Microbial Ecology

Microbial Community Structure and Dynamics in the Largest Natural French Lake (Lake Bourget)

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Abstract

We investigated the dynamics and diversity of heterotrophic bacteria, autotrophic and heterotrophic flagellates, and ciliates from March to July 2002 in the surface waters (0–50 m) of Lake Bourget. The heterotrophic bacteria consisted mainly of "small" cocci, but filaments (>2 μm), commonly considered to be grazing-resistant forms under increased nanoflagellate grazing, were also detected. These elongated cells mainly belonged to the *Cytophaga-Flavobacterium* (CF) cluster, and were most abundant during spring and early summer, when mixotrophic or heterotrophic flagellates were the main bacterial predators. The CF group strongly dominated fluorescent *in situ* hybridization–detected cells from March to June, whereas clear changes were observed in early summer when Beta-proteobacteria and Alpha-proteobacteria increased concomitantly with maximal protist grazing pressures. The analysis of protist community structure revealed that the flagellates consisted mainly of cryptomonad forms. The dynamics of *Cryptomonas* sp. and *Dinobryon* sp. suggested the potential importance of mixotrophs as consumers of bacteria. This point was verified by an experimental approach based on fluorescent microbeads to assess the potential grazing impact of all protist taxa in the epilimnion. From the results, three distinct periods in the functioning of the epilimnetic microbial loop were identified. In early spring, mixotrophic and heterotrophic flagellates constituted the main bacterivores, and were regulated by the availability of their resources mainly during April (phase 1). Once the "clear water phase" was established, the predation pressure of metazooplankton represented a strong top-down force on all microbial compartments. During this period only mixotrophic flagellates occasionally exerted a significant bacterivory pressure (phase 2). Finally, the early summer was characterized by the highest protozoan grazing impact and by a rapid shift in the carbon pathway transfer, with a fast change-over of the main predators contribution, i.e., mixotrophic, heterotrophic flagellates and ciliates in bacterial mortality. The high abundance of ciliates during this period was consistent with the high densities of resources (heterotrophic nanoflagellates, algae, bacteria) in deep layers containing the most chlorophyll. Bacteria, as ciliates, responded clearly to increasing phytoplankton abundance, and although bacterial grazing impact could vary largely, bacterial abundance seemed to be primarily bottom-up regulated (phase 3).

Introduction

Pelagic microbial ecosystems are characterized by a complex set of dynamic interactions between organisms. Competition for nutrients and light, commensalism between autotrophs and heterotrophic bacteria, recycling of material, cell lysis, and predation are typical processes implicated in the ecological interactions between viruses, bacteria, micro-algae, and their predators (flagellates, ciliates, microzooplankton). Top-down (grazing), bottom-up (nutrient availability, amount of prey) controls and viral lysis are primarily responsible for microbial population structure and diversity, and they operate simultaneously rather than separately.

Since the seminal papers of Pomeroy [57] and Azam et al. [6], heterotrophic bacteria have been shown to play a crucial role in aquatic ecosystems, as the principal decomposers of organic matter [79], and as a main food source for microorganisms at the base of the trophic web [28, 37, 70]. The regulation of bacterial biomass, productivity, and community structure by nutrients (both organic and inorganic) and grazing (by single-cell and multicel-

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lular zooplankton) is thus a central issue in aquatic microbial ecology [50, 53, 70]. Heterotrophic nanoflagellates have long been viewed to be mainly responsible for bacterial biomass control [13, 55, 65]. Some studies have also pointed out that ciliates [39, 44, 69], metazoans such as *Daphnia* and copepods [1, 21, 38, 82], as well as lytic viruses [12, 54, 60] may also play key roles in structuring the heterotrophic bacterial community. For example, protist predation has been shown to induce some important shifts in the structure and composition of bacterial communities, as a result of the potential selective predation on particular bacterial size classes or specific groups [28, 29, 37, 41, 55]. To understand the complex interactions in microbial food webs, special attention has to be paid to fine dissection of microbial compartments in order to identify relevant functional groups. Some studies recently stressed the importance of developing a functional partitioning of all planktonic compartments to distinguish possible carbon pathways within the pelagic food webs [23, 32]. For example, it appeared that mixotrophs, which are usually considered unimportant bacterial grazers, could play a significant role in bacterivory and should be introduced in general models of food webs [30]. Bacteria were, until recently, considered to be a homogeneous functional unit, but the development of the fluorescent *in situ* hybridization (FISH) technique has now made it possible to determine the relative abundance of different phylogenetic groups of bacteria. This technique allowed better analyses of seasonal successions within these groups, which aim to identify regulating factors [2, 26, 56]. Several studies have suggested that bacteria belonging to the *Cytophaga-Flauobacterium* (CF) cluster could be preferentially grazed by hetorotrophic nanoflagellates (HNF) or ciliates [37, 43]. The FISH technique has also revealed that grazing-resistant morphotypes (typically filaments and aggregates) can be found in the major phylogenetic groups, such as the alpha-proteobacteria and beta-proteobacteria, and the *Cytophaga-Flavobacterium* phylum [37, 70].

Understanding how the microbial food web works also requires consideration of the seasonal dynamics of microbial communities [17, 31, 56]. The combination and the importance as structuring forces of top-down and bottom-up controls show seasonal variations that play an important role in the structure and dynamics of the bacterial community, as demonstrated by Muylaert et al. [53] for eutrophic lakes.

The present article describes, for the first time, the structure and dynamics of the microbial community (heterotrophic bacteria, picocyanobacteria, flagellates, and ciliates) in Lake Bourget over a period covering the onset of thermal stratification (March to July 2002). Our aims were (1) to investigate the changes in microbial abundance and biomass according to season and depth (we chose not to consider only general functional groups but to dissect all investigated compartments); (2) to examine the changes in bacterial composition and the development of forms resistant to protist grazing; and (3) to analyze the relative importance of mixotrophy within the protist community. This allowed us to propose a conceptual scenario for the succession of bacterial predators in Lake Bourget, and hence of how the microbial food web works over a well-defined period of time. To support our hypotheses on regulation pathways, an assessment of protozoan grazing rates on bacteria was used. These data provided an essential basis from which to develop studies on microbial communities in Lake Bourget; notably, it allowed us to conceptualize further experimental approaches (Domaizon et al., in prep.).

Methods

Study Site and Sampling Strategy. The mesotrophic Lake Bourget (45°44′N, 05°51′W, 231 m altitude) is located in the eastern part of France at the edge of the Alps. It is a monomictic, and elongated (18 km long, 3 km wide), north–south orientated lake, with an area of 42×10^6 m^2, a total volume of 3.5×10^9 m^3, a maximum and average depth of 145 and 80 m, respectively, and a water residence time of approximately 10 years. Water samples were collected from March to July 2002 at five depths (2, 6, 10, 30, and 50 m) at the reference station located in the middle and deepest part of the lake, referred to as point B. This station is more than 1.5 km from each bank, and more than 5 and 10 km from the two main freshwater inputs to the lake. The analysis of bacterial composition by the FISH technique (see below) was only performed at the 2 and 50 m depths.

Physicochemical Variables. Nutrient concentrations (N total, NH_4^+, NO^{3-}, P total, PO_4^{3-}) have been measured at the hydrobiological station Institut National Recherche Agronomique (INRA) [4] (details available at http://www.thonon-inra-chimie.net/pages/public/analyses.asp). The concentration of dissolved organic carbon (DOC) was measured with a carbon analyzer (Labtoc, UV promoted persulfate oxidation, IR detection) in water samples (15 mL) filtered through a 0.2-µm pore size polycarbonate filter (data not shown). A conductivity-temperature-depth measuring device (CTD SEABIRD SBE 19 Seacat profiler) was used to obtain vertical profiles from the surface to the bottom of the water temperature gradient and of the oxygen concentration, using an oxygen probe, a polarographic YSI 5739 electrode (data not shown).

The chlorophyll *a* (Chl*a*) was measured *in situ* using a submersible spectrofluorometer that was calibrated to perform the survey of chlorophyll vertical distribution on the basis of the chlorophyll fluorescencse intensity at 680 nm (due to photosystem II core pigments) [46]. For a detailed description and functioning of this FluoroProbe, see Beutler et al. [8] and Leboulanger et al. [46].

