased until November. The concentration of total nitrogen (Ntot) and N-NO_3 started to decrease in March. In Lake Bourget, total phosphorus (Ptot) and P-PO_4 decreased after reaching a peak in early April and February, respectively, while in Lake Annecy they started to increase only in late summer. Concentrations of N-NH_4 showed a significant increase in spring in Lake Bourget, while it remained relatively stable throughout the year in Lake Annecy. The mean concentration of P and N nutrients was about 1.5–8 times higher in Lake Bourget

Table 2 Minimum, maximum, and mean values for the different biological and environmental variables presented in this study

Variables	Lake Bourget				Lake Annecy			
	Min. (month)	Max. (month)	Mean	SD	Min. (month)	Max. (month)	Mean	SD
Temp (°C)	5.5 (Feb)	16.9 (Sep)	12.5	4	5.2 (Feb)	17.4 (Sep)	12.6	4.5
Light (μmol m^{-2} s^{-1})	12.6 (Jan)	404.6 (Jun)	227	122.7	29.7 (May)	484.9 (Jun)	233.3	144.4
Dis. Oxy (mg l^{-1})	7 (Sep)	12.8 (Apr)	9.5	1.6	9.5 (Jan)	15.4 (Apr)	11.5	1.4
Ptot (μg l^{-1})	7 (Jul)	27 (Apr)	12	5	4 (May)	11 (Aug)	5.9	2
P-PO_4 (μg l^{-1})	2 (Oct)	10 (Feb)	5	2	1 (Oct)	4 (Sep)	2.5	0.9
Ntot (μg l^{-1})	333 (Nov)	758 (Mar)	537	109	167 (Nov)	397 (Mar)	284	65
N-NO_3 (μg l^{-1})	175 (Nov)	510 (Mar)	318	96	10 (Nov)	243 (Mar)	111	67
N-NH_4 (μg l^{-1})	2 (Jan)	57 (Apr)	16	16	1 (Jan)	8 (Oct)	4.6	2.2
SiO_2 (μg l^{-1})	790 (Sep)	3,030 (Feb)	1737	762	907 (Nov)	3,503 (Mar)	2,532	891
Chl*a* (mg l^{-1})	1.47 (Jan)	13.67 (Apr)	4.17	3.1	0.11 (Mar)	0.32 (Aug)	0.24	0.06
Het. bacteria (10^5 cells ml^{-1})	13.8 (Feb)	45 (Apr)	26.4	7.6	5.76 (Jul)	33.9 (Oct)	18.1	6.92
Synechococcus spp. (10^4 cells ml^{-1})	0.22 (May)	16 (Aug)	4.14	4.36	4.45 (Jun)	15.6 (Mar)	9.17	3.35
Cyanobacteria (μg l^{-1})	0.03 (May)	529 (Nov)	78.2	134.4	0 (Feb)	371.2 (Jun)	41.2	97.8
Chlorophyceae (μg l^{-1})	1 (Feb)	306.3 (Oct)	106.7	104.8	0.9 (Jul)	168.8 (May)	24.5	42.7
Chrysophyceae (μg l^{-1})	0 (Mar)	1225.3 (Jun)	251.6	341.4	13.9 (Feb)	354.6 (Apr)	162.6	112.4
Prymnesiophyceae (μg l^{-1})	2 (Mar)	234.3 (Apr)	30.3	53.8	0 (Jun)	17.9 (May)	3.9	5
Cryptophyceae (μg l^{-1})	11.4 (Jun)	2670 (Apr)	368.8	702.6	17.1 (Jun)	108.1 (Apr)	51.7	50.4
Diatomeae (μg l^{-1})	13 (Sep)	636.7 (Nov)	187.2	161.6	20.4 (Oct)	540.8 (Jun)	267.7	178.7
Dinophyceae (μg l^{-1})	0[a]	515.5 (Aug)	135.3	163.3	0 (Nov)	339 (Sep)	71.2	102.9
Zygophyceae (μg l^{-1})	0[a]	271.2 (May)	18	65.5	0[a]	17 (Sep)	2.2	5
VLP1 (10^7 part. ml^{-1})	3.5 (Jan)	27.5 (May)	8.88	5.04	2.75 (Jun)	10 (Sep)	5.14	2.2
VLP2 (10^5 part. ml^{-1})	20.6 (Feb)	66.9 (May)	44.5	13.6	4.3 (Jun)	85 (Sep)	41.4	20.9
Cyanomyovirus (10^3 part. ml^{-1})	9.37 (Mar)	942 (Sep)	108	208	2.79 (Jan)	367 (Sep)	89.5	105

Abbreviations are explained in the text

Min. minimum, *Max*. maximum, *SD* standard deviation

[a] Several months can be implied. For *Dinophyceae* in Lake Bourget: Jan, Feb, Apr, May, and Nov. For *Zygophyceae* in Lake Bourget: Jan, Mar–Jul, Sep and Oct. For *Zygophyceae* in Lake Annecy: Jan–Jun, Oct and Nov

than in Lake Annecy. The concentration of chlorophyll *a* (Chl*a*) increased between early March and early April in both lakes, reaching a higher, sustained level in Lake Bourget.

The temporal dynamics of the major phytoplankton groups, both in terms of biomass and cell abundances, are reported in Fig. 1. Unicellular forms of both prokaryotic and eukaryotic phytoplankton dominated, comprising >80 % of community biomass in Lake Annecy, and >90 % in Lake Bourget. The average proportion of colonial cells was tenfold higher in Lake Annecy (18 % of total cells) than in Lake Bourget (1.7 %). Among the 163 examined phytoplankton taxa observed in the two ecosystems, 33 % were members of the Chrysophyceae, and this group dominated phytoplankton community composition in both lakes. In general, the number of taxa observed was higher in Lake Bourget than in Lake Annecy for all classes of phytoplankton except the Diatomeae and Dinophyceae (Table S1). The average annual phytoplankton biomass in Lake Bourget was 1.9-fold higher than that of Lake Annecy. The filamentous and colonial cyanobacteria accounted for only 7 % of overall phytoplankton biomass for both lakes. The Chrysophyceae, Diatomeae, and/or Cryptophyceae dominated the phytoplankton biomass. The dominant species were *Rhodomonas minuta* and *Dinobryon divergens* for Lake Bourget, and *Cyclotella costei*, *Dinobryon divergens*, and *Cyclotella kuetzingiana* for Lake Annecy. In Lake Bourget, Cryptophyceae (31 %) dominated, while in Lake Annecy it was the Diatomeae (43 %). In terms of abundance, however, Chlorophyceae (30 %) predominated in Lake Bourget, although Prymnesiophyceae were also prevalent (18 %). For these two classes, the dominant species were *Chlorella vulgaris* and *Erkenia subaequiciliata*. By contrast, Diatomeae (33 %) predominated in Lake Annecy, while Prymnesiophyceae (6 %) and Chlorophyceae (11 %) were less abundant. Cyanobacterial abundances were also higher in Lake Annecy than in Lake Bourget. This was confirmed by the FCM counts: picocyanobacteria (e.g., phycoerythrin-rich *Synechococcus* spp.) being twofold higher in Lake Annecy, with a mean of 9.2×10^4 cells ml^{-1}. Likewise, the microscopy examination revealed 1.6-fold fewer filamentous forms (86.8 cells ml^{-1}), and 4.1-fold more colonial forms (334 cells ml^{-1}) in Lake Annecy than in Lake Bourget. By contrast, both the biomass and cell abundances of (eukaryotic) microalgae were on average 1.1–8.2-fold higher in Lake Bourget, with the exception of the diatoms. Likewise, the mean concentrations of heterotrophic bacteria (Hbact), cyanomyoviruses, VLP1 and VLP2 (i.e., the two groups we could detect within the virus-like particles using FCM) were 1.5-, 1.2-, 1.7-, and 1.1-fold higher, respectively, in Lake Bourget than in Lake Annecy.

