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T. Sime-Ngando · J. Colombet · S. Personnic
I. Domaizon · U. Dorigo · P. Perney
J. C. Hustache · E. Viollier · S. Jacquet

Short-term variations in abundances and potential activities of viruses, bacteria and nanoprotists in Lake Bourget

Abstract Samples were collected at four depths every 6 h over a 42-h period during two contrasting seasons (June vs. December) from Lake Bourget, France, for evidence of circadian fluctuations in the concentrations and potential activities of viruses, prokaryotes and protists in relation to environmental conditions: temperature, chlorophyll *a* and dissolved organic carbon (DOC) concentrations. Considerable vertical and temporal fluctuations were observed for all variables. Circadian variations were noted for DOC and chlorophyll *a* concentrations. Despite the external abiotic forcing (light, water movements), the fluctuations of microbial variables (including viruses) in most cases were apparently linked to biotic factors and interactions. Standing stocks and activities, as well as the number and levels of correlations among the microbial components, were, surprisingly, higher in winter than in summer. We speculate that this was because trophic interactions prevailed over the seasonal forcing (i.e. temperature) in shaping the observed differences.

Keywords Lakes · Diurnal cycles · Viruses · Virioplankton · Bacteria · Protists

T. Sime-Ngando (✉) · J. Colombet
UMR CNRS 6023, Biologie des Protistes,
Université Blaise Pascal, Clermont-Ferrand II,
63177 Aubière Cedex, France
E-mail: Telesphore.SIME-NGANDO@univ-bpclermont.fr
Tel.: +33-4-73407836
Fax: +33-4-73407670

S. Personnic · U. Dorigo · P. Perney · J. C. Hustache · S. Jacquet
UMR CARRTEL, Station INRA d'Hydrobiologie Lacustre,
74203 Thonon Cedex, France

I. Domaizon
Université de Savoie, 73376 Le Bourget du Lac Cedex, France

E. Viollier
UMR CNRS 7047, Géochimie des Eaux,
Université Paris 7 et IPGP, 75251 Paris Cedex 05, France

Introduction

Because turnover rates are relatively high, species composition, cell counts, biomass and activities of pelagic microorganisms can vary substantially over short time scales (Sime-Ngando and Hartmann 1991; Amblard et al. 1994; Jugnia et al. 2000; Winter et al. 2004). Some microorganisms, such as ciliates, are motile, chemosensory and photosensitive, and others, such as copepods, can migrate vertically on a diel cycle (see Sime-Ngando and Hartmann 1991). In general, fluctuations in both biotic and abiotic parameters are discernible in pelagic ecosystems at the time scale of diurnal cycles.

Basically, this is because both autotrophic and heterotrophic processes depend on factors as obvious as light. Light can govern cycles of resources, such as phytoplankton exudates, and influence heterotrophic processes. Light can also influence the mortality of pelagic microbes, either directly through DNA damage (Herndl et al. 1993; Jeffrey et al. 1996) or indirectly, since it is a dominant mechanism of viral destruction and inactivation (Suttle and Chen 1992; Wommack et al. 1996; Noble and Fuhrman 1997; Weinbauer et al. 1997). Enhanced solar ultraviolet exposure has also been shown to be an inhibitory factor for nanoflagellate bacterivory (Ochs 1997; Ochs and Eddy 1998). Other studies have concluded that light may stimulate heterotrophic processes, such as photoenzymatic activity or photoreactivation (i.e. light-dependent DNA repair) in a bacterial community (Weinbauer et al. 1997) and may also facilitate digestion, grazing and growth rates of protozooplankton (Strom 2001). The direct impact of light on crustacean zooplankton and ichthyoplankton has also been partially tested (Browman et al. 2000).

However, it is usually difficult to differentiate the causes of short-term variability in natural communities. In addition to external abiotic forcing and day–night cycles, a mixture of factors such as water movements, turbulence, population dynamics and interactions may be involved (Sime-Ngando and Hartmann 1991;

Sime-Ngando et al. 1991), including possibly chaotic fluctuations (Becks et al. 2005; Mandal et al. 2006). Microbial communities in a given sampling point within the water column can be imported or exported horizontally or vertically as a result of advection, thereby, changing the variance resulting from biotic interactions in relation to the diel cycle. Consequently, the effects of diel cycles on the biology of the plankton are usually difficult to isolate because they are obscured by the hydrology of the system. This is well known from lake studies (Sime-Ngando and Hartmann 1991; Sime-Ngando et al. 1991; Amblard et al. 1994; Jugnia et al. 1998, 2000; Tadonléké et al. 1998) compared to the oceans; where the use of free-floating buoy or drifter deployments is more practicable (Weinbauer et al. 1995; Graham et al. 2000; Winter et al. 2004).

The aim of the present study was to examine short-term variations in the abundances and potential activities of viruses, prokaryotes and nanoprotists in Lake Bourget, France, in relation to environmental conditions, i.e. temperature, chlorophyll *a* and dissolved organic carbon (DOC) concentrations. Samples were collected from one station at four different depths representative of the water column every 6 h over a 42-h period during two contrasting seasons (June vs. December) for evidence of circadian fluctuations. High-resolution profiles of temperature and of chlorophyll *a* concentration were determined in an effort to track the vertical structure (i.e. physical and biological) of the whole water column. This is the first attempt to study short-term variability in the plankton of Lake Bourget.

Materials and methods

Study site, sampling and physico-chemical parameters

Lake Bourget is located on the edge of the Alps (45°44′N, 05°51′E). It is an elongated (18 km and 3 km in length and width, respectively) north–south orientated lake, with a surface area of 42×10^6 m^2, a total volume of 3.5×10^9 m^3, maximum and average depths of 145 m and 80 m, respectively and a water residence time of approximately 10 years. It has a catchment area of about 560 km^2, with maximum and average altitudes of 1,845 m and 700 m, respectively. Water quality restoration programmes started in the 1970s have significantly lowered the trophic status of the lake, from highly eutrophic to mesotrophic. In 2005, the water transparency varied between 2.4 m and 14.5 m, the total P concentration in winter was at about 23 μg l^{-1} and the maximum concentration of chlorophyll *a* was less than 13 μg l^{-1} (Jacquet et al. 2005a, b).