Table 1. Oligonucleotide probes used in this study

Probe	*Specificity*	*Sequence of probe*	*Target site*	*%FA*[a]	*Reference*
EUB338	Bacteria	GCTGCCTCCCGTAGGAGT	16S (338–355)	20	[4]
ALF968	Alpha subclass of proteobacteria	GGTAAGGTTCTGCGCGTT	16S (968–986)	20	[45]
BET42a	Beta subclass of proteobacteria	GCCTTCCCACTTCGTTT	23S (1027–1043)	35	[39]
CF319a	*Cytophaga flavobacterium* cluster	TGGTCCGTGTCTCAGTAC	16S (319–336)	35	[40]
ARCH915	Archaea	GTGCTCCCCCGCCAATTCCT	16S (915–935)	35	[64]

[a]Percentage of formamide.

Microorganism Counts. A 1-mL sample for bacteria was fixed with formaldehyde (final concentration: 3.7%), and stored at 4°C until it was analyzed (within 3 days after sampling). Heterotrophic bacteria were concentrated on black polycarbonate membranes (diameter: 47 mm, pore size: 0.2 μm) by filtering under reduced pressure of <150 mm Hg. Cells were stained by covering the filter with Di Aminido Phenyl Indol (DAPI) (final concentration: 0.04 μg mL^{-1}) according to technique of Porter and Feig [58]. Microscopic counting was performed using an epifluorescence microscope (Nikon, Eclipse TE200). Besides the DAPI-stained small cells, we found filamentous bacteria (>2 μm). The biovolume of more than 400 bacteria was measured using a semi-automatic image analysis system (Lucia 4.6, Laboratory Imaging Ltd, Czech Republic) and was converted to carbon units using the allometric relationship proposed by Loferer-Krößbacher et al. [38].

The frequency of dividing cells was determined and used as an estimator of bacterial productivity. We demonstrated through a seasonal study (May to August) that in Lake Bourget the [^{3}H]thymidine incorporation rate into bacterial DNA is significantly correlated with the number of dividing cells (NDC) ($r = 0.74$, $n = 18$, $P < 0.01$): [^{3}H]thymidine incorporation = 0.0084 × NDC − 0.0354 ([^{3}H]thymidine: nmole L^{-1} h^{-1} ; NDC: 10^7 cells L^{-1}) (Domaizon et al., unpublished).

Under green light, the phycoerythrin content of the picocyanobacteria produced a red-orange autofluorescence [74], which made it possible to count the picocyanobacteria collected on 0.8-μm polycarbonate membranes using epifluorescence microscopy. The mean biovolume was determined by the same image-analysis system used for heterotrophic bacteria. The conversion factor of 360 fgC μm^{-3} was used to obtain the biomass of picocyanobacteria [76].

Glutaraldehyde (1% final concentration) was used to fix the flagellates. Samples were filtered (pressure <100 mm Hg) on polycarbonate membranes (diameter: 25 mm, pore size: 0.8 μm), then stained with primulin [14] and stored at the most a few days at −20°C until analysis. Slides were examined under UV light to count the heterotrophic nanoflagellates, and under blue light to count the autotrophic flagellates at 1250 × magnification. Green light (545 nm) was useful to display phycoerythrin-containing flagellates. Flagellate counts were converted into biomass using a conversion factor of 220 fgC μm^{-3} [11]. Ciliates were preserved with mercuric bichloride (25%) and identified and counted (within 15 days after sampling) according to the method Sime Ngando et al. [67]. The sedimentation of samples was carried out over 48 h in an Uthermhöl column, and the observation was recorded with an inverted microscope at a 1250 × magnification. The C content of ciliates was calculated from the biovolume using the conversion factor of 190 fgC μm^{-3} [61].

FISH. Five Carbonindocyanine 3 (CY3)-labeled oligonucleotide probes (Microsynth, Swiss) were used in this study (Table. 1). As described by Alfreider et al. [2], cells fixed with formaldehyde (3.7%) were collected on white polycarbonate membranes (diameter: 25 mm, pore size: 0.2 μm) by applying reduced pressure <150 mm Hg; they were then stored at −20°C until analysis. Each filter was cut into several sections, which were placed on glass slides and covered with 20 μL of a hybridization buffer prewarmed to 46°C. This buffer (0.9 M NaCl, 20 mM Tris/HCl (pH = 7.4), 0.01% sodium dodecyl sulfate [SDS] was used with variable concentrations of formamide depending on the probe (ARC915, EUB338, ALF968: 20%); (BET42a, CF319a, GAM42a: 35%) plus 1 μL of CY3-labeled oligonucleotide probe (50 ng μL^{-1}). The BET42a probe was used with a competitor oligonucleotide. Samples were then incubated for 90 min at 46°C. The filters were rinsed with 48°C prewarmed washing buffer (450 mM NaCl for EUB338, ALF968, ARC915 or 100 mM for BET42a, CF319a, GAM42a, 20 mM Tris HCl pH = 7.4, 5 mM EDTA, 0.01% SDS) and incubated for 15 min at 48°C. After drying, filter sections were stained with 10 μL of DAPI (10 μg mL^{-1}) for 10 min in the dark at ambient temperature. The filters were then rinsed in ethanol (70%) for 30 s, dried, and mounted on slides with Citifluor. The slides were examined under an epifluorescence microscope (Nikon Eclipse TE200) fitted with a camera (Nikon Digital camera DXM 1200) under green light (detection of CY3-labeled bacteria) and under UV light (DAPI-stained cells) at 1250 × magnification. Each filter was counted using the semi-automatic image analysis system (Lucia 4.6, Laboratory Imaging Ltd).

Assessment of Protozoan Bacterivory. Grazing rates of ciliates and flagellates (heterotrophic and potentially mixotrophic) on heterotrophic bacteria were

estimated from the ingestion of fluorescent micro-beads. We conducted three experiments of *in situ* grazing measurement, in surface layers (0–10 m) from March to August 2003 in order to estimate clearance rates for each taxon. The detailed protocol and discussion of the method are presented in Domaizon et al. [23]. Experiments were conducted in duplicate in 250-mL glass containers where 0.5-µm tracer particles (Fluoresbrite Plain Microspheres, Polysciences) were added. The concentration of the microspheres in each experimental bottle was from 5% to 20% of the bacterial concentration in the lake. Based on preliminary measurements of predation kinetics realized in pre-alpine lakes [23; Domaizon unpublished], we chose an incubation time of 15 min. We analyzed samples at 0 min and 15 min in each experimental bottle. Ice-cold glutaraldehyde was added to each sample (2% final concentration) for minimizing the egestion of particles [65]. The microbeads ingested by flagellates were counted after filtering each sample (30 mL) onto an 0.8-µm polycarbonate black membrane (Nucleopore) and staining by primulin according to the protocol described by Caron [14]. Flagellates and microbeads were observed at a 1250 × magnification under UV and blue light. Briefly, UV light allowed display of microbeads and all flagellates that were primulin-stained. On the same microscopic field, pigmented flagellates were distinguished by switching on blue light to observe the autofluorescence of chlorophyll *a*. Taxa that had plastids and ingested microbeads were considered as mixotrophs. Beads ingested by ciliates were observed by inverted microscopy after sedimentation. Clearance rates (nL flagellate^{-1} h^{-1} or nL ciliate^{-1} h^{-1}) were calculated for each taxon by dividing the number of ingested beads per hour by the bead concentration in the bottle. The clearance rates fell within the range of values published for freshwater systems [15, 17, 23, 73, Jacquet et al. submitted] and correlated poorly from one date to another. The mean clearance rate measured for each taxon (Table 2) was used to assess the potential grazing impact of flagellates and ciliates during our seasonal study. Ingestion rates of each taxon (bacteria grazer^{-1} h^{-1}) were calculated by multiplying the corresponding clearance rate by the heterotrophic bacteria concentration. The grazing impact (bacteria h^{-1} L^{-1}) of a taxon was estimated by multiplying its ingestion rate by its actual concentration.

Statistical Analysis. All statistical analyses were performed using the Statistica Ed. ''99 software package (Tulsa, OK). Because of the sample size, we used the non-parametric U-test (Mann-Whitney) and the Kruskal-Wallis test to analyze the differences in the abundance of the phylogenetic groups identified in the study and the abundance and biomass of bacteria and protists, relative to time and space, respectively. Spearman rank correlations were done in order to highlight the relationships between microbial components.

