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Dynamics of auto- and heterotrophic picoplankton and associated viruses in Lake Geneva

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Abstract. Microbial dynamics have rarely been investigated in Lake Geneva, known as the largest lake in western Europe. From a 5-month survey, we report dynamic patterns of free-living virus, bacteria and small phytoplankton abundances in response to a variety of environmental parameters. For the first time, we fractionated the primary production to separate the contribution of different size-related biological compartments and measured both bacterial and viral production in addition to experiments conducted to quantify the virus-induced bacterial mortality. We observed marked seasonal and vertical variations in picocyanobacteria, bacteria and virus abundances and production. The contribution of picoplankton and nanoplankton production to the total primary production was high (reaching up to 76 % of total primary production) in November and the spring–summer transition period, respectively. The impact of viral lysis on both bacteria and picocyanobacteria was significantly higher than grazing activities. Virus-induced picocyanobacterial mortality reached up to 66 % of cell removal compared to virus induced (heterotrophic) bacterial mortality, which reached a maximum of 34 % in July. Statistical analyzes revealed that temperature and top-down control by viruses are among important factors regulating the picocyanobacterial dynamics in this lake. More generally speaking, our results add to the growing evidence and accepted view nowadays that viruses are an important actor of freshwater microbial dynamics and more globally of the functioning of the microbial food webs.

1 Introduction

As is true for any ecosystem, Lake Geneva is changing continuously, posing challenges to ecologists (Anneville et al., 2013). Over the past few decades, water quality monitoring surveys have been performed under the authority of the International Commission for the Protection of Lake Geneva (see reports at http://www.cipel.org/sp/), in order to study the water quality, functioning and evolution of this ecosystem, which is connected to an important catchment area. The detailed analysis of the viral and microbial communities in Lake Geneva during periods of the year such as the spring-to-summer and the summer-to-fall transitions have not been provided yet. The interactions between these microorganisms and their environment within the food webs are, however, a key issue to study for a better understanding of Lake Geneva ecology.

Picoplankton is an integral component of the microbial community which seems to be ubiquitous in all seas and lakes (Azam et al., 1983; Callieri and Stockner, 2002). In aquatic microbial ecology, the term picoplankton traditionally refers to all cells which fall into the size class 0.2–3 µm; that includes picocyanobacteria, heterotrophic bacteria, archaea and small eukaryotic phototrophs referred to as picoeukaryotes (Li et al., 1983; Whitman et al., 1998; Worden and Not, 2008; Auguet et al., 2010). The ubiquitous distribution of the picophytoplankton (cyanobacteria and autotrophic picoeukaryotes) and their importance in terms of biomass and production make them a critical food web component and carbon cycling in a wide variety of aquatic environments

(Worden et al., 2004). Compared to autotrophic picoplankton, heterotrophic bacteria contribute a larger percentage to total plankton biomass and play a central role in the transformation and mineralization of organic matter in the biosphere. These heterotrophs contribute largely to the cycling of carbon and nutrients in aquatic systems (Sarmiento and Gruber, 2006) and also form an important nutrient resource for higher trophic levels (i.e., the heterotrophic nanoflagellates, ciliates, metazooplankton).

Autochthonous and allochthonous factors can impact both auto- and heterotrophic organisms, affecting their distribution, structure, diversity as well as interactions among the organisms. The dynamics of picoplankton in aquatic ecosystems are not only controlled by abiotic factors (temperature, light, and nutrients), but also by biotic factors such as natural death, viral lysis, predation and parasitism. In recent years, top-down control of picoplankton populations has evoked much interests among microbial ecologists with the finding of large numbers of viruses (10^8–10^{11} L^{-1}) in aquatic systems. Studies have now revealed that viral lysis can be a significant source of mortality, as important as bacterivory by protists (Fuhrman and Noble, 1995; Pradeep Ram et al., 2005; Personnic et al., 2009b). Through their lysis activity, viruses also play an important role in regulating carbon and nutrient fluxes, food web dynamics and microbial diversity in aquatic systems (Suttle, 2005; Jacquet et al., 2010; Breitbart, 2012).

The factors that influence viral abundance and dynamics in aquatic environments are complex and are found to vary with aquatic ecosystems (Clasen et al., 2008). Although a large majority of these studies have been focused on marine environments (Weinbauer and Suttle, 1997; Weinbauer, 2004), fewer investigations have been carried out in freshwater systems to study the influence of environmental factors on the dynamics of viral communities associated with autotrophic and heterotrophic picoplankton (Maranger and Bird, 1995; Clasen et al., 2008; Jacquet et al., 2010). Studies have shown that the viral abundance is influenced more by the bacterial abundance in marine environments and by chlorophyll *a* concentration in nutrient-rich lakes (Pradeep Ram et al., 2010). A few studies in the lacustrine environments have included the influence of grazers on the picoplankton and bacterial abundances in studying viral dynamics (Personnic et al., 2009b; Berdjeb et al., 2011).

Lake Geneva is a mesotrophic peri-alpine lake where past studies have suggested that *Synechococcus* is the most predominant species in the autotrophic picoplankton (APP) (Duhamel et al., 2006; Personnic et al., 2009a). Lake Geneva has been poorly investigated in terms of microbial dynamics and diversity, and information on virus-bacteria, flagellates-bacteria and picocyanobacteria-ciliates interactions is still lacking for this lake (Duhamel et al., 2005; Personnic et al., 2009a, b). Our aim was to bring out an understanding about how various environmental and water quality parameters vary over a period of 5 months and how these changes may determine the abundance of autotrophic and heterotrophic plankton and associated viruses and to elucidate the type and extent of relationships of various physical, chemical and biological factors in determining the abundance of various autotrophic and heterotrophic planktonic groups in Lake Geneva. Recently by using a PCR-based molecular approach, we showed that Lake Geneva displays clear seasonal variations in the diversity of viruses (Parvathi et al., 2012). However, information regarding both the phytoplankton (in particular the picophytoplankton) dynamics and production and the dynamics and role of associated viruses is still lacking from this lake. Further, the influence of various chemical and physical parameters on viruses and different groups of plankton has not yet been well documented. Therefore, in the present study we proposed to highlight the seasonal and vertical variations in environmental and water quality parameters and resultant changes in picoplankton abundance, production and different viral parameters in relation to both biotic and abiotic factors in Lake Geneva over a 5-month period including summer and fall.

2 Materials and methods

2.1 Study site and sampling strategy

Lake Geneva, which lies at an altitude of 372 m, is the largest lake in western Europe and forms the border between France and Switzerland at the north of the French Alps. The lake is 72 km long and 13 km wide with an area of 580 km^2. Its catchment area is about 7419 km^2, reaching a maximal altitude of 4634 m (the average altitude is 1670 m), and at least 60 different tributaries aliment the lake (among which the Rhone River as the main one, with on average 180 $m^3 s^{-1}$). In terms of hydrology, annual rainfall has been about 1000 mm for the period 1981–2010. The water circulation of the lake has been clearly less studied (Lemmin et al., 1999; Ishiguro and Balvay, 2003). It is a meromictic lake, never covered by ice, with temperature ranging between 4 and 22 °C. It holds an approximate volume of 89×10^9 m^3 for a maximum depth of 309 m (average depth is 152 m), and this lake was reported as eutrophic during the 1970s. Later during the 1990s, following restoration programs, including measures to reduce phosphorus inputs, the lake changed to a mesotrophic state. In 2011, the lake had a total phosphorus content of 27 µgP L^{-1} (Lazzarotto and Klein, 2012). In our study, samples were collected at the reference station (lat 46°27′ N, long 6°32′ E), corresponding to the deepest part of the lake at monthly or bimonthly intervals from July to November 2011. The summer period extended from the end of July to September, and autumn from October to November. The samples were collected at different depths (2, 7.5, 10, 15, 20, 25, and 30 m) using a Niskin water sampler in two 20 L polycarbonate containers and stored at ambient temperature, protected

from light and heat, and brought to the laboratory within 3 h of collection.