The sampling strategy was decided based on the available logistics and included two short-term sampling series carried out at a reference station known as point I in Gresine Bay (maximum depth $Z_{max} = 35$ m), located in the eastern part of the lake. For each series, four different depths were sampled (2, 10, 15 and 30 m) eight times, every 6 h, from 6:00 p.m. on 9th June to 12:00 noon on 11th June 2004 for the first series, and from 12:00 noon on 1st December to 6:00 a.m. on 3rd December 2004 for the second series. Before each sampling operation, high-resolution vertical profiles (i.e. continuous measurement in the whole water column) of temperature and chlorophyll *a* concentrations (Chl) were monitored using FluoroProbe® (BBE, Moldenke, Germany), a submersible spectrofluorometer configured for in situ measurements of chlorophyll *a* fluorescence, as described and detailed elsewhere (Leboulanger et al. 2002). For the June sampling series, DOC levels were determined in 15-ml aliquots of filtered (pre-combusted glass-fibre filters) samples collected in pre-combusted glass vials. The samples were then acidified to pH < 3 with 2 N HCl and the vials were flame-sealed and stored at 4°C in the dark until analysis. DOC concentrations were measured using a carbon analyser (Labtoc, UV promoted persulphate oxidation, IR detection), as previously described (Comte et al. 2006).

Virus and cell counts

The abundances of viruses, heterotrophic bacteria and picocyanobacteria were estimated immediately after sampling using flow cytometry. A 1-ml sample was analysed without adding any fixative or dye to analyse the autotrophic picoplankton community. In Lake Bourget, this community is typically dominated by Phycoerythrin-rich cyanobacteria (Humbert and Le Berre 2001; Briand et al. 2005; Jacquet et al. 2005a). Another 1-ml sample was fixed with glutaraldehyde (0.5%, v/v, final conc.) and bacterial and viral counts were performed as described elsewhere (Duhamel and Jacquet 2006; Duhamel et al. 2006). We used a FAC-SCalibur flow cytometer (Becton Dickinson) equipped with an air-cooled laser providing 15 mW at 488 nm with the standard filter set-up. Fluorescent microbeads (Molecular Probes) of diameter 1 μm were added to each sample as an internal standard. For heterotrophic bacteria, samples were diluted with 0.2-μm-filtered water from the lake, while for viruses, samples were diluted with 0.02-μm-filtered TE (Tris–EDTA, pH = 8) buffer and heated for 10 min at 75°C. The samples were stained with SYBR Green I (1/10,000 final conc.) for 15 min in the dark and run at medium speed (ca 40 μl min^{-1}). Either a minimal number of 10,000 events were recorded in log mode for each sample or a minimal acquisition time of 4 min when the number of events was < 10,000. Plots of green fluorescence measured at 530 ± 30 nm versus 90° light scatter or forward-angle light scatter were used to discriminate and count the stained items. Bacteria with high DNA content (HDNA) were discriminated (i.e. from those cells with low DNA content) on the basis of their typical FCM signatures within the total community and were considered as the most

dynamic members of the bacterial assemblage (Gasol et al. 1999).

For the nanoflagellate counts, glutaraldehyde (1%, v/v, final conc.) fixed samples were filtered (pressure < 100 mmHg) over polycarbonate membranes (diameter: 25 mm, pore size: 0.8 μm), stained with primulin (Caron 1983), mounted between slides and glass cover slips with a non-fluorescent immersion oil and stored at $-20°C$ until analysis. The slides were examined under UV light to count heterotrophic nanoflagellates and under blue light to count pigmented flagellates at 1,250× magnification. Green light was useful to display phycoerythrin-containing flagellates.

Bacterial production and enzyme activity

Bacterial production (BP) was determined from the incorporation of [methyl-^{3}H] thymidine (Amersham Biosciences, UK, 70–95 Ci/mmol of specific activity) into bacterial DNA (Bell 1993). For each sampling time and depth, three replicates and one formalin-killed control were inoculated with thymidine at a final concentration of 20 nM in glass vials and then incubated in-situ for 2 h. The live incubations were stopped by adding formalin and all samples were extracted in the laboratory using trichloroacetic acid (TCA, 10% final conc.) and kept on ice for 30 min. The samples were then filtered through a 25-mm-diameter, 0.22-μm-pore-size membrane (GTTP). The filters were then rinsed three times with 3 ml of TCA (5% final conc.). The filters were placed in scintillation vials, allowed to dry and solubilised with 1 ml of soluene. After adding 10 ml of scintillation cocktail (Hionic Fluor, Perkin Elmer), the radioactivity was counted with a 2100-TR (Packard Instruments) with counting efficiency corrected for quenching. Bacterial production, calculated in moles of ^{3}H-TdR incorporated into DNA, was converted into the number of cells produced by using a conversion factor (10^{18} cells mol^{-1}) recommended by Bell (1993). In all cases, the within-sample coefficients of variation ($n = 3$) were <15%.

For the December sampling series, the extracellular enzyme activity was determined as an additional proxy of bacterial activity in the winter. The cleavage of a model substrate leucine methyl-coumarin-amide (Leu-MCA; Sigma) was measured according to Somville and Billen (1983). Increasing amounts of the substrate Leu-MCA stock solution (40 mmol l^{-1}) were added to 2-ml bulk water subsamples collected at each depth (2, 10, 15 and 30 m), in order to obtain final concentrations ranging from 0 to 4,000 μmol l^{-1}. Incubation lasted one hour and was performed in the dark at 20°C and under slight movement with gentle stirring. The potential extracellular enzyme activity was then calculated for both unprocessed water and water filtered through the 2-μm filter, collected at each depth, by adding the fluorescent substrate to two replicate subsamples (2 ml) at the saturating concentration. The activities were

similar in both fractions (Students t-test, $p > 0.05$) and were correlated ($r = 0.80$, $p < 0.01$). For this reason, only the activity in the unprocessed water is presented. A blank used in order to assess the non-enzymatic hydrolytic activity of the substrate was prepared by filtering a sufficient amount of water from each depth through a 0.2-μm-pore-size filter, by autoclaving it and by adding 230 μl of glycerine buffer to inhibit the enzyme activity before adding the substrate. After incubation, the fluorescence was determined using a fluorometric and luminometric reader (Fluoroskan Ascent FL, Thermo Labsystem), at excitation and emission wavelengths of 355 nm and 460 nm, respectively. A calibration curve was established by dissolving a 2 mmol l^{-1} stock solution of the standard MCA (Sigma) in Methyl Cellosolve and adding 0.22-μm-filtered and autoclaved water from the sampling site. The final results are expressed in μmol MCAl^{-1} h^{-1}. The variability among replicate incubations was typically small (CV < 5%).