Table 2. Mean clearance rates (SD) of the main protist taxa, based on fluorescent microbead ingestion (n = 6, from March to August in 0–10 m layers of lake Bourget)

		Mean clearance rate (SD) nL grazer^{-1} h^{-1}
Non pigmented Flagellates		
	Spumella	2.2 (0.6)
	Katablepharis	5.0 (2.7)
	Undetermined Cryptomonads	3.5 (1.9)
	Uniflagellated	1.6 (0.3)
Pigmented Flagellates		
	Dinobryon	2.4 (0.4)
	Cryptomonas sp1	1.7 (0.98)
	Cryptomonas sp2	7.5 (3.5)
Ciliates		
	Strobilidium sp1	37.7 (11.2)
	Strobilidium sp2	13.9 (5.4)
	Strobilidium sp3	61.3 (6.1)
	Strobilidium delicatessum	1.8
Oligotrichida	*Lohmanniella*	1.5 (1.1)
	Strombidium sp1	9.1
	Strombidium sp2	11.2
	Halteria sp1	2.0 (1.4)
	Halteria sp2	18.1 (6.5)
Haptorida	*Dinophrya* psp	32.5
	Mesodinium psp	8.1
Prostomatida	*Holophrya* psp1	26.5 (5.2)
	Holophrya psp2	12.3 (2.6)
	Urotricha sp1	21.5 (8.6)
	Urotricha sp2	3.7
	Urotricha psp	7.0 (2.7)
	Coleps sp1	2.7
	Coleps sp2	3.9 (2.5)
Peritrichida	*Vorticella*	18
Hymenostomatida	*Glaucoma*	2
	Tetrahymena	8
Scuticociliatida	*Uronema*	15 (5.6)
	Cyclidium	5.3 (4.1)
Colpoda	*Colpoda*	19

Results

Physicochemical Variables. The temperature fluctuated between 5.8°C and 23.5°C throughout the study period, and thermal stratification appeared from the middle of May, with a thermocline around 10 m depth. The lowest value for water transparency was observed on April 2, whereas the clear water phase appeared at the beginning of May with a maximum transparency value of 12 m and continued until the end of May. A first peak of chlorophyll *a* concentration was recorded on April 2 (13.66 µg L^{-1}). A subsequent decrease was recorded until the end of May, and a gradual increase followed during early summer, with a maximum observed on June 19 (21.61 µg L^{-1}); (Fig. 1).

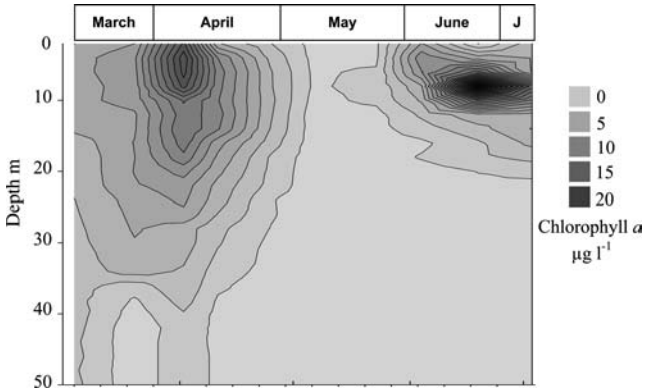

Figure 1. Seasonal changes in chlorophyll *a* (Chl*a*) concentrations from March to July 2002.

Ammonium concentrations fluctuated between 0.001 and 0.037 mg L^{-1}, whereas those of nitrate and total nitrogen (N_t) reached values of 0.70 and 0.88 mg L^{-1}, respectively. Maximum concentrations of PO_4^{3-} and P_{tot} were 0.026 mg L^{-1} on April 2 and 0.049 mg L^{-1} on April 11, respectively. Nutrient concentrations revealed distinct vertical gradients from early stratification to the end of the study. Typically, concentrations of NO_3^-, N_t, PO_4^{3-}, and P_{tot} declined in the epilimnion from the beginning of May. In contrast, NH_4^+ concentrations were higher in the upper layer from May to June (data not shown).

Although a slight increase in dissolved organic carbon (DOC) concentration was observed from March to July at 10, 30, and 50 m depths, these deep layers displayed relatively constant concentrations (mean: 2.78, 2.65, and 2.60 mg L^{-1}at 10, 30, and 50 m, respectively), compared to those in the upper layers, where DOC reached peak values of 4.96 and 4.42 mg L^{-1} (at 2 m and 6 m, respectively) on March 22 (data not shown).

Autotrophic Picoplankton. Picophytoplanktonic cells were massively dominated by phycoerythrin-containing picocyanobacteria. Maximum abundances for picocyanobacteria were recorded in the three upper layers (2 m, 6 m, and 10 m) on July 3, and concentrations ranged from 4.5×10^2 cells mL^{-1}—i.e., 0.1 µgC L^{-1}—on March 22 at 50 m, to 6.9 10^5 cells mL^{-1}, i.e., 101.8 µgC L^{-1}—on July 3 at 10 m (Fig. 2A). Picocyanobacteria were mainly solitary cells; microcolonies were rarely observed during the study. *Synechoccocus*-like cells (mean diameter: 1.05 µm, mean biovolume: 0.39 μm^3), which largely dominated the picocyanobacterial community, accounted for 85.23% of the total picocyanobacteria during the study. Like the observations reported by Crosbie et al. [19] in Lake Mondsee, we found no clear spring peak in solitary picocyanobacteria. The weak stratification in March-April could explain the absence of the spring peak that is observed in others mesotrophic lakes [19].

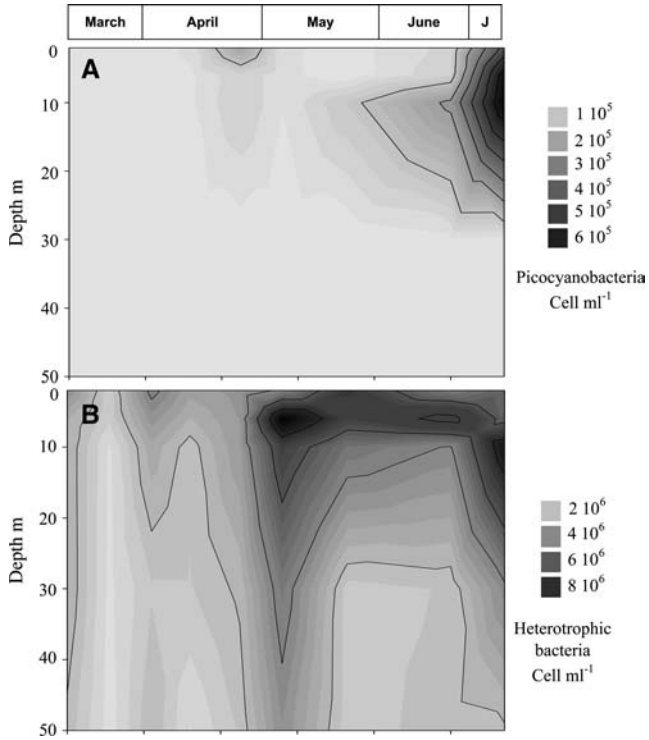

Figure 2. Seasonal changes in picocyanobacteria (A) and heterotrophic bacteria (B) abundances from March to July 2002.

Abundance and Biomass of Heterotrophic Bacteria. The abundance and biomass of heterotrophic bacteria fluctuated between 5.95×10^5 bacteria mL^{-1}, i.e., 15.1 µgC L^{-1} (March 22), and 9.98×10^6 bacteria mL^{-1}, i.e., 161 µgC L^{-1} (May 6); (Fig. 2B). The values recorded for these parameters increased in the epilimnion at the end of the spring bloom and remained high until the end of the study. In contrast, the values at 30 m and 50 m depth were fairly stable. Heterotrophic bacteria consisted mainly of small cocci (mean biovolume: 0.08 μm^3), but filaments (>2 µm length) were regularly observed. Maximum filament concentrations were recorded on June 19 at both depths, and could reach 3.6×10^4 bacteria mL^{-1} (2 m). In the euphotic layer, two filament abundance peaks were recorded on April 25 and June 19. Although the filament abundance values were similar, the size structure within the filaments was different on these two dates. The analysis of the bacterial size structure revealed that on April 25, the dominant size class was the 5–10 µm class, which accounted for 45% of all filaments, and the maximum length of the filaments was about 30 µm. In contrast, on June 19, larger filaments were observed (up to 70 µm), with the "> 30 µm" size class accounting for 7% of the total filaments, whereas the dominant group (44% of filaments) belonged to the smallest size class of filaments (2–5 µm).