2.2 Environmental parameters and plankton analysis

A multiparameter probe (CTD 90M, Sea and Sun Technology) was used to collect different parameters: temperature, light, conductivity, chlorophyll *a* and oxygen profiles. Samples collected at discrete depths (2.5, 5, 7.5, 10, 15, 20, 30, and 50 m) were analyzed for nutrients, namely nitrate ($N\text{-}NO_3$), nitrite ($N\text{-}NO_2$), total nitrogen (N_{tot}), phosphate (PO_4), total phosphorous (P_{tot}) and silicate (SiO_2) using standard methods (Anneville et al., 2005). Raw water samples for the phytoplankton analysis were taken with a patented integrating instrument developed by Pelletier and Orand (1978) integrating the 0–18 m upper water layer and fixed with a few drops of Lugol's solution for phytoplankton and zooplankton analysis. For each sample, 25 mL was poured into an Utermöhl room (cylinder surmounting a blade with sediment chamber; Utermöhl, 1931) and left to form a deposit for at least 12 h away from light and heat. The qualitative and quantitative examination of the phytoplankton was carried out using inverted microscopy (Zeiss). For the zooplankton, vertical sampling from a depth of 50 m to the surface was carried out using a net of 212 µm mesh size. The samples were fixed with formol (5 % v/v). The enumeration of microcrustaceae presented here was achieved by means of a standard microscope (Olympus BX40) following Anneville et al. (2007).

2.3 Flow cytometry analysis

Virus-like particles (VLPs) from discrete depths were counted using a FACSCalibur flow cytometer (FCM) (Becton Dickinson) equipped with an air-cooled laser providing 15 mW at 488 nm. Samples were fixed with glutaraldehyde (0.5 % final concentration, grade I, Merck) for 30 min, then diluted in 0.02 µm filtered Tris-EDTA buffer (referred to as TE, 0.1 mM Tris-HCL and 1 mM EDTA, pH 8), and incubated with SYBR Green I (at a final 10^{-4} dilution of the commercial stock solution; Molecular Probes), for 5 min at ambient temperature in the dark. At last, the sample was incubated for 10 min at 75 °C, and for another 5 min at room temperature prior to FCM analysis (Personnic et al., 2009a). FCM discriminated at least 3 subgroups of viruses, designated as VLP1, VLP2 and VLP3 (virus-like particles, group 1, 2 and 3) (Jacquet et al., 2010), but only VLP1 and VLP2 could be observed throughout the period of analysis and were shown thereafter. The analysis for determining heterotrophic bacterial abundance from different depths was performed as for the viruses but without heating at 75 °C and by using < 0.02 µm filtered lake water instead of TE (details can be found in Jacquet et al., 2013). The picocyanobacteria and other smaller phytoplankters were analyzed without fixing or staining, but by using their natural autofluorescence. During previous experiments or surveys in peri-alpine lakes (e.g., Annecy, Bourget and Geneva), some picocyanobacteria were sorted with flow cytometry and cultured, and both genetic affiliation and size were analyzed in order to confirm their identity. All cultured strains isolated so far with typical phycoerythrin-rich (PE) picocyanobacteria FCM signatures belong to *Synechococcus*-like populations and these PE-rich picocyanobacteria for the French sub-Alpine lakes vary in size between 1.5 and 2.5 µm (Jacquet, unpublished). Analysis was made on samples to which a suspension of 1 µm beads had been added (Molecular Probes).

2.4 Fractionated primary production

Size-fractionated primary production at five discrete depths (2.5, 7.5, 10, 15 and 20 m) was determined by in situ incubations with the isotope ^{14}C. < 200 µm water samples from each depth were filled into three 250 mL glass bottles (two "light" and one "dark" bottle). These bottles were inoculated with 1 ml of radiolabeled $NaH^{14}CO_3$ (5 µCi mL^{-1}) and subsequently incubated for 5 h at respective depths where the water was sampled. At the end of the incubation, samples were sequentially filtered through 20 µm nylon mesh and 3.0 and 0.2 µm polycarbonate filters. The phytoplankton cells concentrated in the 20 µm mesh were washed with filtered lake water and again concentrated on 0.7 µm GF/F filters. This corresponded to the microphytoplankton fraction, whereas the 3.0 and 0.2 µm represented the nano- and picophytoplankton fractions, respectively. The filters were used for subsequent analysis after removing excess dissolved inorganic carbon ($DI^{14}C$) by exposing it to concentrated hydrochloric acid fumes for one minute. The filters were then placed in scintillation vials and a 5 mL scintillation cocktail was added. Radioactivity was measured using a liquid scintillation counter (Beckman Coulter, USA). Production rate was calculated based on the photoperiod of each day and expressed as µg C L^{-1} d^{-1}. Other details can be found elsewhere (Anneville et al., 2002; Tadonléké, 2010).

2.5 Bacterial and viral production

Water samples collected in polycarbonate bottles (in triplicates) were stored in ice and transported to the laboratory. Bacterial production was determined by incorporation of the nucleoside ^3H Thymidine into bacterial DNA (Jugnia et al., 1999) on the integrated 0–18 m water samples. Briefly, a 30 mL water sample (in triplicates) along with trichloroacetic acid (TCA) killed control (1 % final concentration) was incubated with ^3H Thymidine (^3H TdR) at a final concentration of 10 nM in the dark for 1 h at ambient temperature in the laboratory. TdR incorporation was stopped by adding 1 % TCA. The samples were filtered through 0.22 µm (Millipore, USA) membrane filter, extracted in cold 5 % TCA and rinsed with 80 % ethanol. The dried filters were placed in scintillation vials and 0.5 mL of ethyl acetate was added to dissolve

the filter. A 5 mL scintillation cocktail was added and the radioactivity was measured using liquid scintillation counter (LS 6500 Scintillation Counter, Beckman Coulter, USA). The disintegration values per minute (dpm) after correcting for blank were converted to moles TdR of bacterial cells (2×10^{18} cells mol^{-1}) and bacterial carbon (20 fg C cell^{-1}).

Viral production was estimated by the virus reduction method (Wilhelm et al., 2002), similar to a previous study conducted at a peri-Alpine lake by Thomas et al. (2011). Briefly, a 100 mL water sample was diluted with 3 volumes of ultrafiltered sample (< 0.02 µm, free of viruses) to reduce the number of free viruses in the sample significantly. This was divided into three replicates and samples were incubated in the dark for 24 h. Subsamples were drawn at 2-hourly intervals to monitor the abundance of bacteria and viruses. The bacterial and viral abundances were determined using flow cytometry as described above.