Community structure of phytoplankton viruses

We investigated phytoplankton virus community structure using PCR-DGGE targeting the *g20*, *g23*, *psbA*,

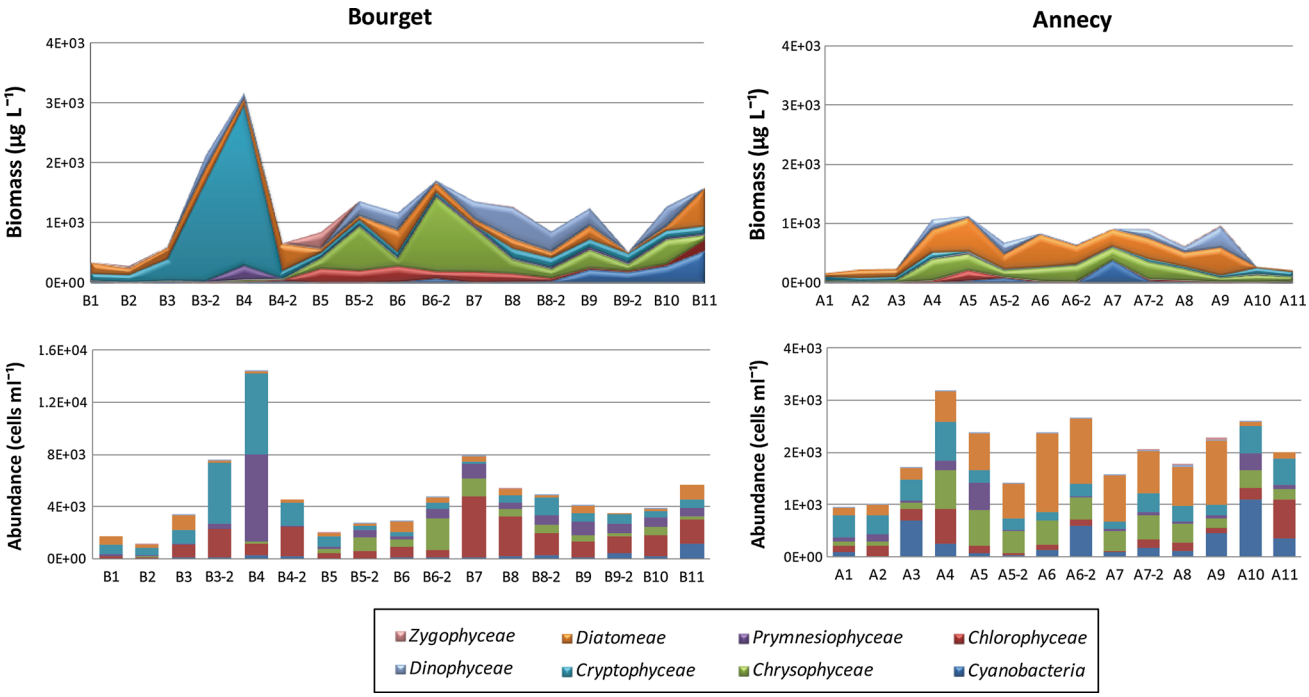

Fig. 1 Temporal dynamics of phytoplankton biomass and cell abundance throughout 2011 in Lakes Bourget and Annecy. Note that the scale is the same for biomass, but not abundance, for the two lakes. The sample codes give the lake (*A* Annecy, *B* Bourget) and the month of sampling (1–11). The "−2" designates the second sampling time. So, for example, A6 and A6-2 are the first and second samples taken in June from Lake Annecy. B6 is the first sample taken in June from Lake Bourget

polB, and *mcp* marker genes. The temporal variability of DGGE bands was examined for each viral group in each lake (Fig. 2; Table 3). The variation could be different between the two lakes and between distinct viral groups. No identical fingerprint could be detected between two samples of either lake or different time points. The maximum number of bands per sample could be 1.5 to 15-fold higher than the minimum (Table 2). In each lake, most of the DGGE bands (82–100 % of the obtained bands) varied with time, while only a few or even none was detected to be present in all samples throughout the year.

The DGGE banding pattern analysis with 70 % similarity (Fig. 3) revealed that *mcp* phycodnaviruses clustered into three distinct groups for Lake Bourget: spring, summer, and autumn to winter. No seasonal clustering pattern was detected for Lake Annecy. With 45 % similarity, the *polB* phycodnaviruses banding pattern in Lake Bourget clustered into three distinct groups: winter to early spring, spring, and summer to autumn. For Lake Annecy, only two clusters emerged, with the similarity percentage set at 60 % containing samples from winter, and summer to autumn. Spring samples did not cluster together. The DGGE banding pattern analysis with 45 % similarity (Fig. 3) revealed that the *psbA*-containing cyanophages clustered into three distinct groups in Lake Bourget (late winter to early spring, mid-spring to early summer, and late summer to mid-winter), and in Lake Annecy (winter to spring, mid-summer, and autumn). The fingerprints of *g20* cyanomyoviruses in Lake Bourget clustered into four distinct groups with similarity percentage set at 45 %: winter to mid-spring, late spring, early autumn, and late autumn. The summer samples did not cluster together. There was no clear seasonal pattern for Lake Annecy. At last, the DGGE banding pattern analysis with 75 % similarity (Fig. 3) revealed that T4-like myoviruses in Lake Bourget clustered into three distinct groups: late winter to mid-spring, late spring, and summer to autumn. For Lake Annecy, samples from late winter to spring, summer to early autumn, and late autumn grouped together.

Viral community composition in relation to biotic and abiotic variables

The canonical correspondence analysis (CCA) showed that 47.3 % of *mcp* phycodnavirus structure variance was explained by seven parameters in Lake Annecy, and

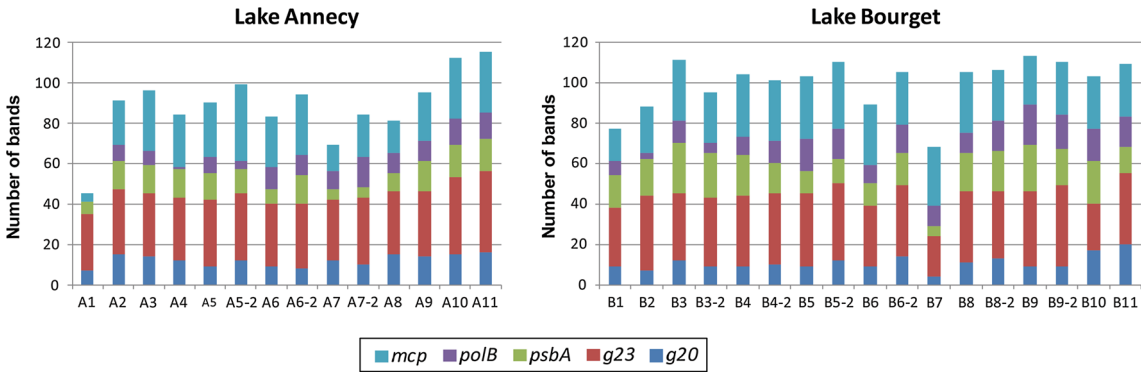

Fig. 2 Temporal changes in the number of DGGE bands throughout 2011 in Lakes Annecy and Bourget for the different viral gene markers investigated during this study. Sample codes are as in Fig. 1

Table 3 Summary of DGGE results

Gene markers	Lake Bourget					Lake Annecy					Annecy and Bourget	
	Min. (month)	Max.	Mean	Sub total	Persisting bands (%)	Min. (month)	Max.	Mean	Sub total	Persisting bands (%)	Bands in common	Total
mcp	16 (Jan)	33 (May)	29	58	1.7	4 (Jan)	38 (May)	24	68	0.0	42	85
polB	3 (Feb)	20 (Sep)	12	42	2.4	1 (Apr)	15 (Jul)	8	24	4.2	12	54
g20	7 (Feb)	17 (Nov)	11	41	2.4	7 (Feb)	16 (Nov)	12	27	18.5	16	52
psbA	5 (Jul)	25 (Mar)	17	33	9.1	5 (Jul)	16 (Nov)	11	24	4.2	18	39
g23	20 (Jul)	40 (Sep)	33	61	1.6	28 (Jan)	40 (Nov)	33	56	10.7	42	75

The minimum, maximum, and mean number of bands for each viral gene marker for each lake are shown. Also shown for each gene marker are: "sub-total", the total number of different band types detected throughout the year (by lake); "persisting bands", the percentage of bands present in all samples taken throughout the year (by lake); "bands in common", the number of bands shared by both lakes; and "total", the total number of bands found, both lakes combined

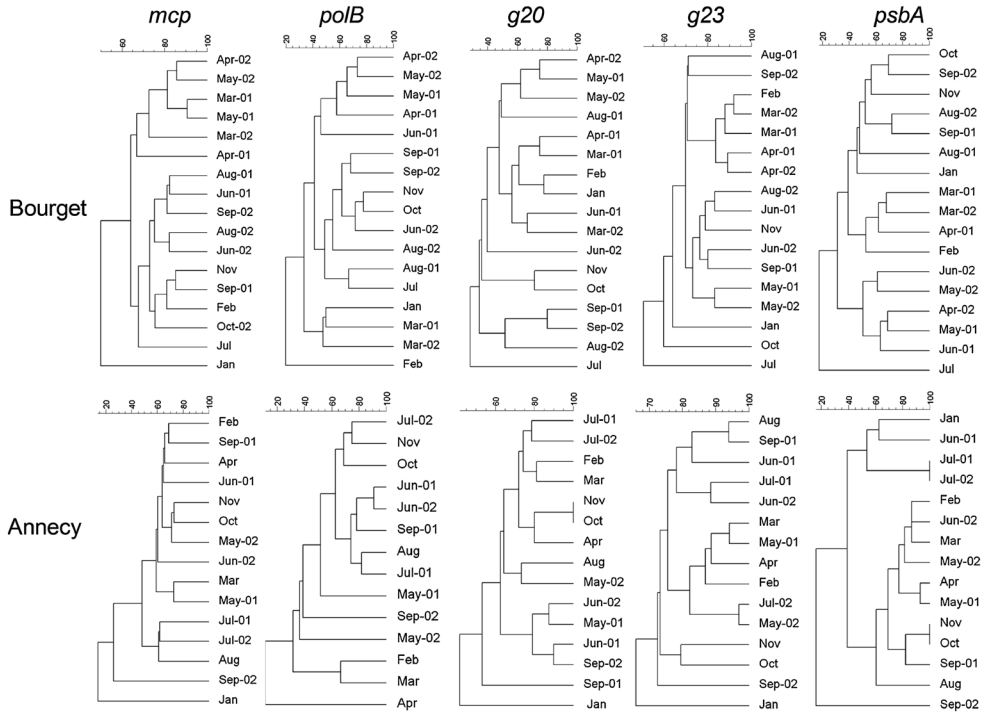

Fig. 3 Clustering analysis of the DGGE fingerprinting patterns obtained for each viral signature gene for Lakes Annecy and Bourget in 2011