FDC. The frequency of dividing cells (FDC) fluctuated between 1.2% and 9.1% and was highest in the

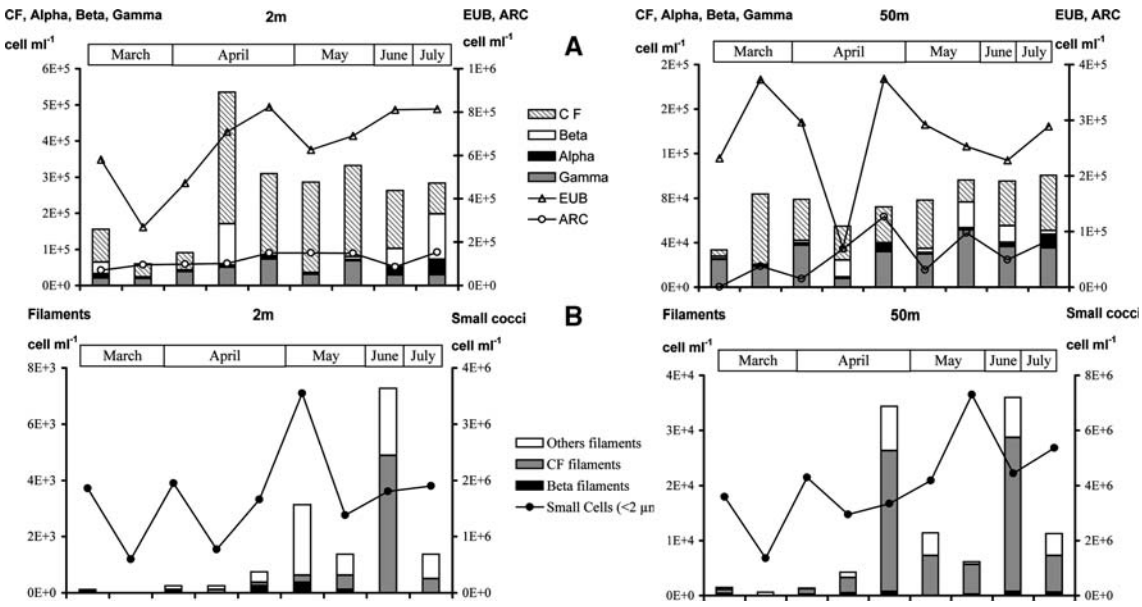

Figure 3. Seasonal changes in the abundance of the different phylogenetic bacterial groups at 2 m and 50 m depths from March to July 2002. (A, B): Alpha-proteobacteria (Alpha), Beta-proteobacteria (Beta), Gamma-proteobacteria (Gamma), and *Cytophaga flavobacterium* (CF); (C, D): small and filamentous cells.

epilimnion and lowest at 30 m and 50 m depths. The highest values for FDC occurred on 22 March, at the beginning of the spring phytoplankton peak (5.5%, 7.5%, and 9.1% at 2 m, 6 m, and 10 m, respectively); another series of high values was recorded later, on 12 April. For all depths, minimal values for FDC were observed during the clear water phase (6 May to 23 May). Based on the relation we established between NDC and [^{3}H]thymidine we could estimate bacterial production varying between 3.2×10^6 and 2.5×10^8 bacteria $L^{-1} \cdot h^{-1}$.

FISH. The efficiency of detection with the eubacterial probe EUB338 relative to DAPI, ranged from 9.2% (March 11, 50 m) to 62.8% (March 22, 50 m), (mean: 23.5%, all depths), whereas the archibacterial probe ARC915 identified between 0.04% and 8.9% of DAPI-stained cells (mean: 3.57%, all depths). Although concentrations of archaebacteria were similar at both depths, their mean relative abundance was higher at 50 m than at 2 m depth (mean: 18.5% and 10.4% of total identified cells (EUB+ARC), respectively at 50 m and 2m, representing 4.7% and 2.4% of DAPI stained cells). In the hypolimnion, local archaebacteria concentrations could be higher than those of *Cytophaga-Flavobacterium* and Beta-proteobacteria (two of the main bacterial groups observed during the study), and could represent up to 49.3% of total detected cells (EUB+ARC) at 50 m (11 April), whereas the maximum relative abundance at 2 m depth was only 17.5% of total detected cells (March 22).

The fraction of bacteria detected by EUB338 was rather low (from 9.2% to 62.8% of DAPI-stained cells), but there was no covariation of the fraction of bacteria detected by EUB338 with bacterial abundance, chlorophyll concentrations, Dissolved Organic Carbon (DOC), or temperature. Maxima and minima of Eubacteria counts were often concomitant with those of DAPI cell counts. The linear regression analysis between the DAPI cell counts and EUB cell counts during this study yielded an r^2 of 0.68 ($n = 18$, $P < 0.02$).

Eubacterial concentrations were significantly higher in the surface layers ($P = 0.001$) (Fig. 3A). Similarly, the mean abundances recorded for the different bacterial groups (Alpha-, Beta-, Gamma-proteobacteria, *Cytophaga-Flavobacterium*) were always higher in the epilimnetic layers than in the hypolimnion (Fig. 3A). These bacterial groups represented from 14.5% to 77.8% of cells detected with EUB338. Alpha-proteobacteria were the minority group at both depths, with a maximum concentration recorded on July 3 at 2 m and 50 m (4.3×10^4 and 1.2×10^4 bacteria mL^{-1}, respectively). However, these highest values accounted for only 5.2% (2 m) and 4.1% (50 m) of Eubacteria (Fig. 3A). At 50 m Beta-proteobacteria abundances remained rather low and stable throughout the study, whereas at 2 m depth, maximum values were recorded on April 11 and July 3. Then, Beta-proteobacteria accounted for 16.1% (April 11) and 15.4% (July 3) of the EUB. Beta filaments were regularly observed, and could account for up to 30% of total Beta-proteobacteria (April 25: 2 m). In the epilimnion, Beta filaments accounted for 0 to 25% (mean: 7.65%) of DAPI-stained filaments, whereas in the deeper layer, these percentages ranged from 0 to 100% (mean: 25.5%); (Fig. 3B). Gamma-proteobacteria represented the second most abundant group, and accounted on average for 6.4% and 11.9% of Eubacteria,

respectively, at 2m and 50 m. Bacterial filamentous forms were rarely detected with Gamma-preoteobacteria probes.

At both depths, CF constituted the main bacterial group, with maximum values of 3.6×10^5 bacteria mL^{-1} on April 11 at 2 m and 6.4×10^4 bacteria mL^{-1} on March 22 at 50 m (Fig. 3A). When maximum abundances were reached, CF represented 51.4% (2 m, April 11) and 43.3% (50 m, April 11) of the EUB338-detected cells. Filaments could account for up to 17.4% of CF cells (maximum on June 19 at 2 m). Peaks of abundance for CF filaments were observed on April 25 and June 19 in the epilimnion, while at 50 m, filament concentrations remained low except on June 19 (Fig. 3B). *Cytophaga-Flavobacterium* filaments accounted for between 0 and 87.8% of the DAPI-stained filaments at 2 m depth (mean: 61.11%), whereas in the hypolimnion, the low proportion of filaments within this group (mean: 23.9%) suggested that most of the DAPI-stained filaments were not identified by FISH.