2.6 Transmission electronic microscopy (TEM) analysis

Viral lytic infections were inferred from the percentage of visibly infected 8 cells (FVIC) according to Sime-Ngando et al. (1996). Bacterial cells contained in milliliters of glutaraldehyde-fixed samples (1 % final concentration), which were stored at 4 °C, were harvested by ultracentrifugation onto 400 mesh NI electron microscope grids with carbon-coated Formvar film, by using a Beckman Coulter SW40 Ti swing-out rotor run at $70\,000 \times g$ for 20 min at 4 °C. Each grid was stained at room temperature (ca. 20 °C) for 30 s with uranyl acetate (2 % wt/wt), rinsed twice with 0.02 µm filtered distilled water and dried on a filter paper. Grids were then examined using a JEOL 1200E × TEM operated at 80 kV at a magnification of × 100 000. At least 600–800 prokaryotic cells per sample were examined to determine the frequency of visibly infected cells (FVIC). Cells were scored as infected if they contained five or more intracellular viruses. For each sample, the mean burst size (viruses bacteria^{-1}) was estimated from the number of viruses in visibly infected cells. Because mature phages are visible only late in the infection cycle, FVIC counts were converted to the frequency of infected cells (FIC) using the equation $\mathrm{FIC} = 9.524 \times \mathrm{FVIC} - 3.256$ (Weinbauer et al., 2002). The FIC was then converted to viral-induced bacterial mortality (VIBM, as a percentage per generation) according to Binder (1999) using the equation $\mathrm{VIBM} = (\mathrm{FIC} + 0.6 \times \mathrm{FIC2})/(1 - 1.2 \times \mathrm{FIC})$.

2.7 Dilution experiments and viral parameters

The modified dilution approach was used to determine the grazing and viral-induced mortality on picoplankton and bacteria (Evans et al., 2003) as previously done by Personnic et al. (2009b) and Thomas et al. (2011). In this method, parallel dilution series (70, 40 and 20 %) of natural lake water was performed with 0.2 µm filtered sample to obtain the grazing rate and with 30 kDa filtered sample to obtain grazing and viral lysis rates. Percentages of grazing and viral mortality were determined from the difference between the two dilution series, provided that the regression slopes were significant (Kimmance et al., 2007). Viral mortality rates (d^{-1}) were also calculated as the ratio of viral production and burst size. The lytic mortality rate was calculated from viral lysis rate and bacterial abundance.

2.8 Statistical analysis

The statistical analysis was carried out for the monthly data for the abundance of heterotrophic bacteria, picocyanobacteria, other phytoplankton, VLP1, VLP2, total VLP and other physicochemical parameters. The dependent variables were the abundance of bacteria, picocyanobacteria, other phytoplankton, VLP1, VLP2 and VLP. The independent variables influencing dependent variables have been considered as explanatory variables (i.e., temperature, pH, turbidity, PO_4, P_{tot}, P_{part}, Mg, COT, Na, Ca, NH_4, Cl, SO_4, Chl *a*, dissolved oxygen, N_{tot}, NO_3-N, NO_2-N, SiO_2-Si). The presence of autocorrelation was checked using the PAST software (PAST version 2.14), which revealed correlations which were not significant. Also, explanatory variables were found to be highly correlated, indicating the presence of multicollinearity. Hence, an alternative method of estimation, the principal component regression, was used to examine the factors that influence the abundances of picoplankton, bacteria and viruses. The principle components were obtained by eigenvalue decomposition of the covariance or correlation matrix of the explanatory variables. For the analysis of variations in VLPs, biological factors such as the bacterial, picocyanobacterial and the other phytoplankton abundances were included as independent variables in factor analysis.

3 Results

3.1 Environmental factors

The mean along with standard deviation values of all the environmental parameters are provided in Table 1, while the dynamics and/or distribution of some of them can be appreciated in Fig. 1. Briefly, the average water temperature during the study period was 14.0 ± 5.2 °C, with minimum and maximum values of 6.2 ° C (November) and 21.5 °C (August), respectively. Vertical profiles showed water temperature to decrease rapidly from surface (19.6 °C) to 30 m (6.9 °C). The vertical stratification was well marked in summer. In the air, the temperature was relatively high during summer, reaching 25 °C with fluctuations between 15 and 25 °C. After August, the air temperature decreased significantly month after month down to 5 °C in November, but the decrease was clearly lower in the upper lit layer of the lake itself. Paralleling the decrease of air temperature, the same trend was observed for the photosynthetic active radiation. The dissolved

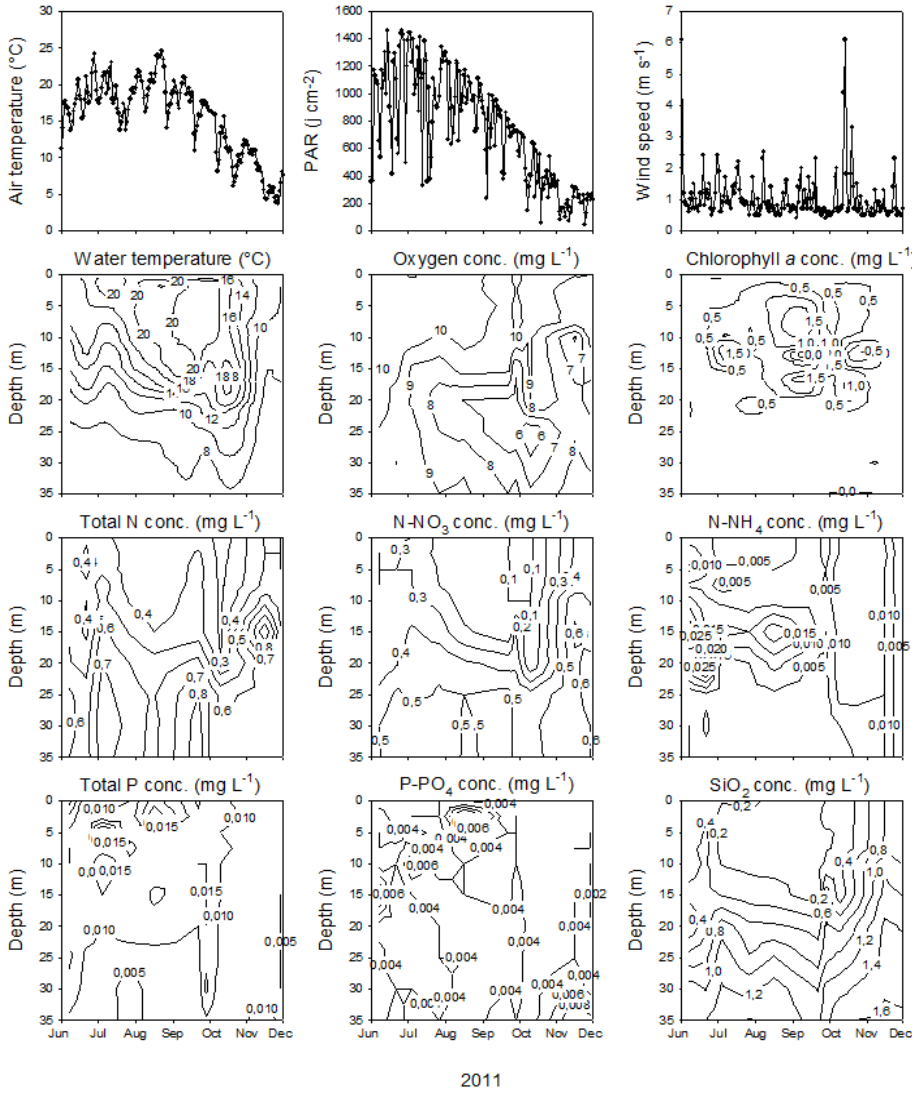

Fig. 1. Time series obtained for the main environmental parameters from June to December 2011. The interpolation between the data was generated automatically by SigmaPlot 12.0.

oxygen concentration varied between 5.2 and 10.9 mg L^{-1}. The highest values were recorded in the 0–10 m surface layer, and values decreased only slightly from summer to fall. During the study period, the concentrations of TP and TN varied by a factor of 15 and 8, respectively. Both nitrogen and phosphorus values varied significantly ($p < 0.05$) with respect to months and depths. Such variations were mainly due to NO_3 for TN. Chlorophyll *a* concentrations ranged from 0.8 to 6.8 µg L^{-1} with the highest and lowest values in July at 7.5 and 30 m depths, respectively. This parameter clearly revealed that the phytoplankton was not distributed homogeneously through the water column and that it was mainly concentrated at 0–20 m. As a matter of proof, water transparency varied between 4.5 m (at the end of July) and 11 m (mid-November) and the estimated euphotic zone thus varied between 11 and 28 m.