Viral bacteriolysis

In formalin-fixed samples, the bacteria contained in 8-ml subsamples were harvested by centrifuging onto 400 mesh copper electron microscope grids with carbon-coated Formvar film using a Centrikon TST 41.14 swing-out rotor (Bettarel et al. 2004). Each grid was then stained for 30 s with uranyl acetate (2% w/w) and examined using a JEOL 1200EX transmission electron microscope operated at 80 kV at magnifications of 20,000–40,000×. At least 1,300–1,500 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 FIC $= 9.524 \times$ FVIC-3.256 (Weinbauer et al. 2002). The FIC was then converted to viral-induced bacterial mortality (VBM, as a percentage per generation) according to Binder (1999) using the equation VBM $= (FIC + 0.6 \times FIC^2)/(1–1.2 \times FIC)$.

Statistical analyses

The data were subjected to one-way analysis of variance (absence of replicates for most variables prevents the use of two-way ANOVA) to test for effects of time or depth, and using correlation analysis to test for significant coupling between variables. In addition, a matrix of data was produced and analysed by means of principal component analysis (PCA) to identify combinations of variables that account for the largest amounts of the total variance observed. For all of the statistical analy-

ses, the data were normalised using logarithmic transformation.

Results

### Temperature and chlorophyll *a* depth–time profiles

In June, surface heating occurred in the late afternoon, increasing the relative thermal resistance to mixing. However, oscillations were apparent at the bottom boundary of the discontinuity layer, with a general cooling phase during the day-time and a deepening of the thermocline during the night (Fig. 1a). For all variables under study, the most significant depth-related difference was from temperatures recorded in June (Table 1). The water column was almost isothermal in December (7.8–8.1°C, Fig. 1b) but the temperatures fluctuated significantly over time during both seasons (Table 1). Chlorophyll *a* concentrations (Chl) were significantly (ANOVA, $p < 0.001$) higher in December (mean 6.9 µg l^{-1}) than in June (3.1 µg l^{-1}). Maxima were recorded in late afternoon and during the night-time in the thermocline in June and in the surface waters in December (Fig. 1b, d).

Short-term fluctuations in biological variables

Changes in concentration and rate measurements are shown in Figs. 2, 3, 4 and 5. For each sampling depth and campaign, the coefficients of variation for standing stocks over the study period ranged from 3% to 70%, apart for the abundance of autotrophic picoplankton recorded at 30 m depth in June, which exhibited a higher CV (211%) due to an exceptional peak recorded at 6:00 a.m., together with a deepening of the thermocline (Figs. 1a and 2c). When eliminating this peak, the CV drops to 35%. The highest CVs were calculated for bacterial production (range: 65–137% in June and 61–105% in December). Values for aminopeptidase activity measured in December ranged from 9% to 23%. Concentrations of the biological variables under study and the viral lytic activity were generally higher in December than in June (ANOVA, $p < 0.001$), similar to Chl. The few exceptions were for the abundance of autotrophic picoplankton and for bacterial production, which were significantly higher in June than in December (Figs. 1, 2, 3, 4 and 5).

ANOVA for the time effect yielded more significant differences in June than in December. Indeed, a significant effect of the sampling time was found for the esti-

Fig. 1 Depth–time diagrams of temperature and chlorophyll *a* concentrations in Lake Bourget, 9th to 11th June and 1st to 3rd December 2004, based on high-resolution vertical measurements (i.e. continuous measurements through the water column) carried out using a submersible spectrophotometer (FluoroProbe®, BBE, Moldenke, Germany)
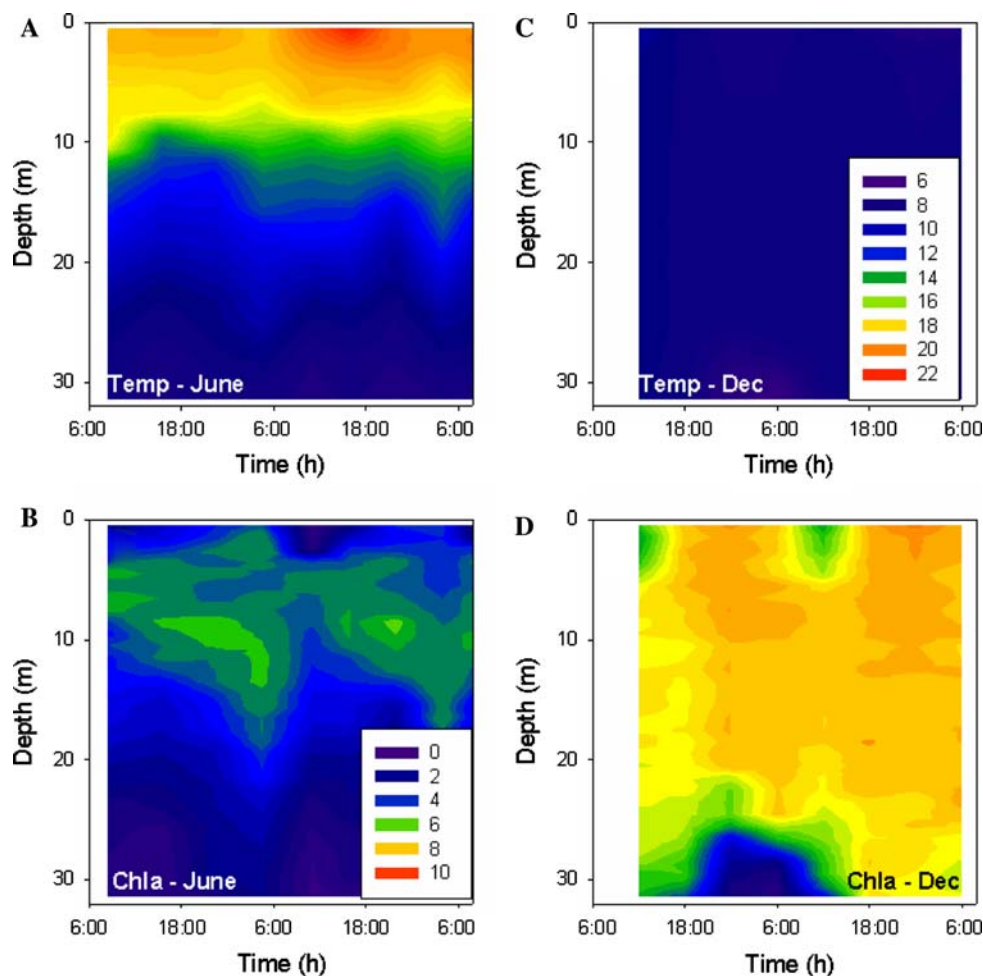
mates of DOC, viral and HNF (heterotrophic nanoflagellates) abundances and bacterial production in June, and for temperature in December (Table 1). Significant effects of time, but also of sampling depth, were also recorded for temperature and Chl in June, and for viral abundance and bacterial production in December. The water column temperature, thus, varied significantly with the sampling time in December, whereas most of the biological variables varied more with depth than with time (Table 1).