46.5 % by ten variables in Lake Bourget. These variables were VLP1, VLP2, Chl*a*, Temp, Chlorophyceae, Diatomeae, and Dinophyceae for both lakes, while Oxy, Ptot, and Ntot were specific to Lake Bourget (Fig. 4). For phycodnavirus structure estimated from *polB* gene, VLP1, VLP2, Ntot, NH_4, Ptot, PO_4, Chl*a*, Temp, and Chlorophyceae were associated to 45.6 and 44.7 % of the community structure variance for Lake Annecy and Bourget, respectively (Fig. 4). In total, 54.7 % of *g20* cyanomyophage structure variance was explained by nine variables in Lake Annecy, and 52.7 % explained by seven variables in Lake Bourget (Fig. 5). VLP1, VLP2, Cyanomyoviruses, Temp, Syn, Ptot, and Cyanobacteria were common to both lakes, while P-PO_4 and Ntot were specific to Lake Annecy. For *psbA*-containing cyanophages, 61.8 and 49.3 % of community structure variance was explained by eight and ten variables in Lake Annecy and Bourget, respectively. VLP1, VLP2, Cyanomyoviruses, Syn, Ptot, Chl*a*, Temp, and Cyanobacteria were common to both lakes, while P-PO_4 and Ntot were, again, specific to only one lake, this time Lake Bourget. For *g23* T4-like myoviruses (including all cyanophages), VLP1, VLP2, Temp, Syn, Ntot, Ptot, and Hbact were associated to 54.4 and 53.1 % of the variance in Lake Annecy and Lake Bourget, respectively (Fig. 5).

We carried out a Pearson's correlation analysis to identify relationships between the number of viral DGGE bands and both the number of phytoplankton taxa and cell abundances. For taxa number, significant correlations (at least $p < 0.05$) were found between *polB* and Chrysophyceae ($r = 0.59$, $n = 14$) and between *mcp* and Chrysophyceae ($r = 0.56$, $n = 14$) in Lake Annecy, and between *polB* and Chlorophyceae ($r = 0.53$, $n = 18$), between *polB* and Chrysophyceae ($r = 0.59$, $n = 18$), and between *mcp* and Diatomeae ($r = -0.64$, $n = 18$) in Lake Bourget. Only the last correlation was negative. For host abundances, positive correlations were only found between DGGE bands number and cyanobacteria: *g20* with *Synechococcus* spp. ($r = 0.681$) and *psbA* with cyanobacteria ($r = 0.549$) in Lake Annecy, and *g20* with cyanobacteria ($r = 0.61$) in Lake Bourget. Further, a more detailed analysis, using again the Pearson's correlation test, was also carried out to examine possible links between the dynamics of each DGGE band (in terms of relative abundance) and phytoplankton taxa. We found that a single band could be related to several phytoplankton taxa (up to 12) as well as a single phytoplankton taxa could be related to several distinct bands (up to 40 % of bands, Table S3).

Discussion

Our study is a comprehensive, in-depth study of phytoplankton virus (both dsDNA algal virus and cyanophage) community structure over a complete year in two large and deep peri-alpine lakes with different trophic states, Lake Annecy (oligotrophic) and Lake Bourget (oligo-mesotrophic). By using a variety of viral gene markers, we obtained an unprecedented portrait of the virus community infecting mainly phototrophic organ-

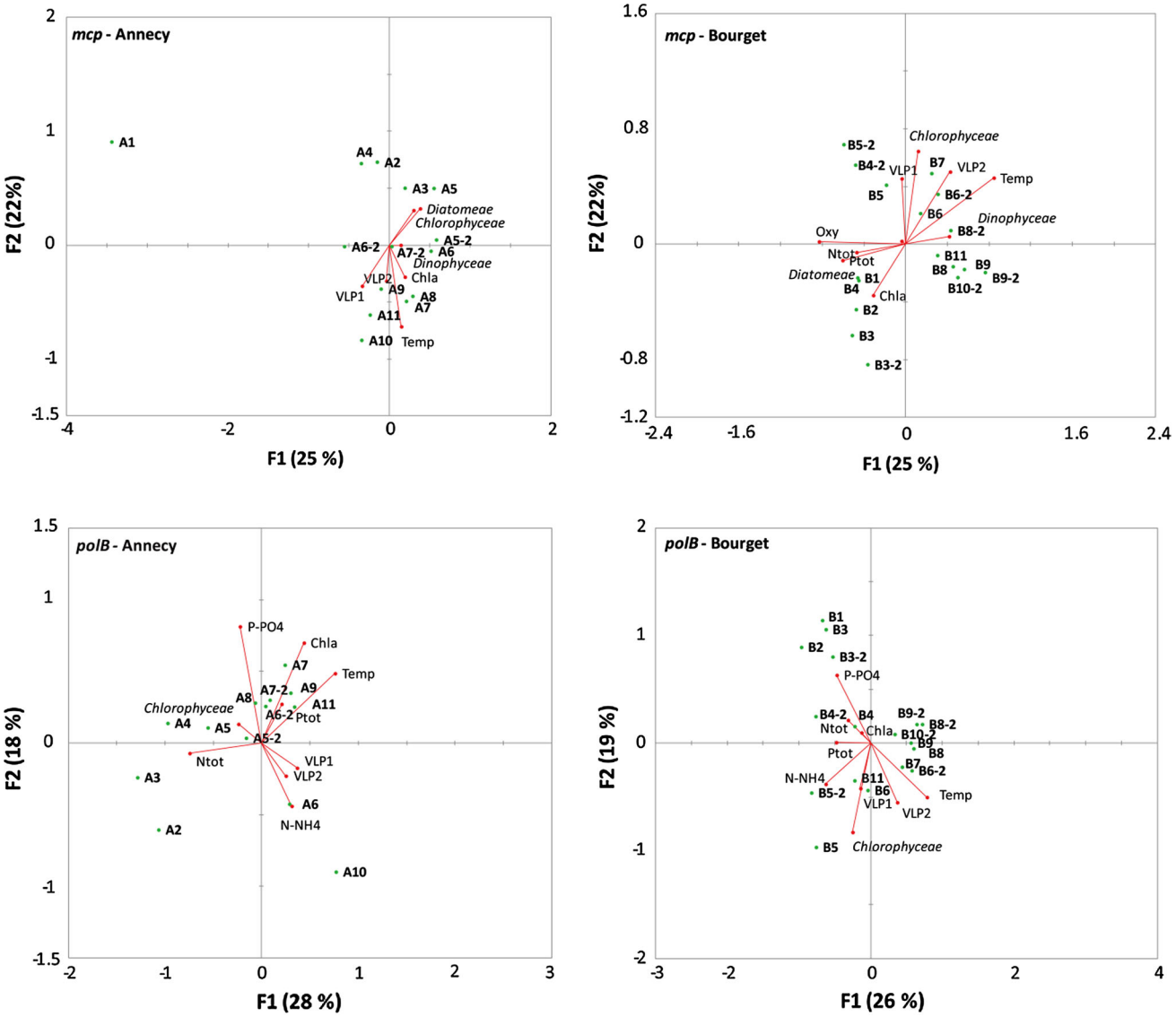

Fig. 4 CCA using both environmental parameters and biological counts as constrained variables to explain temporal changes in algal virus community structure (*mcp* and *polB*). *Red lines* point in the direction of increasing values for each variable. The length of the trait indicates the degree of correlation with the represented axes. The position of samples relative to traits is interpreted by projecting the points on the trait and indicates the extent to which a sample in the community of interest is influenced by the environmental variables represented by that trait

isms in these two major alpine lakes. We also examined the complex relationships that exist between these viruses, their potential hosts, and the physico-chemical environment. Using the PCR-DGGE approach allowed us to fingerprint the "richness" of the most abundant groups and dynamics of a number of specific viral groups through the analysis of PCR-amplified gene fragments. Previous studies using this method have focused mainly on marine systems and only on a single gene, typically *g20* (Wilson et al. 2000; Frederickson et al. 2003; Sandaa and Larsen, 2006), *polB* (Short and Suttle 2002, 2003) or *mcp* (Schroeder et al. 2003; Martínez-Martínez et al. 2007). Only Parvathi et al. (2012) have carried out a comparable study, but they looked at only one ecosystem and over a much shorter time period (i.e., Lake Geneva in summer and fall).