We observed seasonal variations in the relative contributions of the four bacterial groups (Alpha, Beta, Gamma, CF), especially at 2 m depth (Fig. 3A). During the mixing period, the structure within the four groups was quite similar at 2 m and 50 m, and was generally characterized by the marked dominance of the CF group, and the relatively low abundances of Alpha- and Beta-proteobacteria. From March to the end of May only one exception was noted, when, on April 11, the fraction of Beta-proteobacteria increased markedly at both depths. Then, when thermal stratification occurred, and more especially in June and July, at 2 m, the proportion of CF gradually decreased, while the Alpha- and Beta-groups increased to reach their maximum relative abundance on July 3 (16.8% and 49.5% of EUB338-detected cells, respectively). At 50 m depth, the fraction of the Beta-group also increased on 23 May (clear water phase) and 19 June, but it was replaced by Alpha-proteobacteria by the end of the study (Fig. 3A). We noted, at 2 m depth, that the abundances and fractions of Alpha- and Beta-proteobacteria covaried with the abundance of heterotrophic flagellates ($r = 0.81$ and $r = 0.83$, respectively).

Taxonomic Composition, Abundance, and Biomass of Flagellates. In most cases, flagellates could be identified to the genus level. A total of 13 taxa were identified. Within heterotrophic flagellates, major taxa such as Kinetoplastids, Chrysomonads (Heterokonts), and Cryptomonads *Kathablepharis* sp. could be identified under the microscope. Some other flagellates, particularly the colorless Cryptomonads (5–10 μm long) and small uniflagellate cells (2–6 μm long *Oikomonas*-type cells), which could not be identified unequivocally, were referred to as: "unidentified heterotrophic Cryptomonads" (UHC) and "unidentified heterotrophic uniflagellates" (UHU). The cryptomonads *Cryptomonas* and *Rhodomonas* and the Chrysophytes *Dinobryon* and *Chrysidalis* dominated

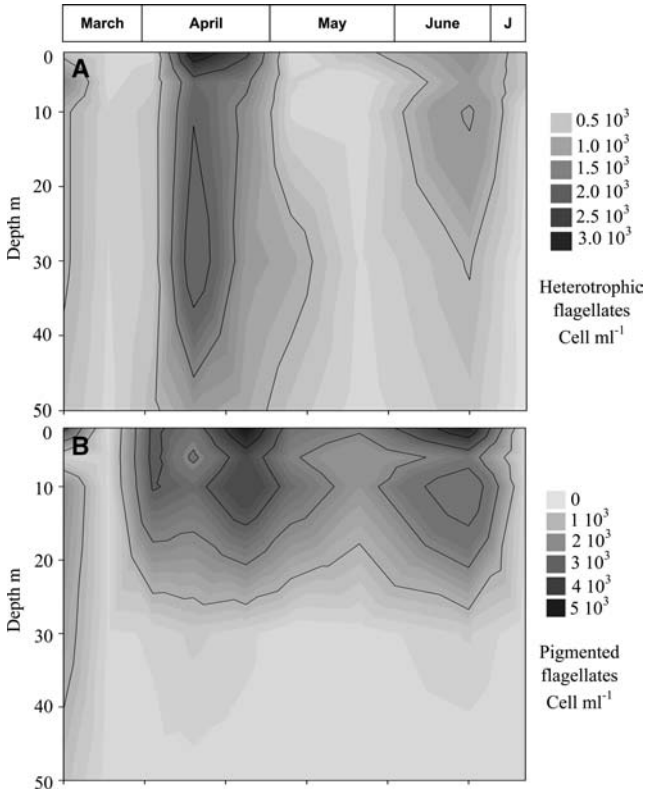

Figure 4. Seasonal changes in of heterotrophic (A) and pigmented flagellate (B) abundances from March to July 2002.

the pigmented flagellate community. More occasionally often, we observed the Chrysophytes *Ochromonas* and *Mallomonas* and the Euchlorophytes *Chlamydomonas*. Of these, *Dinobryon, Ochromonas*, and *Cryptomonas* were the main species known to develop phagotrophic activity [18, 66, 68]. Flagellate abundance and biomass fluctuated from 1.9×10^2 cells mL^{-1}, i.e., 2.4 μgC L^{-1} (April 25, 50 m depth) to 6.6×10^3 cells mL^{-1}, i.e., 8.3×10^1 μgC L^{-1} (April 25 , 2 m depth). In the upper layers, peak abundances were observed during April (max: 6.6×10^3 cells mL^{-1}) and June (max: 5.6×10^3 cells mL^{-1}), while in the bottom layers, no clear variation was observed. In the euphotic zone, at all sampling dates, pigmented flagellates outnumbered the HNF. Heterotrophic flagellate abundance ranged from 7.3×10^1 to 3.2×10^3 cells mL^{-1}. Two abundance peaks were recorded. The first peak occurred in mid-April throughout the depth of the water column, with a maximum value of 2.3×10^3 cells mL^{-1} (April 11, 2 m) and the second, on July 3, was essentially located in the upper layers (max: 3.24×10^3 cells mL^{-1}, 6 m) (Fig. 4A). The heterotrophic flagellate size structure displayed some major seasonal variations. The ">5 μm" size fraction of HNF was composed of Cryptomonads (*Katablepharis*) and some Kinetoplastids (*Bodo*). The small size fraction (from 2 to 5 μm), composed of *Spumella* and several undetermined flagellates represented a large part of the total flagellate community in the bottom layers (45% and

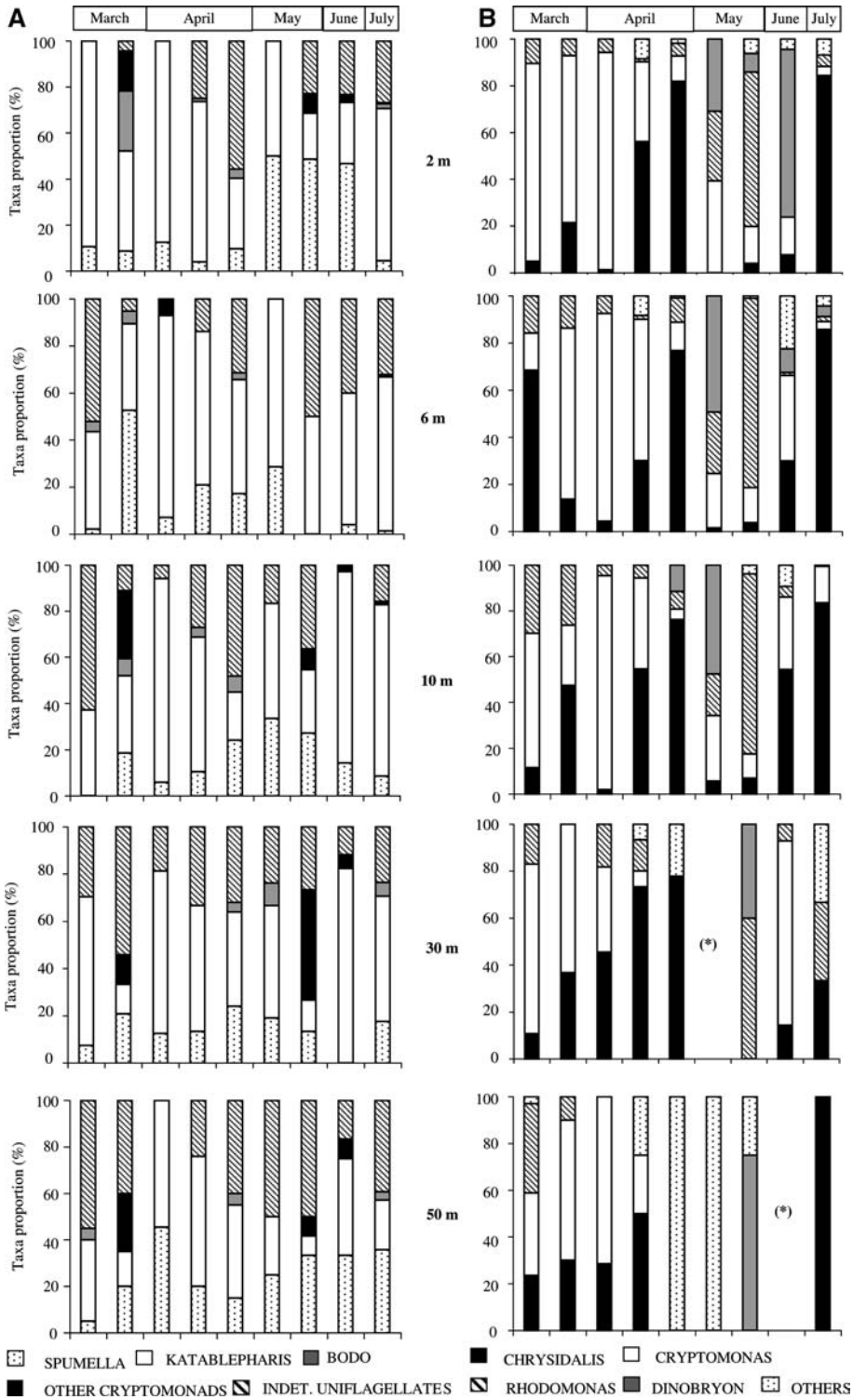

Figure 5. Seasonal changes in the taxonomic composition of heterotrophic (A) and pigmented (B) flagellates at the five sampling depths from March to July 2002. (*): No pigmented flagellates recorded.