During both campaigns, viral abundance increased with time at all depths and this was more marked in December than in June (Fig. 2a, d). Assuming an exponential growth model, viruses accumulated at 0.21 day^{-1} in June ($r^2 = 0.58$) and at 0.31 day^{-1} in December ($r^2 = 0.83$), corresponding to production rates of 0.6×10^7 and 2.4×10^7 viruses 1^{-1} day^{-1}, respectively. These rates were two orders of magnitude lower that those (3.9 and 3.6×10^9 viruses 1^{-1} day^{-1}, respectively) calculated from the viral-induced mortality (see below), bacterial production and burst sizes, which were substantially higher in December (range, mean: 18–81, 41 viruses bacterium^{-1}) than in June (10–48, 29 viruses bacterium^{-1}). A drop in viral and prokaryote

Table 1 Results of analysis of variance (ANOVA) used to test for differences with sampling depth and with time

Variable	Depth		Time	
	F value	p value	F value	p value
June*				
Temperature (T °C)	**546**	**< 0.001**	**2.740**	**0.030**
DOC	2.120	0.120	**2.850**	**0.020**
Chlorophyll (Chl)	**44.020**	**< 0.001**	**4.200**	**0.004**
Viruses (VA)	1.960	0.150	**3.800**	**0.008**
Bacteria (BA)	**6.370**	**0.003**	0.770	0.610
HDNA	**7.400**	**0.001**	0.830	0.570
APP	**9.240**	**< 0.001**	0.060	0.990
HNF	2.010	0.140	**4.170**	**0.005**
PNF	2.220	0.110	1.500	0.220
FVIC	1.430	0.250	2.030	0.090
Bacterial production (BP)	0.100	0.950	**4.460**	**0.003**
December*				
Temperature	2.490	0.080	**3.590**	**0.010**
Chlorophyll	4.700	0.110	0.660	0.700
Viruses	**5.640**	**0.005**	**11.820**	**< 0.001**
Bacteria	**3.750**	**0.020**	0.800	0.590
HDNA	**3.150**	**0.040**	0.920	0.500
APP	**5.820**	**0.004**	0.930	0.500
HNF	**36.400**	**< 0.001**	1.890	0.120
PNF	**10.870**	**< 0.001**	1.700	0.162
FVIC	1.510	0.230	0.250	0.960
Bacterial production	**7.040**	**0.001**	**13.230**	**< 0.001**
Aminopeptidase (MCA)	**3.170**	**0.040**	0.580	0.750

All estimates were log-transformed. Temperature (T °C), concentrations of dissolved organic carbon (DOC), chlorophyll *a* (Chl), viruses (VA), total bacteria (BA), bacteria with high DNA content (HDNA), autotrophic picoplankton (APP), heterotrophic nanoflagellates (HNF), phototrophic nanoflagellates (PNF), rates of bacterial cell production and aminopeptidase extracellular enzyme activity (MCA)
* Differences between June and December were all significant ($p < 0.001$) for all variables. Significant values are in bold

abundances was noted during the first night in December at 30 m (Fig. 2d–f), accounting for the significant variation noted with depth (Table 1). Similar to viruses, the abundance of HNF also generally increased with time. This was clearly related to the pattern during the last 24 h of sampling (Fig. 3a, c), with an associated accumulation rate of 0.28 day^{-1} ($r^2 = 0.67$). In contrast, the significant effect of time on bacterial production in June was related to a general decreasing trend at all depths from the second sampling point (Fig. 4a). Finally, a diel cycle was apparent with regard to DOC concentrations measured in June, with an increasing phase during the day-time at all sampling depths, reaching the highest value at 15 m (Fig. 4c).

Prokaryote production and mortality

Bacterial production ranged from 1 to 10×10^7 cells 1^{-1} h^{-1} (mean 5×10^7 cells 1^{-1} h^{-1}) in June and from 0.5 to 7×10^7 cells 1^{-1} h^{-1} (mean 1×10^7 cells 1^{-1} h^{-1}) in December (Fig. 4a, d). The frequency of visibly infected cells was less than 2.5% (mean = 1.3%) in June and less than 4% (2.3%) in December, corresponding to the destruction of 5–30% (mean 11%) and 10–58% (29%) of bacterial production by viruses, respectively (Fig. 5). We were not able to conduct grazing experiments during this study. However, a grazing study in the same lake carried out in 2002 (Comte et al. 2006) reported taxon-specific HNF clearance rates of 1.6–5 nl ind^{-1} h^{-1} (mean = 3.07 nl ind^{-1} h^{-1}). Relating this to our June campaign, this corresponds to a potential grazing impact ranging from 27% to 84% of bacterial production (mean = 52%). In December, the calculated grazing potential exceeded bacterial production, even when the minimum clearance rate given above was applied.