Methodological drawbacks

Sampling strategy

We are aware that using integrated water samples (i.e., mixed water from the surface to a depth of approximately 20 m) and sampling only every 3–4 weeks may have masked some relationships between phytoplankton and their associated viruses. Indeed, phytoplankton cells are not distributed homogeneously in the water column,

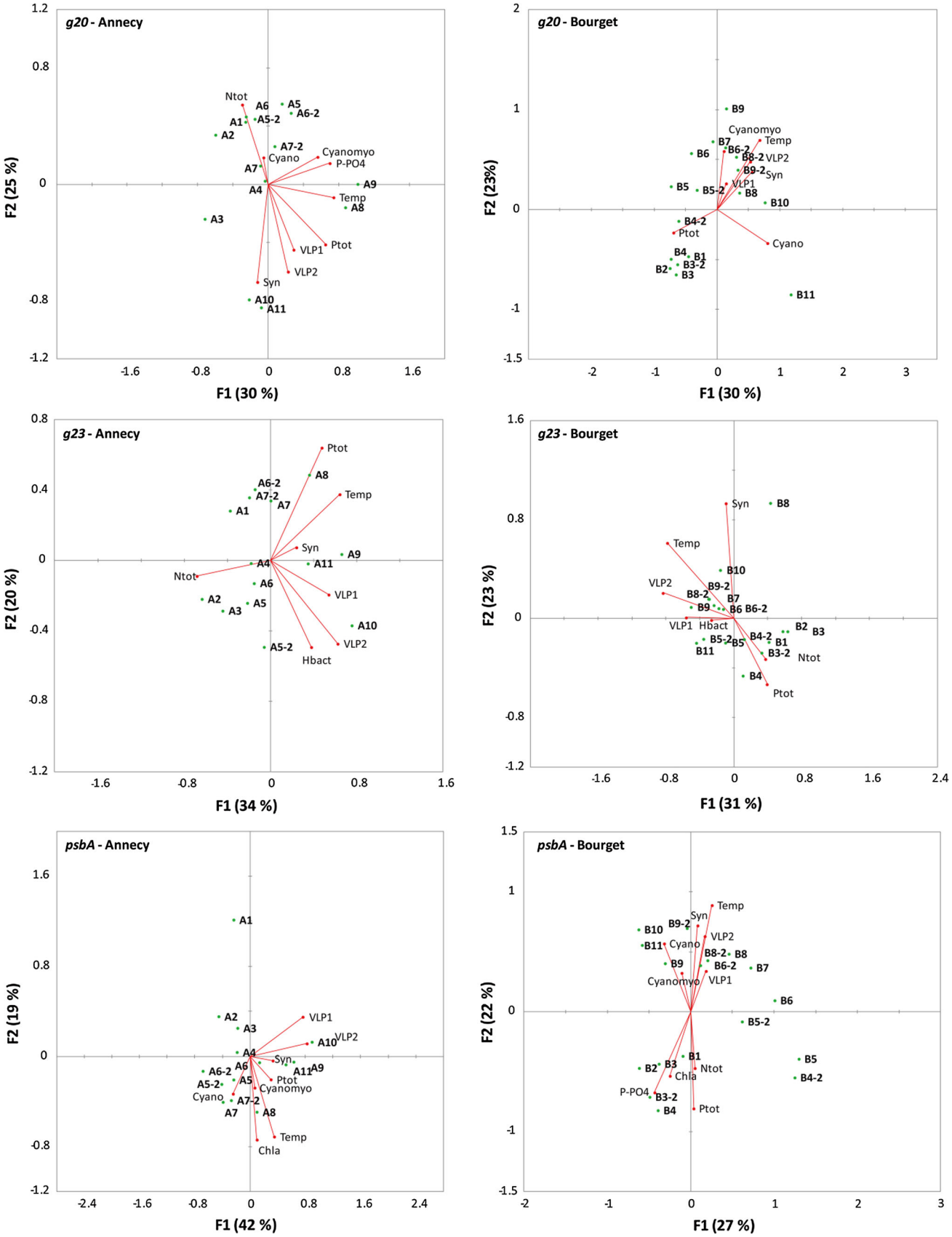

Fig. 5 CCA using both environmental parameters and biological counts as constrained variables to explain temporal changes in cyanophage community structure (*g20*, *g23*, and *psbA*). Legend as in Fig. 4

and our sampling strategy could have obscured fine and/or important interactions between phytoplankton and co-occurring viruses at specific depths. Moreover, it is well known that virus–host interactions may occur at very short time scales, i.e., within a few hours (Heldal and Bratbak 1991), and at microscales (Seymour et al. 2006). This means that the ideal sampling method would, with no doubt, be taking samples at discrete depths and at a higher frequency. However, we wanted to use the data on biological variables (i.e., the phytoplankton) collected by the traditional lake survey carried out on French peri-alpine lakes (Jacquet et al. 2012a), so we decided to sample the viruses using the same regime.

Molecular biology considerations

Hurwitz et al. (2013) have just published a study in which they compared the efficacy of ultrafiltration versus $FeCl_3$ precipitation with the use of DNase sucrose and/or CsCl gradients in concentrating and purifying ocean virus communities. They showed that ultrafiltration probably leads to an underestimation of viral genotype diversity (especially for the Phycodnaviridae and podoviruses) compared to the $FeCl_3$-precipitated virus method. As we used the classical ultrafiltration method, by which we measured on average >65 % loss of viral particles, it is likely that we also have underestimated viral genotype diversity.

Since there is no single universal gene present in all viruses, making inferences about total viral diversity in natural communities is always difficult. Although we made an effort to use several gene markers to circumvent this problem, it is well known that primers used to target specific groups can still miss a large portion of the existing diversity (Short et al. 2010). We tried to mitigate this problem by using primers that target different conserved genes among certain groups of viruses that infect closely related hosts. These primers have been used to amplify key structural or functional viral genes (see Table 1): the *g20* gene from cyanomyoviruses, *g23* from T4-like myoviruses (including cyanomyoviruses), *psbA* from *psbA*-containing cyanophages (including cyanomyoviruses and -podoviruses), and *mcp* and *polB* from phycodnaviruses. Nevertheless, because of the specificity of the primers used, we may not have captured the full viral diversity in the lakes. This is probably particularly true in the case of eukaryotic algal viruses, as evidenced by the results of a phylogenetic analysis conducted in parallel, which revealed that *mcp* and *polB* primers target distinct members of the phycodnaviruses, and both can only detect a limited number of viral groups (Zhong and Jacquet 2013b). It must also be noted that our analysis was restricted to a subset of dsDNA viruses that did not include some viral groups of phytoplankton like the cyanosiphoviruses (for which no primers are yet available), and this study did not consider ssDNA and RNA, which may be quantitatively important (Roux et al. 2012; Steward et al. 2013).

There are a number of limitations with the DGGE fingerprinting method. Theoretically, each band in a DGGE gel represents a different viral population present in the community and, therefore, each PCR of an environmental sample produces a unique fingerprint that reflects the composition of the community amplified. However, such a technique can only provide information on dominant groups (i.e., representing, theoretically, >1 % of the population), so our analysis may be missing some minor groups or groups that were not dominant at the time of the sampling (Berdjeb et al. 2011). Moreover, we are aware that one DGGE band can contain multiple genotypes as long as the sequences possess the same GC content. It is thus possible that these sequences/genotypes varied over time so that their composition inside a single band could also be different from one date to another. Another caveat is that some of the bands that appear in fingerprints may be the result of amplification or electrophoretic artifacts, leading *in fine* to erroneous conclusions because some actually non-existent populations are included in the community analysis. However, this was not the case in our study (e.g., Zhong and Jacquet 2013a, b, 2014). Thus, despite all these caveats, fingerprinting aquatic virus communities using DGGE, which allowed us comparing the community composition for multiple samples, was very useful in the context of this study because of cost-efficiency and rapidity.

Contrasting community structures and dynamics between the two ecosystems

For all gene markers except *mcp*, we found a higher number of different DGGE band types in Lake Bourget than the oligotrophic Lake Annecy (Table 3), suggesting that the oligo-mesotrophic ecosystem can sustain higher virus "diversity" (or at least a higher number of dominant groups). This observation is consistent with our finding that there was a higher number of taxa of their potential hosts in Lake Bourget (Table S1). By contrast, when considering *mcp*, Lake Annecy displayed higher "diversity" than Lake Bourget (68 versus 58 bands). This could be explained by (1) higher "diversity" of the potential hosts of these viruses in Lake Annecy, and/or (2) the amplification of multiple copies of the *mcp* gene from genomes of some specific phycodnaviruses in this lake (Zhong and Jacquet 2013b) as previously suggested or shown for Chloro- (Fitzgerald et al. 2007a, b, c) and Prasinoviruses (Derelle et al. 2008; Moreau et al. 2010; Weynberg et al. 2011). Members of the Diatomeae and Dinophyceae may be potential hosts for these phycodnaviruses in both lakes, as suggested by the relatively high number of taxa (nine for Dinophyceae, 20 for Diatomeae), their significant biomass, and the results of the CCA showing that these two classes were indeed related to *mcp* band pattern (Figs. 1, 4). Our phylogenetic analysis showed that 34 % of obtained *mcp* sequences belonged to phycodnaviruses of unknown hosts, of

which one-third were from Lake Annecy (Zhong and Jacquet 2013b). Strikingly, diatoms dominated the phytoplankton community in Lake Annecy, both in terms of biomass and abundance, especially between May and September, when they accounted for up to 64 % of total phytoplankton abundance. As competitive algal dominants could have more chance to be infected than rarer algae (Fuhrman 1999), it seems likely that diatoms are more likely to be the hosts of these phycodnaviruses. This result is interesting since, to the best of our knowledge, no dsDNA virus infecting (freshwater) diatoms has been reported thus far. To date, only RNA or ssDNA viruses have been isolated from diatoms (e.g., *Rhizosolenia, Chaetoceros*), and exclusively from seawater (Nagasaki 2008). A very promising research avenue would, therefore, be the isolation of dsDNA viruses infecting freshwater diatoms. In Lake Annecy, potential hosts could be *Cyclotella costei* and *Fragilaria crotonensis*, two species dominating the diatom community, especially in spring (Fig. 4). In Lake Bourget, the phycodnaviruses could be associated to other species such as *Asterionella formosa*, *Fragilaria ulna* var. *acus* and *Stephanodiscus minutulus* that dominated the diatom community from January to April (Fig. 4).