55% of the total heterotrophic flagellate community at 30 m and 50 m depth, respectively). We detected seasonal variations in the composition of the HNF community (Fig. 5A). We noticed that *Katablepharis* was relatively important throughout the study; it reported more than 89.5% of the total HNF abundance on March 11 (2 m). *Spumella* ranged from 0 to 52.6% of the total abundance in the upper layers (2 m) during the clear water phase. The relative abundance of unidentified flagellates remained high throughout the study, whereas that of *Bodo* was relatively low. Maximum abundances for pigmented flagellates were recorded during spring (April 25) and early summer (June 19) in the upper layers (5×10^3 and 4.8×10^3 cells mL^{-1}, respectively, at 2 m). We observed a

concentration gradient of pigmented flagellates as a result of their photosynthetic activity (Fig. 4B). Most of the pigmented flagellates ranged in size from 8 to 20 µm. All *Chrysidalis* spp. identified were <5 µm in size, and these small flagellates could occasionally dominate the pigmented flagellates (85.7% and 100%, respectively at 6 m and 50 m on July 3). We noticed a wide seasonal evolution of the composition of the pigmented flagellates (Fig. 5B). The mixotrophic species *Dinobryon* was sporadically recorded from May to June, and could account for up to 71% and 75% of the pigmented community (on June 19 at 2 m and on May 23 at 50 m, respectively), whereas the Cryptomonads *Cryptomonas* and *Rhodomonas* were regularly observed and displayed major variations in their relative abundance throughout the study (Fig. 5B). There was no correlation between heterotrophic flagellates and total bacterial abundance. Only *Dinobryon* and *Bodo* were correlated with bacterial abundance ($P = 0.01$ at 6 m and $P = 0.03$ at 30 m depth, respectively). The bacterivorous activity of these taxa has been reported in different lakes that vary from their trophic status [17, 23, 30, 68].

Statistical analyses indicated that positive correlations existed between specific bacterial groups and heterotrophic flagellate abundances at 2 m depth. Alpha- and Beta-proteobacteria abundances were positively linked with heterotrophic flagellate abundance ($P = 0.01$ and $P = 0.04$, respectively). Filament formation within the Beta and CF groups was also closely correlated with heterotrophic flagellates ($P = 0.04$) and the total flagellate abundances ($P = 0.009$), suggesting grazing resistance toward bacterivores (see Discussion).

Taxonomic Composition, Abundance, and Biomass of Ciliates. We found a total of 28 taxa during the study. Ciliate abundance and biomass fluctuated between 1.62 cells mL^{-1}, i.e., 4.9 µg C L^{-1} (May 23, 6 m), and 1.24×10^2 cells mL^{-1}, i.e., 9.9×10^2 µgC L^{-1} (July 3, 10 m). Ciliates were particularly abundant in the early summer in the upper layers (July 3). Their abundance covaried with the chlorophyll *a* concentration and picocyanobacteria density ($r = 0.62$ and $r = 0.67$ respectively; $P < 0.05$). We defined a group of mixotrophic ciliates composed of three genera: *Strombidium*, *Coleps*, *Mesodinium*. Mixotrophs were always under-represented as compared to the heterotrophic group. The abundance of the former reached 1.4×10^1 cells mL^{-1} on May 6 (10 m), but was generally below 3.4 cells mL^{-1} (Fig. 6A). The abundance of the heterotrophic ciliates ranged from 1.1 cells mL^{-1} (May 23, 2 m) to 1.2×10^2 cells mL^{-1} (July 3, 10 m), with a maximum recorded in early summer in the epilimnetic layers (Fig. 6A). Large seasonal variations in the composition of the ciliate community were observed (Fig. 7). Prostomatids constituted the dominant group during the mixing period. When thermal stratification appeared, we observed an increase of the Scuticociliates, Haptorids, Colpods, and Oligotrichs

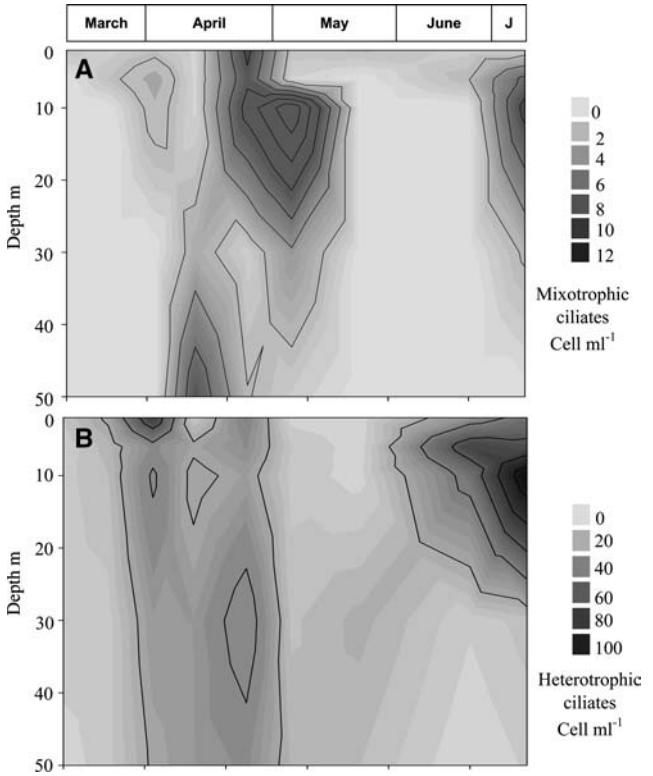

Figure 6. Seasonal changes in mixotrophic (A) and heterotrophic ciliate (B) abundances from March to July 2002.

(particularly in early summer). During this period, we noticed a significant depth-related modification of the structure of the ciliate community. Oligotrichs and Prostomatids were more abundant in the surface waters, while Scuticociliates and Haptorids were dominant deeper down. Ciliates were generally <100 µm in size during the study. We only observed large species during April, and we occasionally recorded a majority of small size ciliates <20 µm (March 11). However, the 20–50–µm class was generally the dominant group at all sampling depths. Within the ciliate community, Colpods and Haptorids were significantly correlated to the bacterial abundance ($P = 0.03$ and $P = 0.02$ at 6 m and 10 m depth, respectively).

Protozoans Grazing on Bacteria. Based on taxon-specific clearance rates assessed by the fluorescent microbeads method, we estimated taxon-specific ingestion rates ranging from 1.2 to 75.3 bacteriaflagellate^{-1}h^{-1} for flagellates and from 2 to 612 bacteriaciliate^{-1}h^{-1} for ciliates. Large variations were observed in per capita predation rates (ciliates + flagellates). The lowest values were measured during March (22 March) and May (23 May) at both depths ($< 1 \times 10^6$ bacteria L^{-1} h^{-1}), whereas the maximal per capita predation rates were recorded during July (7.6×10^7 bacteria L^{-1} h^{-1} at 6 m depth) (Fig. 8). Some high values were also observed during June (both depths) and April (particularly at 2 m depth).