Relationships between variables

The number and the significant levels of empirical relationships between variables were higher in December than in June (Table 2). With a few exceptions in June, temperature, Chl and the abundances of viruses, of total bacteria and of bacteria with high DNA content were positively correlated to each other during both campaigns, as were the abundances of phototrophic and heterotrophic nanoflagellates. Data on bacteria with low DNA content (not shown) were not related to any other variables. In addition, viral abundance during both campaigns was positively correlated to HNF counts. The latter variable was negatively correlated to bacterial production in June, whereas bacterial production and Chl were positively correlated in December. The other correlations were positive and were detected in the December samples, including those between (1) viral abundance vs. bacterial production and enzyme activity, (2) FVIC versus HNF abundance, (3) the abundance of

Fig. 2a–f Spatial and temporal changes in concentrations of virus-like-particles (**a**, **d**), heterotrophic bacteria (**b**, **e**), and autotrophic picoplankton (APP; **c**, **f**) in Lake Bourget between 9th and 11th June and 1st and 3rd December 2004. The *black bars* indicate the night-time period
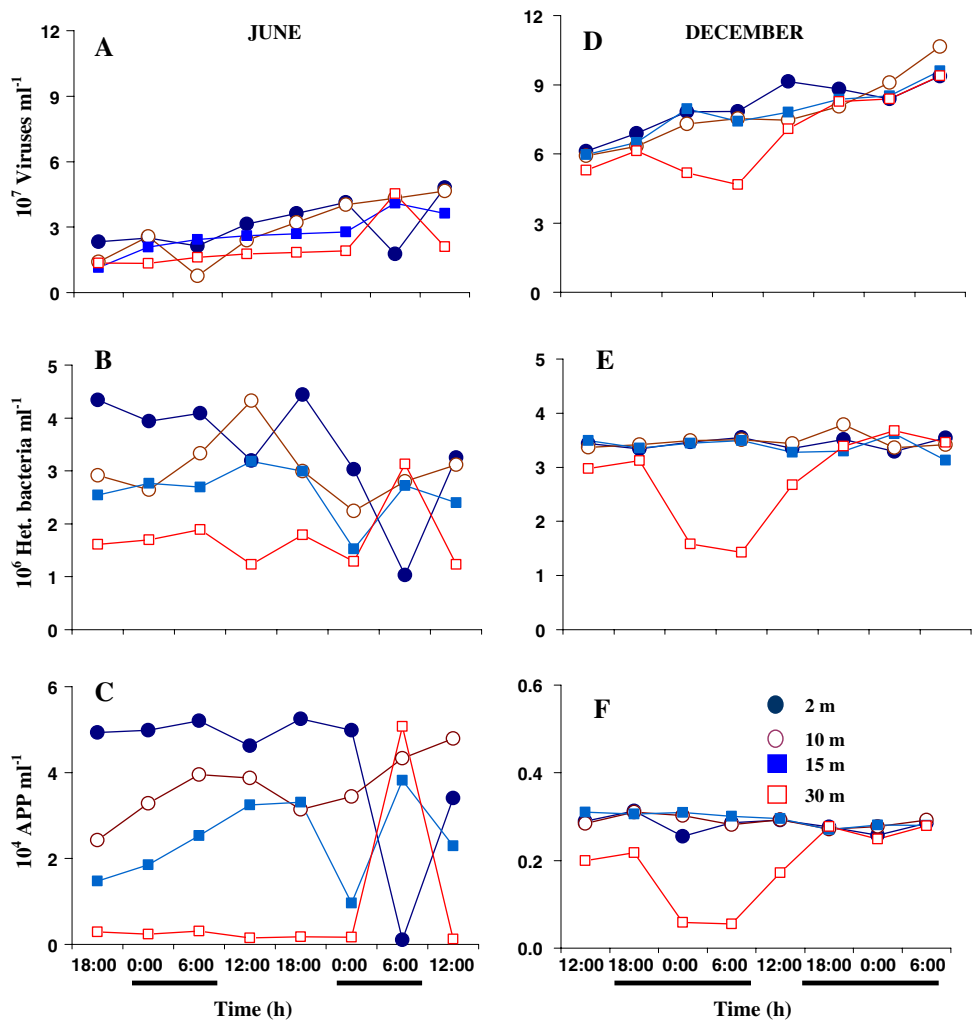

Fig. 3a–d Spatial and temporal changes in concentrations of heterotrophic (HNF; **a**, **c**) and autotrophic (ANF; **b**, **d**) nanoflagellates in Lake Bourget between 9th and 11th June and 1st and 3rd December 2004. The *black bars* indicate the night-time period