For all signature genes examined, the majority of the DGGE band types varied over time in abundance, and only a few persisted throughout the entire year (Table 3). This observation agrees with the Bank model, which asserts that only a small portion of the global pool of viruses is active and abundant at any given time, with the majority remaining rare and/or inactive (Breitbart and Rohwer 2005). Such temporal variation was also observed for phytoplankton abundance and composition (Fig. 1; Table S1), supporting the idea that viral activity is probably directly influenced by the abundance and growth of their phytoplankton hosts (Short et al. 2011). One could argue that our finding that the viruses did not persist throughout the annual cycle is an artifact, a failure of detection associated with using the DGGE method. However, taking *mcp* and *g23* in Lake Bourget as an example, the minimum number of bands detected for these two marker genes remained relatively high (16 and 20 bands, respectively), yet we observed that less than 2 % of these bands persisted throughout the year (Table 3).

The five phytoplankton virus groups examined (Table 1) all exhibited marked seasonal dynamic patterns in Lake Bourget, while this was the case only for *psbA*-containing cyanophages and T4-like myoviruses in Lake Annecy. The patterns were even significantly correlated between the two lakes for T4-like myoviruses (Mantel test, $r = 0.12$, $p < 0.0001$, $\alpha = 0.05$), but not for the *psbA*-containing cyanophages ($r = -0.01$, $p = 0.892$, $\alpha = 0.05$). As these two ecosystems are situated in the same ecoregion, we hypothesize that these differences are not related to climatic fluctuation, but rather to differences in host abundance and diversity, and water chemistry. In Lake Annecy, relative to Lake Bourget, the oligotrophic conditions (with prolonged stratification, P source limitation) result in a relatively reduced phytoplankton biomass and the absence of marked seasonal variation in abundance for some phytoplankton groups (Fig. 1; Zhong et al. 2013). As a result, the band patterns of some viral groups (i.e., the cyanomyophages and the phycodnaviruses) may remain relatively stable throughout the year. Nevertheless, the seasonality recorded for T4-like myoviruses and *psbA*-containing cyanophages band patterns also suggests that these viral groups are sensitive to their environment. The hosts for T4-like myoviruses are likely mainly heterotrophic bacteria as revealed from phylogenetic analysis (Zhong and Jacquet 2014), for which major shifts in abundance and community composition have been observed in these lakes (e.g., Personnic et al. 2009; Berdjeb et al. 2011). We hypothesize that bacterial diversity and/or heterotrophic processes vary seasonally, resulting in marked seasonal patterns in bacteriophage community composition in the two lakes. The dynamic patterns observed for hosts of *psbA*-containing cyanophages may be associated with major shifts in *Synechococcus* strains while the abundance of the whole picocyanobacterial community remains relatively abundant and present throughout the year in both lakes. Note that such shifts in *Synechococcus* strains were recently observed in the neighboring Lake Maggiore by Callieri et al. (2012). Due to the presence of the *psbA* gene in cyanophage genomes, photosynthesis could be maintained or enhanced in both lakes (Mann et al. 2003; Millard et al. 2004; Lindell et al. 2005; Sullivan et al. 2006; Bragg and Chisholm 2008; Brauer et al. 2012).

Phytoplankton virus community structure in relation to biotic and abiotic factors

The CCAs revealed complex relationships between biotic and environmental parameters. Overall the biotic environmental variables examined explained between 45 and 62 % of variance in phytoplankton virus community structure. For both lakes, the band patterns of *psbA*-containing cyanophages and cyanomyoviruses (*g20*), but not T4-like phages (*g23*) and phycodnaviruses (*mcp* and *polB*), were related to the abundance of cyanomyoviruses. This result is consistent with the taxonomic identity of *g20* and *psbA* genes. By contrast, VLP1 and VLP2 were involved in the community structuring of all viral groups, suggesting that these two viral groups could contain both phycodnaviruses and cyanophages. This contrasts to what has been proposed previously, i.e., that VLP1 and VLP2 may be strongly associated with bacteriophages and cyanophages, respectively (Personnic et al. 2009). We tried to address this question by regularly sorting VLP1 and VLP2 over the course of our study and by testing all primers on the sorted particles. We found on several occasions that the signature genes of phycodnavirus (*polB*) and cyanophage (*g20, psbA*) could be amplified from sorted VLP1

(Table S2). Thus, it is likely that VLP1 mainly contains bacteriophages, but this does not entirely rule out the presence of cyanophages and/or phycodnaviruses in this group.

Among the biotic factors explaining *g20* and *psbA* band patterns, *Synechococcus* spp. were important (Fig. 5). This result is consistent with the finding that the PE-rich picocyanobacteria dominate the cyanobacterial community in peri-alpine lakes (Personnic et al. 2009; Domaizon et al. 2013), and the finding that all *psbA* sequences obtained were of *Synechococcus* phage origin (Zhong and Jacquet 2013a). Chlorophyll *a* (a proxy for phytoplankton biomass) could explain both *mcp* and *polB* band patterns from January to April in Lake Bourget, and in summer in Lake Annecy, suggesting that major shifts of the phytoplankton were responsible for the changes in the composition of phycodnavirus communities. More specifically, Chlorophyceae, diatoms and dinoflagellates were related to *mcp* band patterns at different periods of the year in both lakes, while only Chlorophyceae was associated with *polB* band patterns. These results are in agreement with our phylogenetic analysis (Zhong and Jacquet 2013b), which showed that the primer targeting *polB* can only amplify sequences from viruses infecting Chlorophyta, while *mcp* targets a larger span of phycodnaviruses. While 60 % of the *mcp* sequences belonged to viruses infecting Prymnesiophyceae (Zhong and Jacquet 2013b), the CCA did not detect a significant role for Prymnesiophyceae in determining *mcp* band patterns for either lake (Fig. 3). This result could be due to the fact that Prymnesiophyceae are small unicellular cells (difficult to identify) and that only one species, *Erkenia subaequiciliata*, was thus unambiguously reported. Although this taxon was quantitatively important, accounting for up to 46 % of the phytoplankton abundance in Lake Bourget in April and 22 % in Lake Annecy in May, the correlation test analysis with *mcp* bands showed that its dynamic only coupled with two bands in Lake Bourget but no band in Lake Annecy. Therefore, this study suggests that *E. subaequiciliata* may, in fact, not be the main cause of Prymnesiophyceae virus production, at least at the annual and/or community scales. The CCA did not reveal Chrysophyceae to be related to either *polB* nor *mcp* structuring, contrasting thus with the Chrysophyceae in Lake Annecy. A more detailed analysis revealed in fact that 80 % of *mcp* or *polB* bands were coupled with the abundance of at least one examined Chrysophyceae taxa (Table S3): 25 % to *Kephyrion* sp. in Lake Annecy and around 15 % to *Dinobryon divergens*, *Dinobryon cylindricum* and *Kephyrion* sp. in Lake Bourget. Chrysophyceae (e.g., *Dinobryon divergens*) dominated the phytoplankton biomass in both lakes but at relatively low abundance. This result is interesting since only less than ten viruses have been isolated from Chrysophyceae: *Hydrurus* (Hoffman 1978), *Aureococcus* (Gastrich et al. 1998), *Paraphysomonas* and *Chromophysomonas* (Preisig and Hibberd 1984) and none have been characterized so far (Van Etten et al. 1991). With the absence of available genome sequences, our phylogenetic analysis from either *polB* or *mcp* sequences were unable to identify viruses infecting Chrysophyceae but the statistical analysis supported that some *polB* or *mcp* sequences could be related to this phytoplankton group.