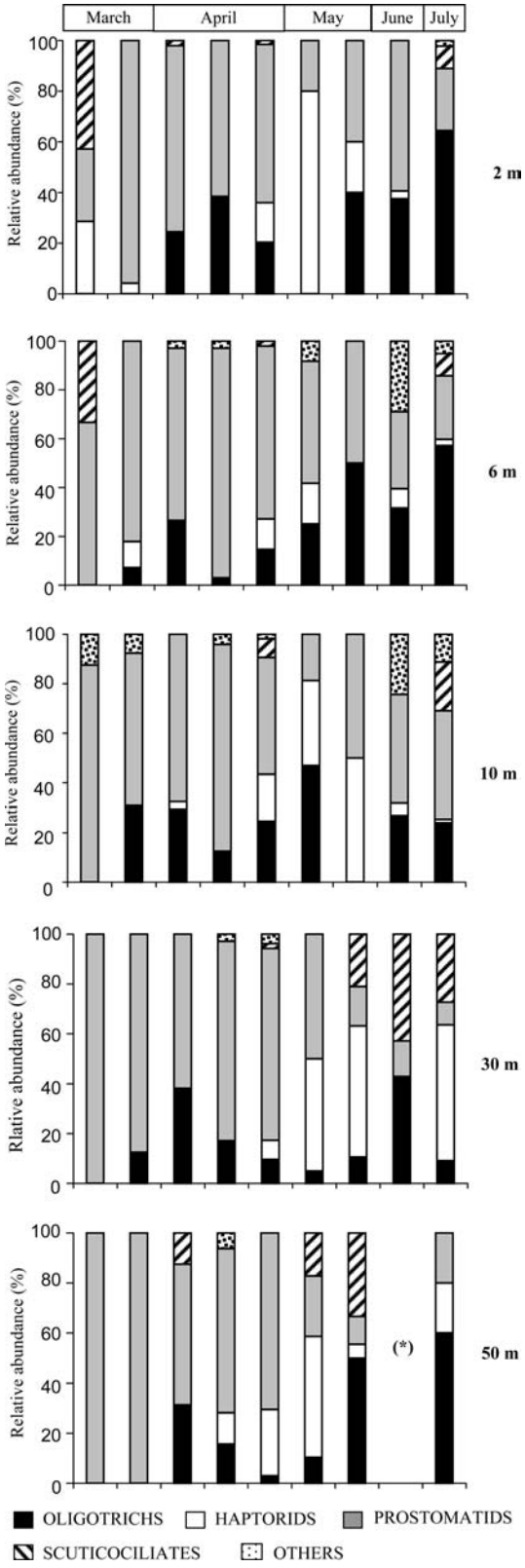

Figure 7. Seasonal changes in the taxonomic composition of ciliates at the five sampling depths from March to July 2002. (*): missing data.

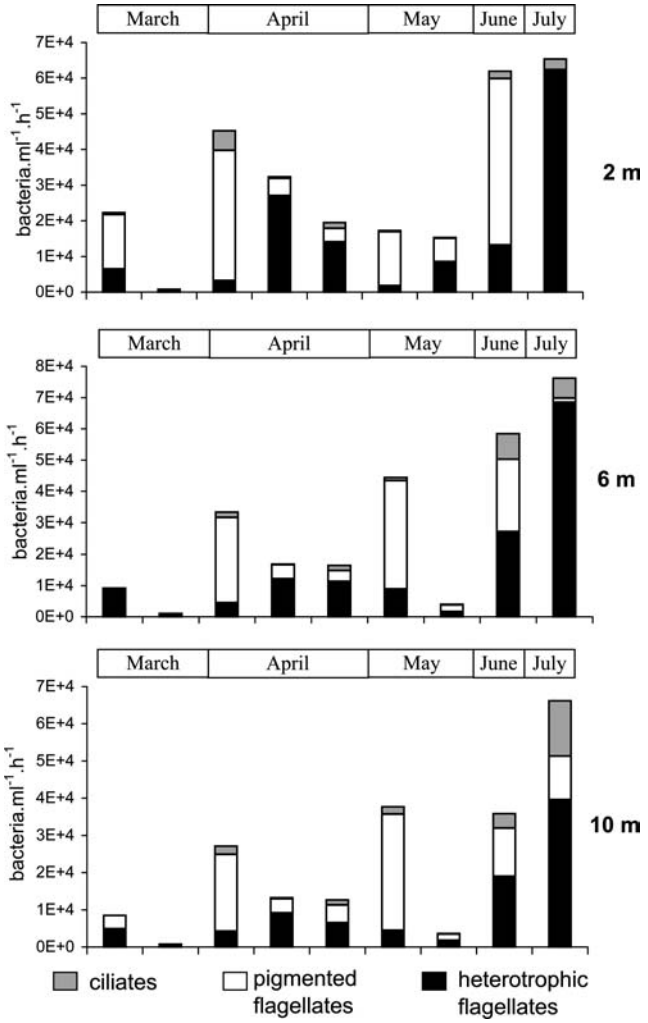

Figure 8. Assessment of protozoan grazing rates (bacteria mL^{-1} h^{-1}) at 2 m, 6 m, and 10 m depth.

Flagellates predation rates (mixotrohs+heterotrophs) fluctuated between 0.7×10^6 bacteria L^{-1} $h^{-1}$and 7.0×10^7 bacteria L^{-1} h^{-1}. The highest grazing rates were recorded on April, June and July (maximum on 3 July at 6 m depth) (Fig. 8). *Katablepharis* and small uniflagellates (UHU) generally represented the main bacterial grazers within heterotrophic flagellates, while *Spumella* had a more sporadic impact. On account of the relatively high ingestion rates of *Dinobryon* and some *Cryptomonas*, the grazing impact of mixotrophs could exceed over that of heterotrophs, for example on 2 April (2 m, 6 m, and 10 m) or during the clear water phase (on 6 May, especially at 2 m depth).

Grazing impact of ciliates was lower than that assessed for flagellates (Fig. 8). The maximal ciliate predation rate was recorded on 3 July at 10 m depth and reached 1.5×10^7 bacteria L^{-1} h^{-1}. High predation rates were also measured at 6 m depth on 19 June and 3 July. According to fluo-

rescent microbeads technique, the highest predation rates at 6 m and 10 m depths were due primarily to grazing pressure of Scuticociliates (mainly *Uronema*), Oligotrichs (mainly *Strobilidium, Lohmaniella, and Halteria*), some Prostomatids (*Urotricha*), and Colpods (*Colpoda*). At 2 m depth the predation impact of ciliates was generally lower than that measured at 6 m and 10 m depth, except on 2 April when Prostomatids (*Urotricha*) and Oligotrichs (*Strobilidium*) were responsible for per-capita grazing rates of 5.5×10^6 bacteria L^{-1} h^{-1}.

Discussion

Limitations of the FISH Technique. One of the limitations of the FISH method is that the efficiency of the detection of target cells varies widely. Bouvier and del Giorgio [10] reported that the percentage of cells detected using the common EUB338 probe (% EUB) varies from 29% to 79% in lakes and ponds. This calculation matches the range in the proportion of cells hybridized using any Eubacterial probe for lakes, which ranges typically from 30% to 80% (reviewed in Glöckner et al. [26] and Pernthaler et al. [56]). On average, during the course of the present study, 23.6 (2 m) and 27.2% (50 m) of DAPI-stained cells were identified as bacteria, with a range from 12.5% to 69.1%. Zwisler et al. [83] recently reported similar low percentages of detection during a study on spatio-temporal dynamics of bacterioplankton in Lake Constance. The detection of cells by means of specific rRNA oligonucleotide probes may depend on (1) the number of ribosome per cell, (2) the accessibility of the target and the penetration of the probe into the cell, and (3) the signal intensity. Many studies have highlighted these problems [10, 20, 51] and this topic will not be further developed here.

Many improvements have recently been obtained: the fixation (4% paraformaldehyde) and the use of enzymatic treatment (lysosyme), for example, were proposed to increase the permeability of Gram-positive bacteria [9]. The application of polyribonucleotide and helper probes significantly improved the probe signal [22, 27]. During the present study attempts were made to improve the detection of cells by increasing cell permeability (use of lysosyme), but that procedure failed (data not shown). We used CY3, one of the most intense fluorochromes, but we did not apply helper probes; therefore, it is not surprising that detection rate by EUB probe was low [83]. Moreover, in our study, the low detection efficiency may be related to the small amount of ribosome linked to a low bacterial activity, as suggested by Kemp et al. [42]. Indeed, a previous study conducted between 1998 and 2002 on Lake Bourget showed that the percentage of active bacteria (as revealed by CTC methods) was always significantly lower than the percentages found in other subalpine lakes (Viboud, unpublished). Although fractions of FISH-detected bacteria were rater low during this study, the modification in bacterial group proportions were useful to highlight changes in bacterial structure. Groups such as Actinobacteria, which may be an important bacterioplankton group in freshwater ecosystems [27], were not counted in this study.