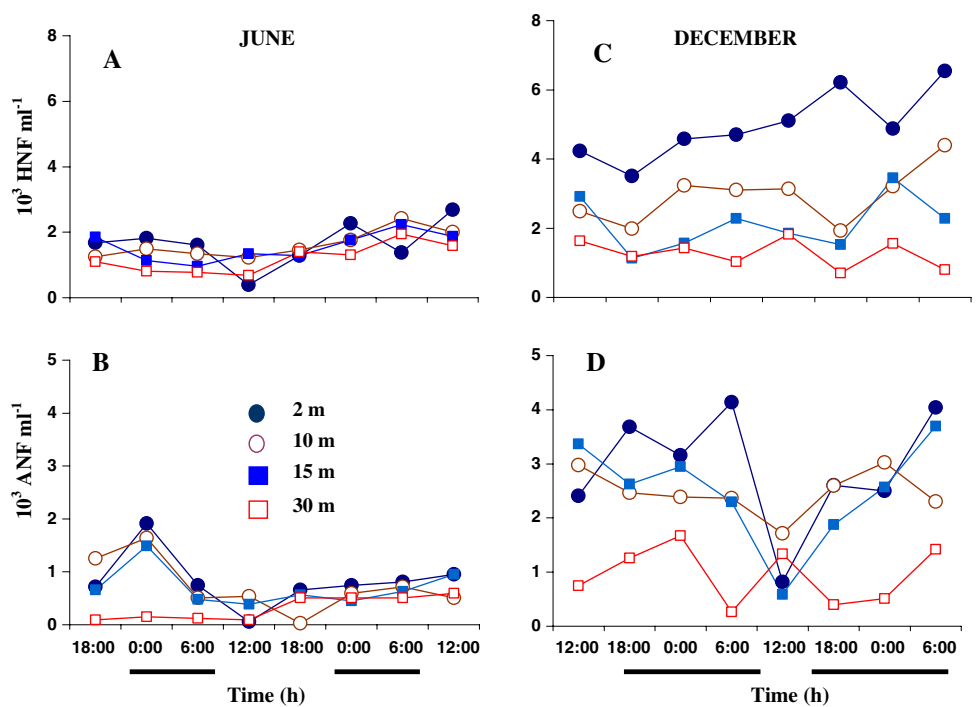**Fig. 4a–f** Spatial and temporal changes in bacterial production (BP; **a**, **d**), percentage of heterotrophic bacteria with high DNA content (HDNA; **b**, **e**—% of total abundance), dissolved organic carbon concentration (DOC (**c**, measured in June) and aminopeptidase extracellular activity (MCA; **f**, measured in December) in Lake Bourget between 9th and 11th June and 1st and 3rd December 2004. The *black bars* indicate the night-time period
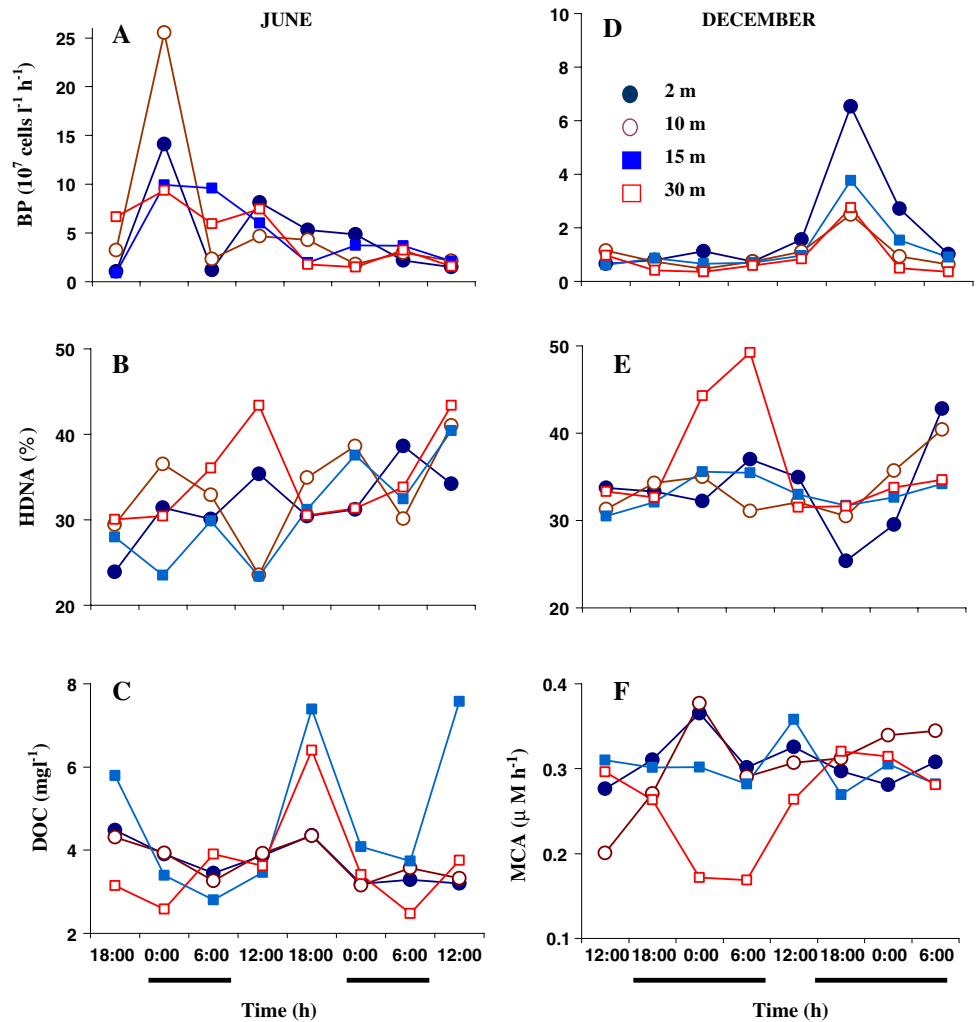
total and high-DNA bacteria versus enzyme activity and (4) enzyme activity vs. temperature, Chl and the abundance of autotrophic picoplankton (Table 2).

For both sampling campaigns, the plots of the descriptors of the PCA grouped most of the variables along axis 1, the relationships between variables being apparently more numerous and closer in December than in June (Fig. 6a, c). In June, burst size (for which no correlation was noted) and the number of infected cells were isolated and opposed to the other variables. The majority of these variables were selected positively by the major principal component in June and negatively in December. The plots of the field observations showed that this selection was mainly related to the samples collected at 2, 10 and 15 m, which were opposed to those collected at 30 m (Fig. 6b, d).

Discussion

The present study is an attempt to analyse planktonic microorganisms on a time scale that is realistic in terms of their probable generation times. High-resolution temperature profiles recorded in the stratified water column in June showed internal oscillations with a period on the order of 12–18 h (Fig. 1a). Physical, chemical and the related biological isolines were shifting with the passage of these internal waves. For example, the abundances of viruses, bacteria and autotrophic picoplankton shifted markedly in the water column on June 11 at 6:00 a.m., coinciding with the deepening of the thermocline (Figs. 1a, 2a–c). From these observations, we conclude that wind-forced internal waves have effects on the plankton dynamics in Lake Bourget (Vinçon-Leite et al. 1989), mainly as a result of the import/export advection of microbial stocks from the target sampling points.

Besides advection, the abundances of HNF in June were similar at all sampling depths, where they accumulated late in the sampling period at a rate (about 0.3 day^{-1}) that was typical of those of planktonic protists in temperate lakes during the spring growing season (Carrias et al. 2001). Similar patterns were also noted for viruses during the two seasons. Sharp increases in the abundances of bacteria and autotrophic picoplankton between 6:00 a.m. and 6:00 p.m. in the deepest water masses on 2nd December would require a