Abiotic factors may also be responsible for determining virus community structure, through direct effects like viral decay, or through indirect effects via viral hosts. We found that temperature was an important factor, since it was linked to band patterns for all five viral groups examined. It is likely that temperature is the primary factor driving host growth and temporal changes in host availability, thereby acting indirectly on viral community structure (Grover and Chrzanowski 2006; Callieri et al. 2012). P and N concentrations could explain *mcp* band patterns in Lake Bourget, but not in Lake Annecy, suggesting a weak bottom-up control of the *mcp*-primer-targeted phycodnaviruses by these resources in the oligotrophic lake. From January to April in Lake Bourget, *g20*, *mcp* and *g23* band patterns were related to Ptot, while *psbA* and *polB* were related to PO_4. The fact that different sources of P available at the same time in the lake were responsible for the community structuring of different viral groups, could reflect differences in host-virus relationships vis-à-vis the resource. The difference could even be observed between *psbA*-containing-cyanophages and cyanomyoviruses, which may infect the same host species, *Synechococcus* spp. (Zhong and Jacquet 2013a). It is suggested that different strains of *Synechococcus* were likely involved in producing *psbA*-containing-cyanophages that could be cyanomyo- and/or cyanopodoviruses. It is well known that cyanomyoviruses have a broad host range, while cyanopodoviruses have a narrow host range, and only a few studies have reported that a single *Synechococcus* stain can produce myovirus and podovirus at the same time (Sullivan et al. 2006, 2008; Wang and Chen 2008). It is thus likely that most *psbA* sequences were of podovirus origin and, indeed, we found that 82 % of the obtained *psbA* sequences had closer phylogenetic proximity to *Synechococcus* podovirus (S-CBP1 and S-CBP3) than to myovirus (Zhong and Jacquet 2013a).

At last, we observed that NH_4 was also an environmental variable explaining *polB* band pattern in both lakes. Previous studies reported that members of Chlorophyta have ammonium uptake abilities (Taylor and Rees 1999; Watanabe and Miyazaki 1996; Fujita et al. 1988). In Lake Bourget, significant enrichment of NH_4 was detected for March to June, peaking in late April (data not shown), a phenomenon known to be associated with zooplankton excretion at this period of the year (Jacquet et al. 2012b). The CCA showed that NH_4 could explain the *polB* community structuring in May and June, when the concentrations of NH_4 decreased. It is possible that the decline in NH_4 supply had a strong impact on the hosts of these phycodnaviruses. Moreover, *polB* band patterns seemed associated with a restricted number of nutrients (NH_4, Ntot, PO_4 and Ptot) at different periods of the year (Fig. 3), indicating that

Chlorophyta and its viruses were greatly affected by nutrient availability in both lakes. No relationship was found with NO_3 for any of the viral groups examined.

Conclusions

Our investigation of Lakes Annecy and Bourget shows that the community structure of phytoplankton viruses is diverse, varies with time and ecosystem and it provides new evidence for the Bank model proposed by Breitbart and Rohwer (2005). In general, the number and relative contributions of the parameters shaping community structure in the five viral groups were not dramatically different between the two lakes. Rather, these parameters acted differently on each viral group at different times of the year in each lake. A more detailed analysis would be now necessary to refine specific host community dynamics. Also, to avoid biases associated to DGGE, other molecular approaches such as the deep-sequencing (using next-generation sequencing technologies to sequence amplicons of marker genes from both viruses and hosts) could provide a better resolution to assess diversity and virus-host interactions. It is worthy to note that obtaining good and specific primers remains also crucial. For such a goal, some efforts have still to be invested in the isolation and characterization of new viruses and hosts, to valid current primers and/or design new ones. At last, in the context of such a survey, working at different discrete depths and with a higher time scale resolution should considerably increase our knowledge on viral ecology and diversity.

Acknowledgments This work was supported by a fellowship from the region Rhône-Alpes (France) awarded to XZ. We thank Susan Lemprière for correcting the English and two anonymous reviewers who helped us to improve this article.

References

Bellas CM, Anesio AM (2013) High diversity and potential origins of T4-type bacteriophages on the surface of Arctic glaciers. Extreme Life Extreme Cond 17:861–870

Berdjeb L, Ghiglione JF, Domaizon I, Jacquet S (2011) A 2-year assessment of the main environmental factors driving the free-living bacterial community structure in Lake Bourget (France). Microb Ecol 61:941–954

Boyce DG, Lewis MR, Worm B (2010) Global phytoplankton decline over the past century. Nature 466:591–596

Bragg JG, Chisholm SW (2008) Modeling the fitness consequences of a cyanophage-encoded photosynthesis gene. PLoS ONE 3:e3550

Brauer VS, Stomp M, Huisman J (2012) The nutrient-load hypothesis: patterns of resource limitation and community structure driven by competition for nutrients and light. Am Nat 179:721–740

Breitbart M, Rohwer F (2005) Here a virus, there a virus, everywhere the same virus? Trends Microbiol 13:278–284

Breitbart M, Salamon P, Andresen B, Mahaffy JM, Segall AM, Mead D, Azam F, Rohwer F (2002) Genomic analysis of uncultured marine viral communities. Proc Natl Acad Sci USA 99:14250–14255

Breitbart M, Thompson LR, Suttle CA, Sullivan MB (2007) Exploring the vast diversity of marine viruses. Oceanography (Wash DC) 20:135–139

Brussaard CPD (2004) Viral control of phytoplankton populations—a review. J Eukaryot Microbiol 51:125–138

Butina TV, Belykh OI, Maksimenko SY, Belikov SI (2010) Phylogenetic diversity of T4-like bacteriophages in Lake Baikal, East Siberia. FEMS Microbiol Lett 309:122–129

Butina TV, Belykh OI, Potapov SA, Sorokovikova EG (2013) Diversity of the major capsid genes (*g23*) of T4-like bacteriophages in the eutrophic Lake Kotokel in East Siberia, Russia. Arch Microbiol 195:513–520

Callieri C, Caravati E, Corno G, Bertoni R (2012) Picocyanobacterial community structure and space-time dynamics in the subalpine Lake Maggiore (N Italy). J Limnol 71:95–103

Chambouvet A, Morin P, Marie D, Guillou L (2008) Control of toxic marine dinoflagellate blooms by serial parasitic killers. Science 322:1254–1257

Chen F, Suttle CA, Short SM (1996) Genetic diversity in marine algal virus communities as revealed by sequence analysis of DNA polymerase genes. Appl Environ Microbiol 62:2869–2874

Chen F, Wang K, Huang S, Cai H, Zhao M, Jiao N, Wommack KE (2009) Diverse and dynamic populations of cyanobacterial podoviruses in the Chesapeake Bay unveiled through DNA polymerase gene sequences. Environ Microbiol 11:2884–2892

Chénard C, Suttle CA (2008) Phylogenetic diversity of sequences of cyanophage photosynthetic gene psbA in marine and freshwaters. Appl Environ Microbiol 74:5317–5324

Clasen JL, Suttle CA (2009) Identification of freshwater Phycodnaviridae and their potential phytoplankton hosts, using DNA pol sequence fragments and a genetic distance analysis. Appl Environ Microbiol 75:991–997

Clasen JL, Hanson CA, Ibrahim Y, Weihe C, Marston MF, Martiny JBH (2013) Diversity and temporal dynamics of southern California coastal marine cyanophage isolates. Aquat Microb Ecol 69:17–31

Clokie MRJ, Millard AD, Mann NH (2010) T4 genes in the marine ecosystem: studies of the T4 like cyanophages and their role in marine ecology. Virol J 7:291

Culley AI, Lang AS, Suttle CA (2006) Metagenomic analysis of coastal RNA virus communities. Science 312:1795–1798

Culley AI, Lang AS, Suttle CA (2007) The complete genomes of three viruses assembled from shotgun libraries of marine RNA virus communities. Virol J 4:69

Dekel-Bird N, Avrani S, Sabehi G, Pekarsky I, Marston MF, Kirzner S, Lindell D (2013) Diversity and evolutionary relationships of T7-like podoviruses infecting marine cyanobacteria. Environ Microbiol 15:1476–1491

Derelle E, Ferraz C, Escande ML, Eychenié S, Cooke R, Piganeau G (2008) Life-cycle and genome of OtV5, a large DNA virus of the pelagic marine unicellular green alga *Ostreococcus tauri*. PLoS ONE 3:e2250

Domaizon I, Savichtcheva O, Debroas D, Arnaud F, Villar C, Pignol C, Alric B, Perga ME (2013) DNA from lake sediments reveals the long-term dynamics and diversity of *Synechococcus* assemblages. Biogeosci Discuss 10:2515–2564

Dorigo U, Jacquet S, Humbert J-F (2004) Cyanophage diversity, inferred from *g20* gene analyses, in the largest natural lake in France, Lake Bourget. Appl Environ Microbiol 70:1017–1022

Duhamel S, Domaizon-Pialat I, Personnic S, Jacquet S (2006) Assessing the microbial community dynamics and the role of bacteriophages in bacterial mortality in Lake Geneva. Water Sci 19:115–126

Filée J, Tétart F, Suttle CA, Krisch HM (2005) Marine T4-type bacteriophages, a ubiquitous component of the dark matter of the biosphere. Proc Natl Acad Sci USA 102:12471–12476

Fitzgerald LA, Graves MV, Li X, Feldblyum T, Hartigan J, Van Etten JL (2007a) Sequence and annotation of the 314-kb MT325 and the 321-kb FR483 viruses that infect *Chlorella* Pbi. Virol 358:459–471

Fitzgerald LA, Graves MV, Li X, Feldblyum T, Nierman WC, Van Etten JL (2007b) Sequence and annotation of the 369-kb NY-2A and the 345-kb AR158 viruses that infect *Chlorella* NC64A. Virology 358:472–484