3.2 Abundances of heterotrophic bacteria, picocyanobacteria and other phytoplankton

The heterotrophic bacterial population showed strong month-to-month and vertical variability, with maximum abundance (5.76×10^6 cells mL^{-1}) observed in August at 10 m (Table 1). Bacterial abundance varied up to 5- and 11-fold with month and depth, respectively. Similarly, the picocyanobacterial abundance was the highest at 10 m, with an average of $1.1 \pm 0.7 \times 10^5$ cells mL^{-1}, and the lowest at 30 m ($2.2 \pm 1.4 \times 10^3$ cells mL^{-1}). The picocyanobacterial abundance maximum was recorded in August (1.9×10^5 cells mL^{-1}) and minimum in November (2.1×10^2 cells mL^{-1}). Like bacterial and picocyanobacterial abundances, other FCM phytoplanktonic groups considered all together also displayed vertical

Table 1. Minimum, maximum, mean and standard deviation values for all the variables measured in Lake Geneva in this study from July to November 2011. Abbreviations are mentioned in the text.

Variables	Min	Max	Mean	SD
Bacteria (cells mL^{-1})	5.27×10^5	5.76×10^6	2.15×10^6	1.33×10^6
Picocyanobacteria (cells mL^{-1})	2.11×10^2	1.94×10^5	5.72×10^4	5.82×10^4
Other phytoplankton (cells mL^{-1})	1.48×10^1	3.21×10^4	4.05×10^3	7.64×10^3
VLP1 mL^{-1}	2.92×10^7	1.21×10^8	7.16×10^7	2.92×10^7
VLP2 mL^{-1}	4.83×10^5	1.30×10^7	5.25×10^6	3.41×10^6
VLP mL^{-1}	3.06×10^7	1.29×10^8	7.69×10^7	3.23×10^7
VPR	13.18	170.35	36.5	25.4
Temperature (°C)	6.22	21.53	14.0	5.15
Turbidity (FTU)	0.71	2.01	1.16	0.30
PAH ($\mu g\,L^{-1}$)	23	35	28.5	3.84
PC ($\mu g\,L^{-1}$)	0.14	1.48	0.66	0.417
pH	7.71	8.73	8.14	0.34
Par_w (µE)	0.58	730.2	92.8	164
O_2 ($\mu g\,L^{-1}$)	5.19	10.92	8.69	1.43
NO_2 ($\mu g\,L^{-1}$)	0.0	11.0	3.0	2.0
PO_4 ($\mu g\,L^{-1}$)	2.0	14	4.0	1.9
P_{part} ($\mu g\,L^{-1}$)	1.0	11.2	4.0	2.0
COT ($mgC\,L^{-1}$)	0.23	1.61	1.05	0.23
NO_3 ($\mu g\,L^{-1}$)	80	610	340	187
SiO_2 ($\mu g\,L^{-1}$)	9.0	159	63.2	48.4
P_{tot} ($\mu gP\,L^{-1}$)	2.0	29.0	10	4.0
NH_4 ($\mu g\,L^{-1}$)	1.0	24.0	8.0	5.0
N_{tot} ($\mu gN\,L^{-1}$)	170	980	529	173
SO_4 ($mg\,L^{-1}$)	0.67	49.54	48.4	0.674
TAC ($meg\,L^{-1}$)	0.13	1.79	1.61	0.135
CHLA	0.80	6.778	3.62	1.48
Microplankton production ($mgC\,m^{-3}\,h^{-1}$)	0.0025	5.30	1.41	1.62
Nanoplankton production ($mgC\,m^{-3}\,h^{-1}$)	0.0076	2.41	0.68	0.74
Picoplankton production ($mgC\,m^{-3}\,h^{-1}$)	0.05	4.40	1.55	1.38

variations, with the highest concentrations observed at 7.5 m ($8.0 \pm 1.45 \times 10^3$ cells mL^{-1}) in August and the lowest at 30 m ($3.6 \pm 2.1 \times 10^2$ cells mL^{-1}) in November (Fig. 2).

3.3 Virus-like particle abundances and lytic infection rates

Maximum abundances of the virus-like particles (VLPs) were observed at 2.5 m ($11.0 \pm 3.1 \times 10^7$ particles mL^{-1}) and the minimum at 30 m ($4.4 \pm 0.74 \times 10^7$ particles mL^{-1}). Highest and lowest VLP abundances were observed in September (1.3×10^8 particles mL^{-1}) and November (3.1×10^7 particles mL^{-1}), respectively (Fig. 2). VLPs could be discriminated into two major groups, referred to as VLP1 and VLP2. Average VLP1 and VLP2 abundances were $7.2 \pm 2.9 \times 10^7$ particles mL^{-1} and $0.53 \pm 0.34 \times 10^7$ particles mL^{-1}, respectively. The highest and lowest VLP1 and VLP2 abundances were 1.2×10^8 and 2.9×10^7 particles mL^{-1} and 1.3×10^7 and 4.8×10^5 particles mL^{-1}, respectively, and they were both measured at 2.5 and 30 m depth. The virus-to-bacteria ratio was highest at 20 m depth (56.6 ± 5.6) and lowest at 10 m (27.6 ± 12.2). The highest (170) and lowest (13.8) ratios were observed in July at different depths.

TEM analysis revealed that phages were mainly associated with oval and short rod morphotypes with an occurrence of 28 %, followed by thin rods (25 %) and cocci (19 %). The burst size of these morphotypes was on average 46 for oval morphotypes and less than 15 for the short rods. The burst size ranged from 15 to 132 in July, whereas the range was 21 to 35 in November. The average burst size ranged from 28 to 44 (mean = 32.6, Table 2). The frequency of virus-infected cells ranged from 1.1 % in November to 2.7 % in July. Similarly the FIC was relatively low in November (7.2 %) and high in July (22.5 %). The virus-induced bacterial mortality was calculated to vary between 8.2 % (November) and 34.9 % (July), and there was a clear trend of decreased mortality from early summer to the end of fall (Fig. 3a).