Fig. 5a–d Spatial and temporal changes in the frequency of visibly infected bacterial cells (FVIC; **a**, **c**—% of total counts) and in the virus-mediated bacterial mortality (VBM; **b**, **d**—% of bacterial production) in Lake Bourget between 9th and 11th June and 1st and 3rd December 2004. The *black bars* indicate the night-time period
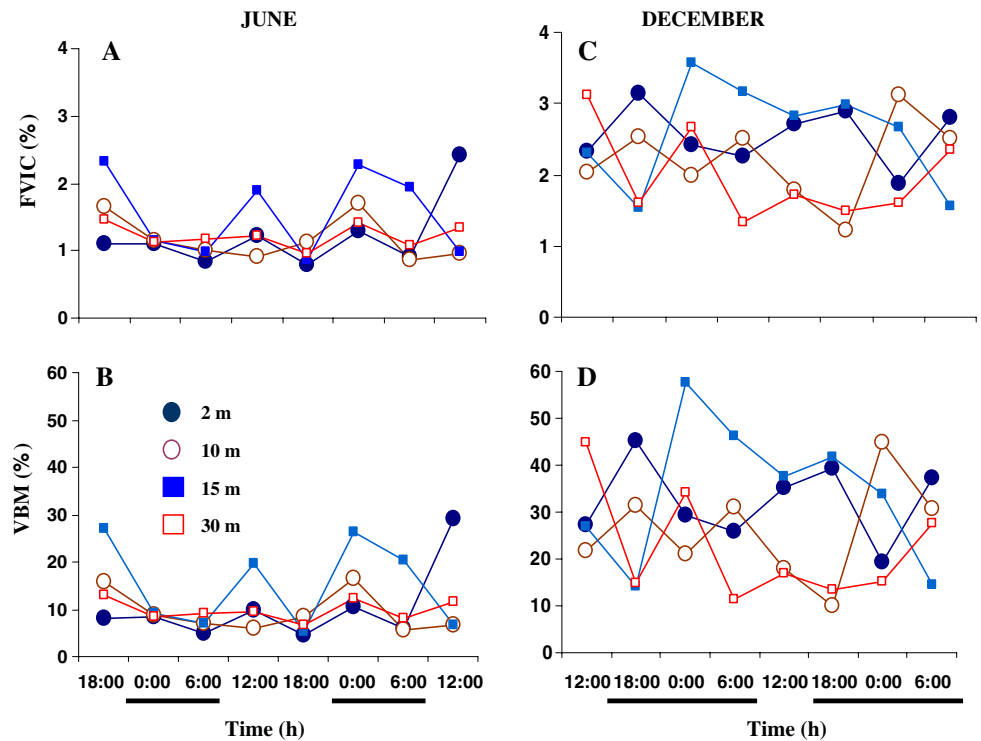

Table 2 Results of Pearson's correlation analysis used to test for empiric correspondence among estimated variables

	VA	FVIC	BA	HDNA	BP	MCA	APP	HNF	PNF	Chl
June										
HDNA	**0.41***	−0.21	**0.91***							
BP	−0.02	−0.15	0.04	0.02						
APP	**0.46****	−0.05	**0.92***	**0.91***	0.05	ND				
HNF	**0.75***	0.23	0.22	0.25	**−0.45****	ND	**0.34***			
PNF	0.27	0.01	0.23	0.18	−0.14	ND	0.25	**0.57***		
Chl	0.17	−0.08	**0.50****	**0.56***	0.16	ND	**0.62***	0.23	**0.34***	
T °C	0.24	−0.11	**0.62***	**0.65***	−0.08	ND	**0.66***	0.27	0.28	**0.65***
December										
BA	**0.61***	0.18								
HDNA	**0.62***	0.25	**0.80***							
BP	**0.35**	0.02	0.29	−0.07						
MCA	**0.61***	0.18	**0.74***	**0.68***	0.17					
APP	**0.58***	0.23	**0.97***	**0.79***	0.26	**0.84***				
HNF	**0.35***	**0.36***	**0.36***	**0.36***	0.27	**0.38***	**0.35***			
PNF	0.26	0.31	**0.45****	**0.41***	0.05	0.29	0.26	**0.58***		
Chl	**0.64***	0.22	**0.94***	**0.73***	**0.34***	**0.78***	**0.64***	0.37	**0.49****	
T °C	0.21	0.16	**0.80***	**0.54****	0.16	**0.72***	0.21	0.16	0.29	**0.69***

See Table 1 for an explanation of the abbreviations. Significant correlations are in bold. Asterisks denote the levels of correlation as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. The underlined coefficients denote common correlations for the two sampling periods

net community doubling time of 5–8 h. Moreover, bacterial production estimated via bottle incubations was higher and fluctuated more in June than in December, the values (10^7–10^8 cells 1^{-1} h^{-1}) being typical of oligotrophic to mesotrophic lakes (Bettarel et al. 2004; Colombet et al. 2006). These observations suggest that part of the variations were intrinsic to microbial compartments during this study, despite noise from water movements.

Only a few of these variations were related to the day–night cycles, perhaps because of the relatively low resolution in our sampling intervals. A diurnal cycle was noted for DOC measured in the water column in June, with maxima occurring during the day (Fig. 4c). In contrast to DOC, chlorophyll *a* concentrations in the surface waters peaked in the late afternoon and during the night, and were correlated to several variables during the two study seasons, as was the abundance of viruses