Fitzgerald LA, Graves MV, Li X, Hartigan J, Pfitzner AJP, Hoffart E (2007c) Sequence and annotation of the 288-kb ATCV-1 virus that infects an endosymbiotic *Chlorella* strain of the heliozoon *Acanthocystis turfacea*. Virology 362:350–361

Frederickson CM, Short SM, Suttle CA (2003) The physical environment affects cyanophage communities in British Columbia inlets. Microb Ecol 46:348–357

Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399:541–548

Fujita RM, Wheeler PA, Edwards RL (1988) Metabolic regulation of ammonium uptake by *Ulva rigida* (Chlorophyta): a compartmental analysis of the rate-limiting step for uptake. J Phycol 24:560–566

Fuller NJ, Wilson WH, Joint IR, Mann NH (1998) Occurrence of a sequence in marine cyanophages similar to that of T4 g20 and its application to PCR-based detection and quantification techniques. Appl Environ Microbiol 64:2051–2060

Gastrich MD, Anderson OR, Benmayor SS, Cosper EM (1998) Ultrastructural analysis of viral infection in the brown-tide alga, *Aureococcus anophagefferens* (Pelagophyceae). Phycology 37:300–306

Gastrich MD, Leigh-Bell JA, Gobler CJ, Anderson OR, Wilhelm SW, Bryan M (2004) Viruses as potential regulators of regional brown tide blooms caused by the alga, *Aureococcus anophagefferens*. Estuaries 27:112–119

Gimenes MV, Zanotto PM, Suttle CA, Da Cunha HB, Mehnert DU (2012) Phylodynamics and movement of phycodnaviruses among aquatic environments. ISME J 6:237–247

Goldsmith DB, Crosti G, Dwivedi B et al (2011) Development of phoH as a novel signature gene for assessing marine phage diversity. Appl Environ Microbiol 77:7730–7739

Grover JP, Chrzanowski TH (2006) Seasonal dynamics of phytoplankton in two warm temperate reservoirs: association of taxonomic composition with temperature. J Plankton Res 28:1–17

Heldal M, Bratbak G (1991) Production and decay of viruses in marine waters. Mar Ecol Prog Ser 72:205–212

Hoffman LR (1978) Virus-like particles in *Hydrurus* (Chrysophyceae). J Phycol 14:110–114

Huang S, Wilhelm SW, Jiao N, Chen F (2010) Ubiquitous cyanobacterial podoviruses in the global oceans unveiled through viral DNA polymerase gene sequences. ISME J 4:1243–1251

Huang HZ, Cheng K, Xu M, Zhao YJ (2011) Genetic diversity of T4 virioplankton, inferred from *g23* gene, in Wuhan Donghu Lake China. Environ Sci 31:443–447

Huang S, Wang K, Jiao N, Chen F (2012) Genome sequences of siphoviruses infecting marine *Synechococcus* unveil a diverse cyanophage group and extensive phage-host genetic exchanges. Environ Microbiol 14:540–558

Hurwitz BL, Deng L, Polos BT, Sullivan MB (2013) Evaluation of methods to concentrate and purify ocean virus communities through comparative, replicated metagenomics. Environ Microbiol 15:1428–1440

Jacquet S, Heldal M, Iglesias-Rodriguez D, Larsen A, Wilson WH, Bratbak G (2002) Flow cytometric analysis of an *Emiliania huxleyi* bloom terminated by viral infection. Aquat Microb Ecol 27:111–124

Jacquet S, Barbet D, Cachera S et al (2012a) Suivi environnemental des eaux du lac du Bourget pour l'année 2011. Rapport INRA-CISALB, pp 220

Jacquet S, Domaizon I, Anneville O (2012b) Evolution de paramètres clés indicateurs de la qualité des eaux et du fonctionnement écologique des grands lacs péri-alpins (Léman, Annecy, Bourget): Etude comparative de trajectoires de restauration post-eutrophisation. Arch Sci 65:225–242

Jamindar S, Polson SW, Srinivasiah S, Waidner L, Wommack KE (2012) Evaluation of two approaches for assessing the genetic similarity of virioplankton populations as defined by genome size. Appl Environ Microbiol 78:8773–8783

Kagami M, De Bruin A, Ibelings BW, van Donk E (2007) Parasitic chytrids: their effects on phytoplankton community and food-web dynamics. Hydrobiologia 578:113–129

Karl DM (2007) Microbial oceanography: paradigms, processes and promise. Nat Rev Microbiol 5:759–769

Kirchman DL, Morán XAG, Ducklow H (2009) Microbial growth in the polar oceans—role of temperature and potential impact of climate change. Nat Rev Microbiol 7:451–459

Larsen JB, Larsen A, Bratbak G, Sandaa RA (2008) Phylogenetic analysis of members of the Phycodnaviridae virus family, using amplified fragments of the major capsid protein gene. Appl Environ Microbiol 74:3048–3057

Lindell D, Jaffe JD, Johnson ZI, Church GM, Chisholm SW (2005) Photosynthesis genes in marine viruses yield proteins during host infection. Nature 438:86–89

López-Bueno A, Tamames J, Velázquez D, Moya A, Quesada A, Alcamí A (2009) High diversity of the viral community from an Antarctic lake. Science 326:858–861

Mann NH (2003) Phages of the marine cyanobacterial picophytoplankton. FEMS Microbiol Rev 27:17–34

Mann NH, Cook A, Millard A, Bailey S, Clokie MRJ (2003) Marine ecosystems: bacterial photosynthesis genes in a virus. Nature 424:741

Marie D, Brussaard CPD, Thyrhaug R, Bratbak G, Vaulot D (1999) Enumeration of marine viruses in culture and natural samples by flow cytometry. Appl Environ Microbiol 65:45–52

Marie D, Partensky F, Simon N, Guillou L, Vaulot D (2000) Flow cytometry analysis of marine picoplankton. In: Diamond RA, DeMaggio S (eds) In living colors: protocols in flow cytometry and cell sorting. Springer, Berlin Heidelberg New York, pp 421–454

Marston MF, Sallee JL (2003) Genetic diversity and temporal variation in the cyanophage community infecting marine *Synechococcus* species in Rhode Island's coastal waters. Appl Environ Microbiol 69:4639–4647

Martínez-Martínez J, Schroeder DC, Larsen A, Bratbak G, Wilson WH (2007) Molecular dynamics of *Emiliania huxleyi* and co-occurring viruses during two separate mesocosm studies. Appl Environ Microbiol 73:554–562

Millard A, Clokie MRJ, Shub DA, Mann NH (2004) Genetic organization of the *psbAD* region in phages infecting marine Synechococcus. Proc Natl Acad Sci USA 101:11007–11012

Mizuno CM, Rodriguez-Valera F, Garcia-Heredia I, Martin-Cuadrado A-B, Ghai R (2013) Reconstruction of novel cyanobacterial siphovirus genomes from Mediterranean metagenomic fosmids. Appl Environ Microbiol 79:688–695

Moreau H, Piganeau G, Desdevises Y, Cooke R, Derelle E, Grimsley N (2010) Marine prasinovirus genomes show low evolutionary divergence and acquisition of protein metabolism genes by horizontal gene transfer. J Virol 84:12555–12563

Mühling M, Fuller NJ, Millard A et al (2005) Genetic diversity of marine *Synechococcus* and co-occurring cyanophage communities: evidence for viral control of phytoplankton. Environ Microbiol 7:499–508

Nagasaki K (2008) Dinoflagellates, diatoms, and their viruses. J Microbiol 46:235–243

Nagasaki K, Bratbak G (2010) Isolation of viruses infecting photosynthetic and nonphotosynthetic protists. In: Wilhelm SW, Weinbauer MG, Suttle CA (eds) Manual of aquatic viral ecology. ALSO, pp 92–101

Nagasaki K, Ando M, Itakura S, Imai I, Ishida Y (1994) Viral mortality in the final stage of *Heterosigma akashiwo* (Raphidophyceae) red tide. J Plankton Res 16:1595–1599

Nagasaki K, Tomaru Y, Katanozaka N et al (2004) Isolation and characterization of a novel single-stranded RNA virus infecting the bloom-forming diatom *Rhizosolenia setigera*. Appl Environ Microbiol 70:704–711

Park Y, Lee K, Lee YS, Kim SW, Choi TJ (2011) Detection of diverse marine algal viruses in the South Sea regions of Korea by PCR amplification of the DNA polymerase and major capsid protein genes. Virus Res 159:43–50

Parvathi A, Zhong X, Jacquet S (2012) Dynamics of various viral groups infecting autotrophic plankton in Lake Geneva. Adv Oceanogr Limnol 3:171–191

Personnic S, Domaizon I, Dorigo U, Berdjeb L, Jacquet S (2009) Seasonal and spatial variability of virio, bacterio- and picophytoplanktonic abundances in three peri-alpine lakes. Hydrobiologia 627:99–111

Ponsero AJ, Chen F, Lennon JT, Wilhelm SW (2013) Complete genome sequence of cyanobacterial siphovirus KBS2A. Genome Announc 1:4

Preisig HR, Hibberd DJ (1984) Virus-like particles and endophytic bacteria in *Paraphysomonas* and *Chromophysomonas* (Chrysophyceae). Nord J Bot 4:279–285