Virus-induced mortality on picocyanobacteria, assessed using the modified dilution method, was also found to be

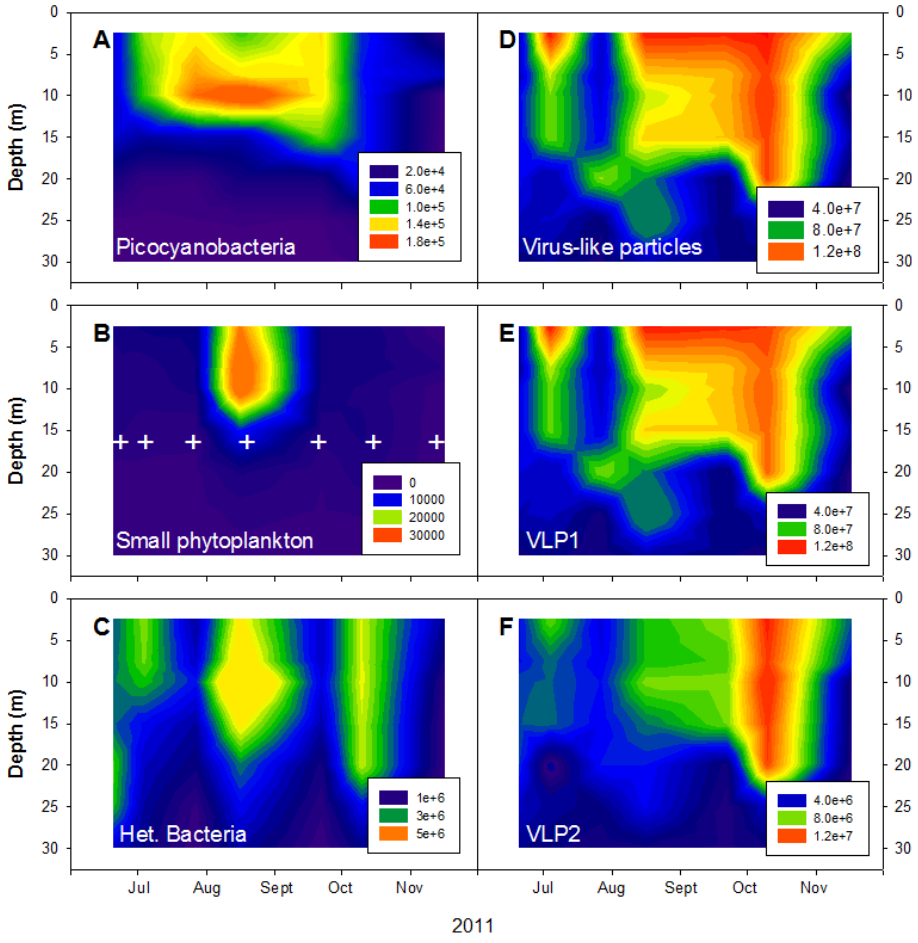

Fig. 2. Vertical distribution of picocyanobacteria **(A)**, small phytoplankton populations **(B)**, heterotrophic bacteria **(C)**, total virus-like particles **(D)**, VLP1 **(E)** and VLP2 **(F)** from July to November 2011 using flow cytometry. The white crosses in **(B)** refer to sampling dates. The interpolation between the data was generated automatically by SigmaPlot 12.0.

relatively high in summer (reaching 66 % in August), while it could be insignificant at other periods, i.e., in November (Table 2). Comparatively, the virus-mediated bacterial mortality ranged from 18.3 % (August) to 33.5 % (July), and no trend was recorded as for TEM-estimated values (Fig. 3b). The grazing impact varied also a lot during the period of study (from 0 to 50 %), and it was globally lower than the viral impact for both the picocyanobacteria and the heterotrophic bacteria.

3.4 Bacterial and viral production

Bacterial production (BP) ranged from 9.1 to 36.9 $\mu gC\,L^{-1}\,d^{-1}$. The lowest BP was measured in September and the highest in July. The BP values slowly recovered during October–November, reaching a value of 15.5 $\mu gC\,L^{-1}\,d^{-1}$ (Fig. 4). The lowest viral production rate was found to be 1.24×10^7 particles $mL^{-1}\,d^{-1}$ in July. Thereafter it increased from August to October, reaching up to 6.28×10^8 particles $mL^{-1}\,d^{-1}$ in October. Viral production decreased to approximately 5.1×10^8 particles $mL^{-1}\,d^{-1}$ in November (Fig. 4).

3.5 Size-fractionated primary production

Primary production measured at 5 different depths between 2.5 and 20 m revealed marked seasonal and depthwise variations with all possible contributions of micro-, nano- and picoplankton to the total primary production. The total primary production maximum was estimated at 7.5 m depth in summer and at 2.5 m in fall. A maximum production rate of 18.5 $mgC\,m^{-3}\,h^{-1}$ was reported at 7.5 m in August. With 6.6 $mgC\,m^{-3}\,h^{-1}$ at 2.5 m, November was the least productive month. The production rates decreased rapidly below 10 m depth. The picophytoplanktonic contribution was relatively high in October and November (Fig. 5), reaching up to 76 %, while it was only 33 % in August. The depth at which the maximum picophytoplankton contribution was reported was 15 m throughout the sampling period.

Table 2. Mean values for the frequency of virus-infected cells (FVIC), frequency of infected cells (FIC), virus-induced bacterial mortality (VIBM) and average burst size (BS) as estimated using transmission electron microscopy. PCGM refers to picocyanobacterial mortality due to grazing, while PCVM refers to the virus-mediated mortality of this community. BGM and BVM are as above but for the heterotrophic bacteria.

Sample	TEM analysis				Dilution experiments			
	FVIC	FIC	VIBM	BS	PCGM (%)	PCVM (%)	BGM (%)	BVM (%)
4 Jul	2.7 ± 0.1	22.5 ± 2.7	34.9 ± 11.2	41 ± 4	6.8 ± 5.6	23 ± 8.2	17.4 ± 6.8	19.9 ± 8.1
27 Jul	2.1 ± 0.3	16.7 ± 8.7	23.1 ± 5.7	30 ± 11	12.1 ± 10.2	16.2 ± 12.4	28.9 ± 13.7	33.5 ± 15.4
16 Aug	1.4 ± 0.6	10.1 ± 2.4	12.2 ± 3.4	32 ± 7	50.2 ± 23.8	66 ± 31.7	NS	18.3 ± 9.3
22 Sep	1.7 ± 0.2	12.9 ± 4.1	16.5 ± 2.6	44 ± 14	NS	35.7 ± 11.6	11.6 ± 3.7	19.8 ± 8.5
10 Oct	1.2 ± 0.1	8.2 ± 2.1	9.5 ± 3.3	28 ± 8	NS	19.1 ± 6.9	18.1 ± 5.9	23.5 ± 13.9
17 Nov	1.1 ± 0.2	7.2 ± 3.1	8.2 ± 1.9	28 ± 6	16.9 ± 9.5	NS	11 ± 4.6	21.1 ± 10.8

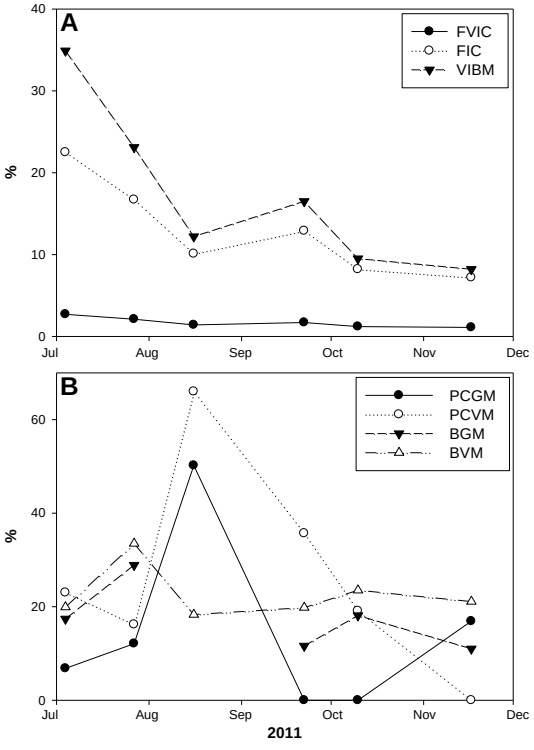

Fig. 3. Patterns of the frequency of virus-infected bacterial cells (FVIC), the frequency of infected bacterial cells (FIC) as estimated using transmission electron microscopy and the virus-induced bacterial mortality (VIBM) **(A)**. Grazing mortality and viral lysis on picocyanobacteria (PCGM and PCVM, respectively) and on heterotrophic bacteria (BGM and BVM) estimated using the modified dilution technique **(B)**.