Fig. 6a–d Results of the principal component analysis (PCA) obtained from the main variables under study (**a**, **c**) and from the observations made for each sampling depth and time (**b**, **d**) in Lake Bourget between 9th and 11th June and 1st and 3rd December 2004. See Table 1 for an explanation of the previous abbreviations; BS = burst size, T1–T8 in panels **b** and **d** refer to the eight sampling hours for each of the four sampling depths. Horizontal X-axis = Axe 1, vertical Y-axis = Axe 2. *Inset* histograms in **a** and **c** are graduated from 0 to 0.5 and represent the fractions of variances (Y) explained by the different PCA axes (X, from Axe 1 onwards)
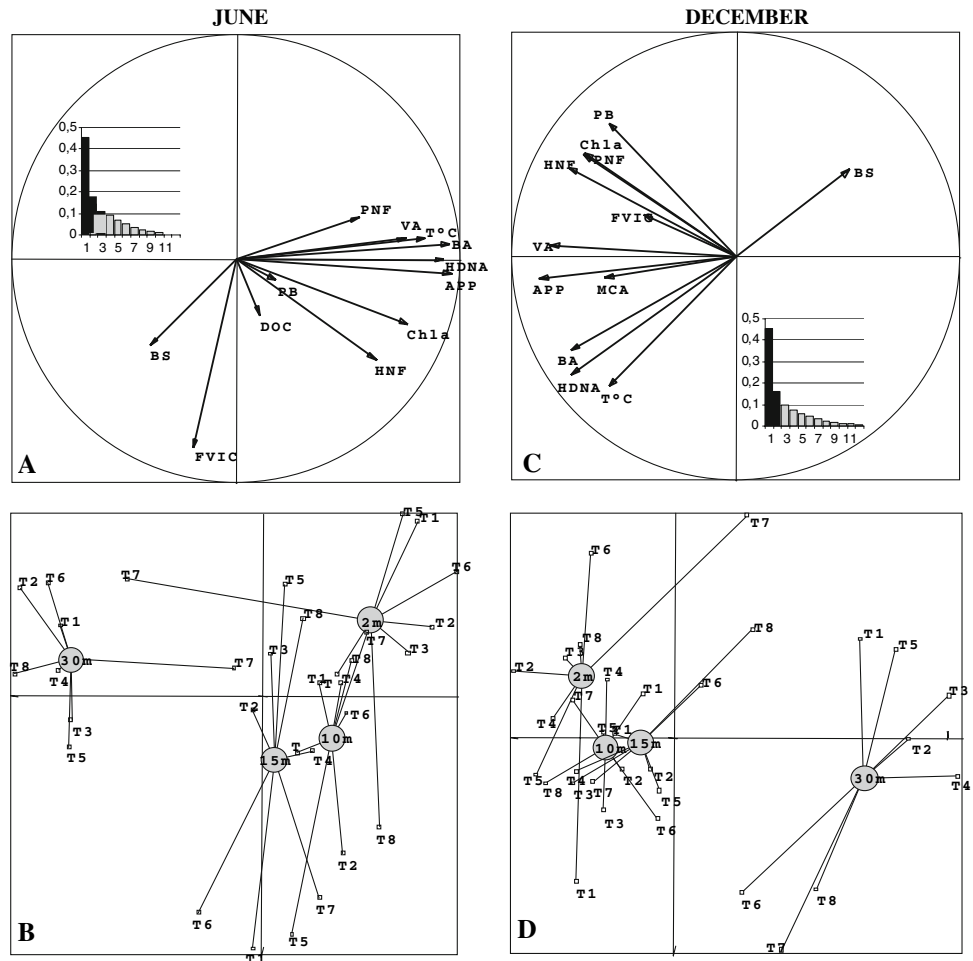
with that of autotrophic picoplankton (APP) (Table 2). The latter community was exclusively represented by Phycoerythrin-rich cyanobacteria, which usually appear to be easier to infect with viruses (Wilhelm et al. 2006). Phytoplankton communities were, thus, likely to be actively implicated in trophic interactions. From the temporal fluctuations of chlorophyll a, it is difficult to know whether phytoplankton cells were accumulating or possibly dividing in the late afternoon or during the night, as we have no data on diel patterns or light-related autoecology of phytoplankton populations, primarily of *Planktothrix rubescens*, the dominant cyanobacterium in Lake Bourget (Humbert and Le Berre 2001; Briand et al. 2005; Jacquet et al. 2005a). However, tentative explanations from the freshwater literature include (1) algal avoidance of exposure to high light, which is a common situation in aquatic systems (Tadonléké et al. 1998) and (2) increasing chlorophyll cell content with decreasing light intensity in some algal species, primarily cyanobacteria (Tadonléké et al. 1998).

Apart from two exceptions (i.e. bacterial production and autotrophic picoplankton abundance), all biological variables under study were significantly higher in the winter than in the summer (Table 2). In Lake Bourget, the phytoplankton biomass is typically dominated

($>90\%$ of the total) by the cyanobacterium *P. rubescens* for a significant part of the year (Jacquet et al. 2005a). Using both probe fluorescent measurements (i.e. the same fluoroprobe used in this study, calibrated with *P. rubescens*) and cell counts, it has recently been shown from depth-integrated seasonal data that this cyanobacterium typically peaks in early December (see Fig. 5 in Leboulanger et al. 2002). From our data and those from multi-year reports on Lake Bourget (Briand et al. 2005; Jacquet et al. 2005a), this is partly related to the fact that, in late autumn and winter, *P. rubescens* colonises the entire water column, whereas during the other seasons, its development is more restricted to specific zones in the water column. Multivariate analysis clustered most of the variables along axis 1 in June and December, isolating burst size and samples collected at 30 m from other variables collected in the upper water column. Correlations among these variables were higher in December than in June, in both their intensity and number, indicating that biological variables were trophically more dependent on each other in December than in June. This could be related to the grazing pressure. During our study, the abundance of zooplankton (dominant species *Daphnia hyalina* and *D. galatea*) was largely higher in spring/summer than in

winter (Laine and Anneville, unpublished) but no general diel pattern is available for metazooplankton in Lake Bourget.

We speculate that microbial interactions and the related cascading effects were more enhanced in winter than in summer. This was indicated by the couplings (1) between chlorophyll *a* and bacterial production (Ducklow and Carlson 1992), (2) between viral abundance and bacterial abundance production and enzymatic activity (Bettarel et al. 2003, 2004) and (3) between the abundances of viruses and bacteria and their potential HNF grazers (Gonzalez and Suttle 1993; Bettarel et al. 2005) in winter. It is tempting to attribute the higher abundance and potential bacterivory (that considerably exceeded bacterial production in all samples) of HNF in December (compared to June) to the reduction of grazing pressure from zooplankton, resulting in higher competition for food in HNF communities, a diversification of their diet and the occurrence of non-bacterivorous species. In contrast, higher zooplankton grazing pressure on HNF in June could have given a competitive advantage to the tiniest HNF cells, i.e. the dominant bacterivores in aquatic systems (Strom 2000), which are known to be strong stimulators of bacterial production (Posch et al. 1999; Simek et al. 2001; Sime-Ngando and Ram 2005). This may, thus, also help explain the two exceptions to the detected 'anomaly' (i.e. the fact that most biological variables under study were significantly higher in the winter than in the summer) noted for autotrophic picoplankton and bacterial production. We consider that the detected 'anomaly' was likely to be forced by the seasonal behaviour of the major phytoplankton population, in relation to the grazing environment and the related trophic interactions.

In conclusion, microbial parameters under study varied considerably over a short-term period in Lake Bourget. Circadian variations noted for DOC and chlorophyll *a* concentrations were found to match the day–night cycle to some extent. However, despite the external abiotic forcing (light, water movements), the fluctuations in biological variables in most cases were apparently forced by trophic interactions. However, we think that a much tighter vertical and temporal sampling programme than ours would be required to better observe general patterns of diel changes in Lake Bourget. Clearly, our study could not distinguish all of the factors relating to short-term variations in the microbial components under study. It, therefore, left unresolved the question of the impact of micro- and mesozooplankton (such as ciliates and rotifers), the role of a variety of sources of food and predation, and possibly chaotic population fluctuations. Nevertheless, we believe that, short-term, in-situ models to study microbial components constitute a rather promising approach to understanding the overall functioning of the planktonic food web in natural lakes such as Lake Bourget.

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