Proctor LM, Fuhrman JA (1990) Viral mortality of marine bacteria and cyanobacteria. Nature 343:60–62

Rimet F, Druart JC, Anneville O (2009) Exploring the dynamics of plankton diatom communities in Lake Geneva using emergent self-organizing maps (1974–2007). Ecol Inform 4:99–101

Roux S, Enault F, Robin A, Ravet V, Personnic S, Theil S, Colombet J, Sime-Ngando T, Debroas D (2012) Assessing the diversity and specificity of two freshwater viral communities through metagenomics. PLoS ONE 7:e33641

Sandaa R-A, Larsen A (2006) Seasonal variations in virus-host populations in Norwegian coastal waters: focusing on the cyanophage community infecting marine *Synechococcus* spp. Appl Environ Microbiol 72:4610–4618

Sandaa R-A, Clokie M, Mann NH (2008) Photosynthetic genes in viral populations with a large genomic size range from Norwegian coastal waters. FEMS Microbiol Ecol 63:2–11

Sandaa R-A, Short SM, Schroeder DC (2010) Fingerprinting aquatic virus communities. In: Wilhelm SW, Weinbauer MG, Suttle CA (eds) Manual of aquatic viral ecology. ALSO, pp 9–18

Schroeder DC, Oke J, Hall M, Malin G, Wilson WH (2003) Virus succession observed during an *Emiliana huxleyi* bloom. Appl Environ Microbiol 69:2484–2490

Seymour JR, Seuront L, Doubell PM, Waters RL, Mitchell JG (2006) Microscale patchiness of virioplankton. J Mar Biol Ass UK 86:551–561

Sharon I, Tzahor S, Williamson S et al (2007) Viral photosynthetic reaction center genes and transcripts in the marine environment. ISME J 1:492–501

Short SM (2012) The ecology of viruses that infect eukaryotic algae. Environ Microbiol 14:2253–2271

Short SM, Short CM (2008) Diversity of algal viruses in various North American freshwater environments. Aquat Microb Ecol 51:13–21

Short SM, Suttle CA (2002) Sequence analysis of marine virus communities reveals that groups of related algal viruses are widely distributed in nature. Appl Environ Microbiol 68:1290–1296

Short SM, Suttle CA (2003) Temporal dynamics of natural communities of marine algal viruses and eukaryotes. Aquat Microb Ecol 32:107–119

Short CM, Suttle CA (2005) Nearly identical bacteriophage structural gene sequences are widely distributed in both marine and freshwater environments. Appl Environ Microbiol 71:480–486

Short S, Chen F, Wilhelm SW (2010) The construction and analysis of marker gene libraries. In: Wilhelm SW, Weinbauer MG, Suttle CA (eds) Manual of aquatic viral ecology. ALSO, pp 82–91

Short CM, Rusanova O, Short SM (2011) Quantification of virus genes provides evidence for seed-bank populations of phycodnaviruses in Lake Ontario, Canada. ISME J 5:810–821

Steward GF, Culley AI, Mueller JA, Wood-Charlson EM, Belcaid M, Poisson G (2013) Are we missing half of the viruses in the ocean? ISME J 7:672–679

Sullivan MB, Lindell D, Lee JA, Thompson LR, Bielawski JP, Chisholm SW (2006) Prevalence and evolution of core photosystem II genes in marine cyanobacterial viruses and their hosts. PLoS Biol 4:e234

Sullivan MB, Coleman ML, Quinlivan V et al (2008) Portal protein diversity and phage ecology. Environ Microbiol 10:2810–2823

Sullivan MB, Krastins B, Hughes JL et al (2009) The genome and structural proteome of an ocean siphovirus: a new window into the cyanobacterial 'mobilome'. Environ Microbiol 11:2935–2951

Suttle CA (2000) Ecological, evolutionary, and geochemical consequences of viral infection of cyanobacteria and eukaryotic algae. In: Hurst C (ed) Viral ecology. Academic Press, San Diego, pp 247–296

Suttle CA (2007) Marine viruses—major players in the global ecosystem. Nat Rev Microbiol 5:801–812

Suttle CA, Chan AM, Cottrell MT (1990) Infection of phytoplankton by viruses and reduction of primary productivity. Nature 347:467–469

Taylor MW, Rees TAV (1999) Kinetics of ammonium assimilation in two seaweeds, *Enteromorpha* sp (Chlorophyceae) and *Osmundaria colensoi* (Rhodophyceae). J Phycol 35:740–746

Thomas R, Jacquet S, Grimsley N, Moreau H (2012) Strategies and mechanisms of resistance to viruses in photosynthetic aquatic microorganisms. Adv Oceanogr Limnol 3:1–15

Tomaru Y, Shirai Y, Nagasaki K (2008) Ecology, physiology and genetics of a phycoDNAvirus infecting the noxious bloom-forming raphidophyte *Heterosigma akashiwo*. Fish Sci 74:701–711

Van Etten JL, Lane LC, Meints RH (1991) Viruses and virus-like particles of eukaryotic algae. Microbiol Mol Biol Rev 55:586–620

Vaulot D (1989) CYTOPC: processing software for flow cytometric data. Signal Noise 2:8

Wang K, Chen F (2004) Genetic diversity and population dynamics of cyanophage communities in the Chesapeake Bay. Aquat Microb Ecol 34:105–116

Wang K, Chen F (2008) Prevalence of highly host-specific cyanophages in the estuarine environment. Environ Microbiol 10:300–312

Wang G, Murase J, Asakawa S, Kimura M (2009) Novel cyanophage photosynthetic gene *psbA* in the floodwater of a Japanese rice field. FEMS Microbiol Ecol 70:79–86

Wang G, Murase J, Asakawa S, Kimura M (2010) Unique viral capsid assembly protein gene (*g20*) of cyanophages in the floodwater of a Japanese paddy field. Biol Fert Soils 46:93–102

Watanabe T, Miyazaki T (1996) Maximum ammonium uptake rates of *Scenedesmus quadricauda* (Chlorophyta) and *Microcystis novacekii* (Cyanobacteria) grown under nitrogen limitation and implications for competition. J Phycol 32:243–249

Wetzel RG (2001) Limnology: lake and river ecosystems, 3rd edn. Academic Press, San Diego

Weynberg KD, Allen MJ, Gilg IC, Scanlan DJ, Wilson WH (2011) Genome sequence of *Ostreococcus tauri* virus OtV-2 throws light on the role of picoeukaryote niche separation in the ocean. J Virol 85:4520–4529

Wilhelm SW, Matteson AR (2008) Freshwater and marine virioplankton: a brief overview of commonalities and differences. Freshw Biol 53:1076–1089

Wilhelm SW, Suttle CA (1999) Viruses and nutrient cycles in the sea. Bioscience 49:781–788

Wilhelm SW, Carberry MJ, Eldridge ML, Poorvin L, Saxton MA, Doblin MA (2006) Marine and freshwater cyanophages in a Laurentian Great Lake: evidence from infectivity assays and molecular analyses of g20 genes. Appl Environ Microbiol 72:4957–4963

Wilson WH, Fuller NJ, Joint IR, Mann NH (1999) Analysis of cyanophage diversity and population structure in a south-north transect of the Atlantic Ocean. In: Sharpy L, Larkum AWD (eds) Marine cyanobacteria. Bulletin Institute of Oceanography, Monaco, pp 209–216

Wilson WH, Fuller NJ, Joint IR, Mann NH (2000) Analysis of cyanophage diversity in the marine environment using denaturing gradient gel electrophoresis. In: Bell CR, Brylinsky M, Johnson-Green P (eds) Microbial biosystems: new frontier. Proceedings of the 8th international symposium on microbial ecology, Halifax, pp 565–570

Wilson WH, Van Etten JL, Allen MJ (2009) The Phycodnaviridae: the story of how tiny giants rule the world. Curr Topics Microbiol Immunol 328:1–42

Zheng C, Wang G, Liu J, Song C, Gao H, Liu X (2013) Characterization of the Major Capsid Genes (*g23*) of T4-type bacteriophages in the wetlands of northeast China. Microb Ecol 65:616–625

Zhong X, Jacquet S (2013a) Prevalence of viral photosynthetic and capsid protein genes in two large and deep peri-alpine lakes: a new insight on the diversity of freshwater cyanophages. Appl Environ Microbiol 79:7169–7178

Zhong X, Jacquet S (2013b) Contrasting diversity of phycodnavirus signature genes in two large and deep western European lakes. Environ Microbiol. doi:10.1111/1462-2920.12201

Zhong X, Jacquet S (2014) T4-like myophages of two close neighboring large and deep sub-alpine lakes are characterized by different community composition and dynamic patterns. Freshw Biol (in revision)

Zhong Y, Chen F, Wilhelm SW, Poorvin L, Hodson RE (2002) Phylogenetic diversity of marine cyanophage isolates and natural virus communities as revealed by sequences of viral capsid assembly protein gene g20. Appl Environ Microbiol 68:1576–1584

Zhong X, Berdjeb L, Jacquet S (2013) Temporal dynamics and structure of picocyanobacteria and cyanomyoviruses in two large and deep peri-alpine lakes. FEMS Microbiol Ecol 86:312–326