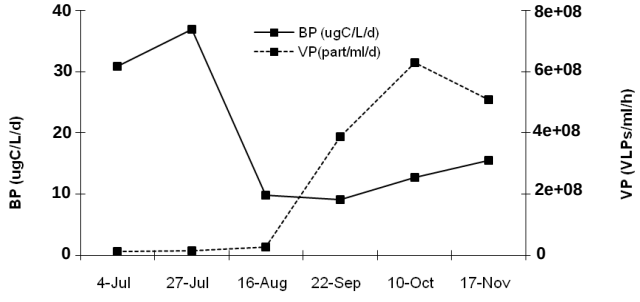

Fig. 4. Bacterial production (BP) and viral production (VP) measured from July to November 2011.

3.6 Statistical analysis

The various environmental and biological factors showed significant seasonal variations. Summer months behaved differently with complex interactions between biological variables and physicochemical parameters. The bacterial abundance was significantly correlated with picocyanobacteria, while picocyanobacteria were positively correlated with temperature, pH and O_2, and negatively with NO_3, SiO_2 and total phosphorus. In summer, VLP1 did not show any correlation with any of the biological factors including the bacterial abundance, but had significant correlations with physicochemical factors like pH and total phosphorus and negative correlation with NO_3, SiO_2, total phosphorus and total nitrogen. VLP2 on the other hand displayed a significant positive correlation with picocyanobacteria and other phytoplankton. At first sight, temperature seems to be the most determining factor for planktonic abundance when compared to phosphorus. But over the months, the relationships were more complex with significant correlations between important measured biotic and abiotic factors, except for Chl *a* (Table 3).

A principal component regression analysis was then performed to examine the environmental factors determining the dynamics of different planktonic communities in the take. The two main factors (axes) explained more than 70 % of the total variance. Temperature and pH were important significant factors influencing the abundance not only of bacteria and picocyanobacteria but also of the primary production. For bacteria, factor 1 contributed to an eigenvalue of 4.34, which included temperature (0.87), pH (0.94), NO_3 (-0.87), SiO_2 (-0.93) and P_{tot} (0.70). For the picocyanobacteria,

Table 3. Results of Pearson's correlation analysis to test for empirical correspondence among estimated variables. Abbreviations are explained in the main text. Significant correlations are in bold at $P < 0.01$ at $n = 42$.

	Bact	PC	PP	VLP1	VLP2	VLP	Temp	NO_3	SiO_2	P_{tot}	N_{tot}	pH
PC	**0.78**											
PP	**0.79**	**0.82**										
VLP1	**0.83**	**0.74**	**0.65**									
VLP2	**0.79**	**0.81**	**0.66**	**0.88**								
VLP	**0.83**	**0.75**	**0.66**	**0.99**	**0.90**							
Temp	**0.56**	**0.79**	**0.60**	**0.61**	**0.67**	**0.63**						
NO_3	**−0.78**	**−0.88**	**−0.76**	**−0.81**	**−0.83**	**−0.82**	**−0.77**					
SiO_2	**−0.72**	**−0.94**	**−0.80**	**−0.73**	**−0.75**	**−0.74**	**−0.79**	**0.86**				
P_{tot}	**0.62**	**0.54**	**0.60**	**0.57**	**0.42**	**0.56**	**0.44**	**−0.51**	**−0.61**			
N_{tot}	**−0.77**	**−0.78**	**−0.66**	**−0.70**	**−0.78**	**−0.72**	**−0.70**	**0.89**	**0.72**	**−0.44**		
pH	**0.72**	**0.84**	**0.76**	**0.69**	**0.66**	**0.69**	**0.77**	**−0.84**	**−0.87**	**0.59**	**−0.74**	
Chl *a*	0.17	0.32	0.20	0.14	0.09	0.13	0.34	−0.12	−0.31	0.32	−0.20	0.27

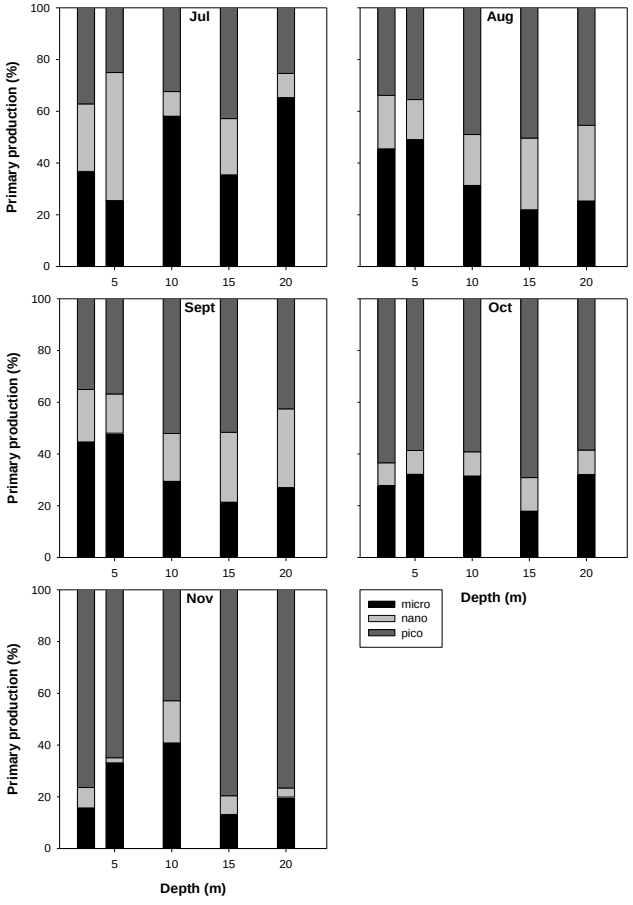

Fig. 5. Fractionated primary production representing the contribution of the various size fractions of the phytoplankton (i.e., the pico-, nano- and microphytoplankton) at the different periods sampled.

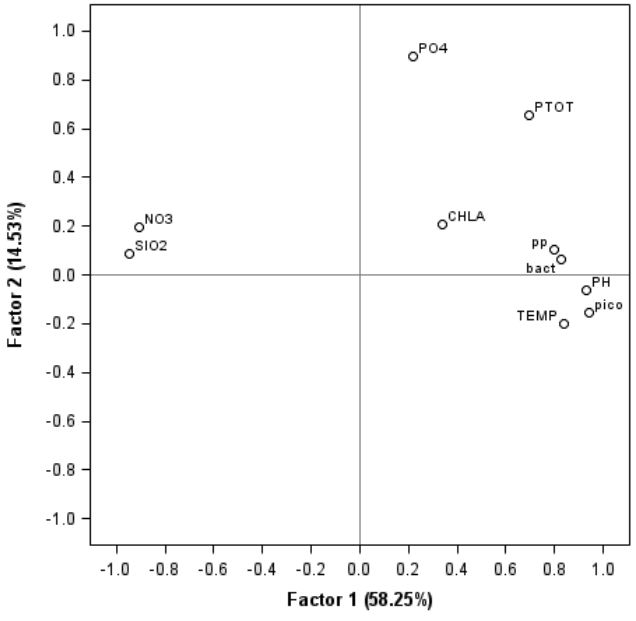

Fig. 6. Principal component regression analysis for bacteria, picocyanobacteria and other phytoplankton with the rest of the environmental variables.

PO_4 (0.89) and P_{tot} (0.64) were the major components in addition to temperature and pH. For VLPs, bacteria (0.83), picocyanobacteria (0.94) and phytoplankton (0.79) were the significant determinants, with an eigenvalue of 6.41. Temperature, pH, NO_3, P_{tot}, SiO_2 and Chl *a* explained 65 % of the variability in bacterial abundance (Fig. 6). In the case of picocyanobacteria and other phytoplankton groups, the abovementioned factors accounted for a variability of 90 and 60 %, respectively. In the case of VLPs, host abundances (bacteria, picocyanobacteria and potentially all other phytoplanktonic cells) also played an important role in determining the variations observed for viral abundances. Principle component

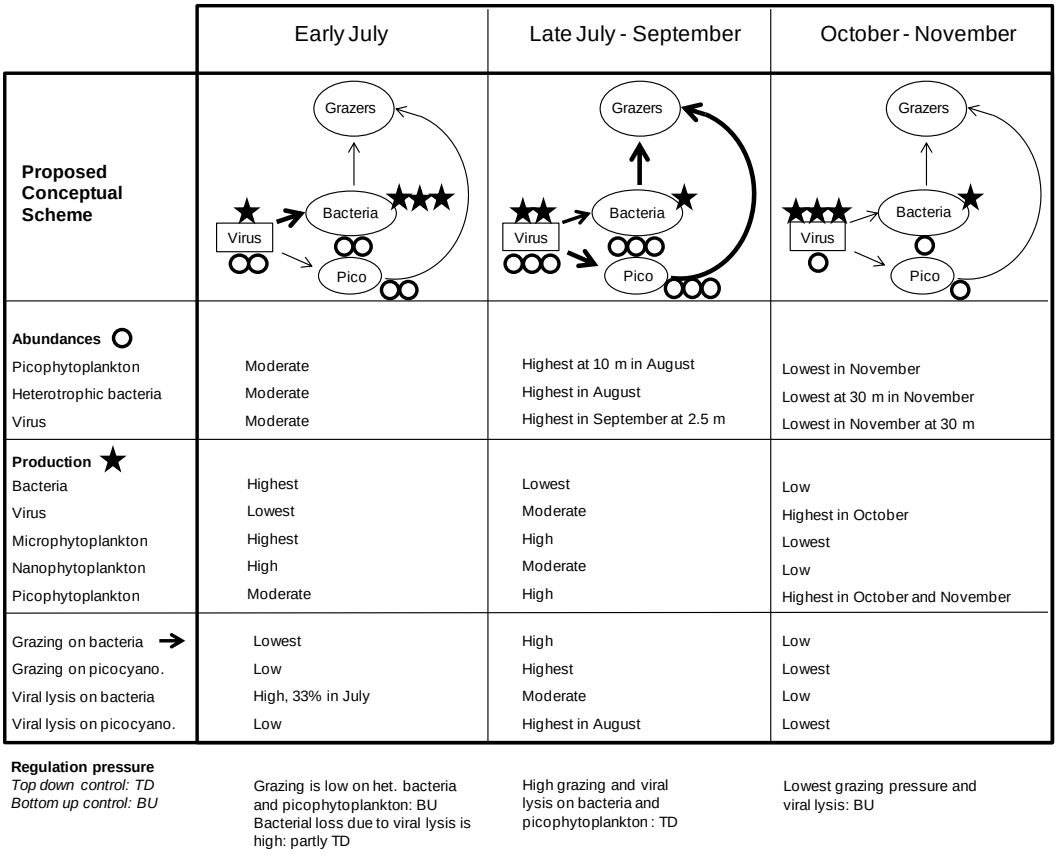

Fig. 7. Conceptual scenario for the seasonal succession of two major constituents of the microbial food web (the heterotrophic bacteria and the picophytoplankton) in upper layers (0–20 m) of Lake Geneva between July and November 2011.

regression analysis revealed that temperature, pH, NO_3, P_{tot}, SiO_2 and Chl *a* together with host abundances (bacteria, picocyanobacteria and other phytoplankton) contributed 77 % variability in VLP1, 72 % in VLP2 and 78 % in total VLP abundances (not shown).

4 Discussion

The main aim of the present study was to shed light on ecological changes occurring in Lake Geneva from early summer to the end of fall. How environmental changes and specific biotic interactions could influence different microbial components (both in terms of abundance and activity) helped us to highlight the importance of some key parameters and viral or grazing pressure in the functioning of the microbial food web of Lake Geneva. In particular, a conceptual model could be proposed for the seasonal succession of key components of the microbial food web structure in the upper lit layer (0–20 m) of Lake Geneva between July and November 2011 (Fig. 7).

We observed clear variations in the environmental factors such as air and water temperature, light conditions and nutrient concentrations. These variations impacted the abundance of various microbial communities (bacteria, viruses, and picocyanobacteria), which were comparable to what has been reported earlier for Lake Geneva (Duhamel, 2006; Personnic et al., 2009a; Parvathi et al., 2012). The abundances of viruses, bacteria and autotrophic picoplankton changed markedly with months and depths (Table 4). The phytoplankton distribution through the water column was not homogeneous, which could be due to the vertical temperature stratification and light availability. Higher plankton abundance in summer was attributed to higher temperature, availability of light and nutrients that are key determinants for their growth. This is further emphasized by the fact that the transparency was lower during summer compared to fall.

The role of nutrients and their role in controlling the temporal fluctuations in abundance and activity of planktonic algal communities have been considered since the earliest days of phytoplankton ecology (Hutchinson, 1967). The planktonic community structure of an aquatic system largely depends on the lake's trophic state and contribution of picoplankton production to the total autotrophic production, which could vary depending on the nutrient concentrations of the lakes (Stockner and Porter, 1988). These seasonal and vertical variations in total nitrogen and phosphorus have

Table 4. Results of the two-way ANOVA to test significant differences in the abundance of bacteria, picocyanobacteria, other phytoplankton and VLPs. Degrees of freedom are indicated as df. S = significant; NS = not significant; $P < 0.01$.

Parameter	Difference	df	F value	Significance
Bacteria	months	5, 30	31.2	S
	depth	6, 30	12.3	S
Picocyanobacteria	months	5, 30	4.5	S
	depth	6, 30	14.3	S
Other phytoplankton	months	5, 30	7.1	S
	depth	6, 30	2.3	NS
VLP1	months	5, 30	8.7	S
	depth	6, 30	8.7	S
VLP2	months	5, 30	13.7	S
	depth	6, 30	9.4	S
VLP	months	5, 30	9.2	S
	depth	6, 30	9.0	S

reflected in the distribution and activity of various planktonic fractions. The relationship between phosphorus concentration and chlorophyll (Dillon and Rigler, 1974) suggests that phosphorus, and at times nitrogen and silicon, are limiting resources. Previous studies in Lake Geneva suggested that P_{tot} is a critical component determining dynamics of planktonic components in this lacustrine ecosystem (Anneville et al., 2002). The abundance of picocyanobacteria was high during summer months (August–September) when the nutrient concentrations were higher (P_{tot} being $11.0 \pm 4.9\,\mu gP\,L^{-1}$ in summer) compared to autumn ($8.2 \pm 1.9\,\mu gP\,L^{-1}$). The abundance of planktonic communities positively correlated with P_{tot} and negatively correlated with NO_3. P is known as an important component in the dynamics of planktonic communities in lakes (Wetzel, 2000), but the significance of N in regulating the plankton dynamics especially in Lake Geneva has only been recently reported (Tadonléké et al., 2009). In this regard, the capacity of picocyanobacteria to use N sources like NH_4/NO_3 or switch over from P or N subject to the nutrient availability could be considered as an important factor in determining the plankton dynamics in this lake. It is reported that NO_3 is taken up by picocyanobacteria in culture when NH_4 is depleted (Bird and Wyman, 2003). Even though the abundance was higher in summer, picoplankton production (constituted mainly by picocyanobacteria in Lake Geneva) contributed to a high percentage (76 %) of the total primary production during autumn (i.e., October and November), suggesting a significant functional role of the "smalls" in Lake Geneva. This could be explained by lower nutrient concentration of total nitrogen ($280\,\mu g\,L^{-1}$) and total phosphorus ($10\,\mu g\,L^{-1}$) during the fall period. It is reported that, when nutrients become a limiting factor, autotrophic picoplankton cells strongly compete with the bigger phototrophic organisms (Raven, 1988; Callieri, 2008). Previous studies reported picocyanobacteria to be the major contributors to total primary production, with their contribution increasing with depth (Platt et al., 1983) due to greater efficiency of their auxiliary pigments (typically phycoerythrin) to utilize the blue-green light (Glover et al., 1985). Long-term changes in phytoplankton composition due to P loading in Lake Geneva have been found in the past (Anneville et al., 2002). However, to interpret these long-term changes, it is important to gather information on a monthly or seasonal timescale. This study reveals significant seasonal variations in the plankton dynamics which could largely be contributed to temperature, nutrient availability and the wind-induced waves as has previously been reported for peri-Alpine lakes (Vincon-Leite et al., 1989). The results obtained in the present investigation clearly suggest that picocyanobacteria play a crucial role in the trophic status and ecosystem productivity of Lake Geneva as suggested in the past studies for this lake (Duhamel et al., 2006; Personnic et al., 2009a) and elsewhere (Callieri, 2010).

Among the abiotic factors, temperature and pH were the most influential factors in determining the abundance of bacteria, picocyanobacterial and other phytoplankton which contributed to their monthly variability. Both these parameters were positively correlated and hence play an important role in the structuring of planktonic communities in the lake. Phytoplankton reproduction rates are closely linked to temperature. The maximum rate of cell division doubles for each 10 °C increase in temperature. The upper limit of growth is therefore determined by temperature (Harris, 1986). Temperature has been reported to be crucial to *Prochlorococcus* (Olson et al., 1990) and indicated as a dominant factor influencing the seasonal dynamics of both picocyanobacteria and picoeukaryotes in Lake Kinneret (Rushansky et al., 2002). Note that we also found that temperature was among the best predictors of *Synechococcus* spp. abundance in Lake Bourget (Jacquet et al., 2012). Several phytoplankton species (e.g., the diatom *Skeletonema costatum*), however, increase their assimilation rates of nutrients at lower temperatures and subsequently increase biomass (Goldman, 1977). The different responses to temperature exhibited by phytoplankton species can lead to a strong seasonal change in species composition and biomass. Most studies of pH effects on algae have been conducted in freshwater systems where the carbonate buffering system is weaker than in seawater and pH may fluctuate dramatically (Chen and Durbin, 1994). It can change the distribution of carbon dioxide species and carbon availability, alter the availability of trace metals and essential nutrients, and at extreme pH levels potentially cause direct physiological effects. It was suggested that, because the solubility and availability of CO_2 decrease at high temperature, growth of cyanobacteria with high affinity to CO_2 is enhanced (Shapiro, 1990). Variations in viral and bacterial abundances suggested that environmental factors had strong influence on the planktonic communities, as reported in other freshwater systems (Pradeep Ram et al., 2005). Our study

thus clearly demonstrates that the temperature is a key factor in determining the higher abundance and distribution of phytoplankton in the euphotic layer, which in turn could influence pH.

VLP abundances are linked to the physical and chemical characteristics through their dependence on their heterotrophic and autotrophic hosts. VLP abundances were significantly higher in the top 20 m (euphotic) layer, and the abundances correlated with bacterial and picocyanobacterial abundances. High VBR in autumn (October and November) and its increase with depth suggested important phage–host interactions. This may also indicate that the impact of viruses on bacteria is more significant in deeper waters than at the surface, as previously observed in this lake and other ecosystems (Weinbauer and Hofle, 1998; Colombet et al., 2006; Personnic et al., 2009a). Such relationships between these groups were clearly confirmed with the measurements of high virus induced mortality on both heterotrophic bacteria and picocyanobacteria. Previous studies have shown a tight coupling of VLP1 and VLP2 with bacterial and picocyanobacterial abundance, respectively (Duhamel et al., 2006; Personnic et al., 2009b). Higher VLP abundance in the upper 20 m depth could be due to bacterial growth which was stimulated by high temperatures, pH, organic and inorganic nutrients as reported elsewhere (Weinbauer, 2004). The relationship of the viruses with bacteria and picocyanobacteria varied with respect to sampled months, suggesting shifts in the succession of hosts and viruses (Parvathi et al., 2012; Zhong et al., 2013). It is also possible that there were larger initial virioplankton and bacterioplankton populations in the summer months and at the beginning of autumn (Personnic et al., 2009a). A quite similar seasonal pattern was observed for the virioplankton in other temperate lakes, where highest viral abundance occurred in autumn (Bettarel al., 2005; Padeep-Ram et al., 2010). Chl *a* did not have significant correlation with viruses, suggesting that phytoplankton viruses did not contribute significantly to the total virus pool, and that the positive effect of an increase in chlorophyll *a* with heterotrophic bacteria is not directly beneficial to viral production. However, we are aware that Chl *a* represents only a crude approximation of the algal biomass and thus is probably not the best parameter to use while attempting to identify virus–host relationships (Gasol and Duarte, 2000). It is also possible that picocyanobacteria were the most dominant phytoplanktonic group, thus making them more available for viral attachment (Jacquet et al., 2002). The dynamics of larger phytoplankton and their contribution to primary production showed seasonal variations induced by the environmental factors. High viral production rates (up to 2.6×10^7 particles $mL^{-1}\,h^{-1}$) corroborated this strong viral impact and high viral production coincided indeed with high virus induced bacterial (23 %) and picocyanobacterial (19 %) mortality. Thus, it was not surprising that for both bacteria and picocyanobacteria the loss percentage due to grazing was always lower than viral lysis (as estimated by the dilution method when applicable). These mortality rates were comparable with earlier reports of grazing mortality and viral lysis reported for Lake Geneva and other lakes (Weinbauer and Hofle, 1998; Bettarel et al., 2004; Duhamel et al., 2006; Personnic et al., 2009b). All in all, it was found that viral activity was particularly high in autumn, and covaried with the picoplankton production during the same time period, a result already suggested for these peri-Alpine lakes (Personnic et al., 2009a, b).

All together, results obtained in the context of this study made possible the construction of a conceptual scenario for the seasonal succession of viral and plankton abundance and production as well as the importance of the abiotic and biotic parameters in the upper layers (0–20 m) of Lake Geneva between July and November 2011 (Fig. 7). The validity of such a model remains to be tested for other years.

5 Conclusions

The present study highlighted complex relationships among the microbial components of Lake Geneva where physical, chemical and biotic interactions intervene in the dynamics and activity of the picoplankton size community. The results clearly suggest that the picophytoplanktonic size fraction can be responsible for a significant part of the production of this lake and also show how viral action can be a driving force in the dynamics of the picoplankton (through virus-induced picocyanobacterial and heterotrophic bacterial mortality). Lake Geneva can be considered as a model ecosystem for large and deep temperate lakes, and our analysis, even though limited to only a few months during a single year, strongly highlights the importance of considering the viral component in freshwater plankton ecology.

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