Composition and Structure of Microbial Communities. The concentrations and seasonal dynamics of heterotrophic bacteria in Lake Bourget were similar to those reported for mesotrophic Lake Constance [83] and Lake (Léman) Geneva (Jacquet, unpublished). The identification of the phylogenetic groups, as revealed by FISH, showed clearly differing periods in the structure of the bacterial community. Several studies have reported that the Beta-proteobacteria constitute a dominant fraction in freshwater systems [26, 83]. According to Glöckner et al. [26], this subclass may account for 16% (range 3–32%) of total DAPI counts in lakes, whereas the CF phylum only appears to account for 0 to 18% (mean: 7%). In contrast, in Lake Bourget, we observed a considerable dominance of the CF group, and, Beta-proteobacteria or Gamma-proteobacteria constituted the second most abundant bacterial group. The CF cluster also constituted the largest fraction of filamentous morphotypes, as previously observed by other authors [56, 70]. These elongated cells are generally considered to be resistant to nanoplanktonic grazers [29, 37, 59, 71]. Interestingly, the highest concentrations of these filaments were indeed recorded during spring and in early summer, when flagellates were the main grazers. It is noteworthy that FISH revealed that grazing-resistant morphotypes can be found in the major phylogenetic groups, as previously reported [37, 70]. The proportions of the different bacterial groups usually reveal relatively low seasonal and spatial variations [56, 83]. In contrast, we observed clear modifications in the structure of the bacterial communities, especially in the upper layers. From March to May, CF dominated considerably; in contrast, during early summer (July 3), the abundance of CF dropped, whereas that of Beta-proteobacteria increased. This modification in the bacterial structure could be linked either to a modification in nutrient quality or to selected predation pressure on the CF cluster. Indeed, it is well known that the seasonal changes in organic matter quality constitute a strong structuring factor for microbial communities [54, 55]. Similar to the study by Zwisler et al. [83], we observed the highest abundance of CF during the spring phytoplankton bloom, when polymeric substrates and labile organic substrates were dominant (data not shown). *Cytophaga-Flaobacterium* are known to degrade polymeric substrates of a labile and rather recalcitrant nature [18, 43]. Concerning the possible impact of predation, we hypothesized that, although filament formation should allow CF cells to escape flagellate grazing, this strategy was less efficient in early summer, when ciliates

predation pressure increased. During that period, the ciliates were mainly represented by *Halteria* and *Strobilidium*, which are known to be important bacterial consumers in mesotrophic to eutrophic freshwater systems, with a strong grazing impact on a wide prey spectrum [39, 44, 69]. Our grazing assessment using the microbead technique allowed us to confirm the significant role of Oligotrichs in ciliate predation pressure on bacteria. Moreover, the CF cells, which are characterized by having a higher mean biovolume than other groups, are probably more susceptible to ciliate predation [39]. In laboratory experiments of enhanced protozoan grazing, several authors have previously demonstrated that CF are preferentially grazed by bacterivores [37, 43, 56]. Studies in freshwater and marine systems demonstrated that Alpha-proteobacteria show higher resistance to grazing pressure exerted by flagellates compared to other eubacterial subgroups [37, 45]. In contrast, Jardillier et al. [33] showed that, in the epilimnion of the reservoir Sep, Alpha-proteobacteria were subjected to the highest grazing rates when compared to Beta-proetobacteria and CF. The apparent contradictions in the literature as regards the selectivity of bacterivores on eubacterial subgroups (Alpha-, Beta-, Gamma-proteobacteria; *Cytophaga Flavobacterium*) are probably due to the differences existing in the bacterivores' community composition in the various studies. Our results showed that in the epilimnion (1) the decrease in CF abundance was concomitant with highest protozoan grazing rates and (2) the abundances and fractions of Alpha- and Beta-proteobacteria covaried with heterotrophic flagellates abundance, suggesting that these groups were not highly susceptible to high predation rates of flagellates. This kind of link (grazers–bacterial groups) appeared clearly only in the epilimnion.

Both the relatively high richness of ciliate taxa (28 genera) and their concentrations were similar to those reported during previous studies performed in other mesotrophic lakes [51]. The values recorded in Lake Bourget for HNF abundance and biomass were also in the usual range observed in mesotrophic or eutrophic lakes [5]. In fact, our results revealed that the values for Lake Bourget were closer to those measured in slightly eutrophic lakes. In mesotrophic systems, HNF biomass is usually very low in spring and reaches its maximum in early summer, while in slightly eutrophic lakes, the HNF population peaks in spring and declines sharply in early summer [5]. In our study, the marked dominance of *Cryptomonad* forms within the heterotrophic flagellate community was rather unusual, and contrasted with the results of several authors, who all reported *Chrysomonads* as being the main group [64, 73]. In Lake Bourget, *Cryptomonads* dominated the pigmented flagellates, and these include some taxa classified as mixotrophs, such as *Cryptomonas*. Potentially mixotrophic flagellates were largely present during our survey. *Dinobryon*, which is known to exhibit bacterivore behaviour, was abundant during the clear water phase and on June 19, while *Cryptomonas* (which dominated the flagellate community during spring) constituted a potentially important bacterial grazer. Our estimation based on fluorescent microbeads allowed us to support these hypotheses; however, as discussed below, the importance of mixotrophic species as bacterial grazers was lower than that previously observed in the epilimnion of the oligotrophic Lake Annecy [23].

Regulation of Bacteria. The distribution of the heterotrophic bacteria and protist abundance, as well as our estimation of protozoan grazing rates, indicated that the availability of resources and the increase in temperature seemed to be the main structuring factors for heterotrophic bacteria compared to protist predation. Peak concentrations of heterotrophic bacteria were recorded in the epilimnion during the spring (April) and early summer phytoplankton bloom (July). Increases in protist abundance induced no drop in bacterial abundance, suggesting that predation was not of major importance in the regulation of bacterial abundance at that time. The assessment of the protozoan grazing impact allowed us to confirm this hypothesis, showing that the highest per capita grazing rates recorded in April, June, and July induced no reduction in total bacterial abundance and that potential protozoan grazing represented from 1% to 48% of bacterial production, which revealed a moderate impact when compared to similar measurements from in other lakes [7, 15, 23].

Obviously, the absence of direct measurements of bacterial production using radiolabeled thymidine or leucine incorporation during this study prevents us from drawing firm conclusions about these estimated values, and our results should be considered in that light. However, the moderate and highly variable impact of protozoan grazing on bacteria regulation in the epilimnion of Lake Bourget is not surprising. Three *in situ* experiments performed in 2003 in order to compare protozoan and virus-induced mortality of heterotrophic bacteria in this lake showed similar variations in flagellates grazing rates and demonstrated that the virus-mediated mortality (measured by the dilution method) could occasionally exceed flagellate grazing (Jacquet et al., submitted). Viruses are known to be potential important controlling agents in planktonic community composition, diversity, and succession, playing a key role in cell mortality and nutrient cycles [7, 12, 60, 78], however, these points will be not further developed here.

Protozoan grazing did not appear as the main regulator of bacterial abundance in this study, but grazing-induced modifications were observed in both the structure of the phylogenetic bacterial groups, as previously discussed, and in the bacterial size structure. We assumed that the increase in the proportion of filamen-

tous forms within the heterotrophic bacteria was one of the consequences of the highest bacterivore pressures by flagellates. Mixotrophic and heterotrophic flagellates alternately represented the main bacterivores during this study, ciliates played a significant role as bacterivores only in July. The maximum biomass of filaments could amount to 16.6% of the total bacterial biomass (19 June). This percentage was smaller than that reported in other field studies in mesotrophic to eutrophic lakes, in which the elongated cell biomass proportion fluctuated between 20% and 50% [36, 55, 67]. The mechanism that controls a grazing-mediated switch from single-celled bacterial communities to filamentous-dominated bacterial communities is still under discussion [41, 68]. Although the upper grazing size limit is still not well defined, filamentous bacteria are considered by numerous authors to be protected against nanoflagellate predation [28, 29, 37, 41, 68, 71]. However, according to Wu et al. [81], size alone is not sufficient to define a refuge for filamentous bacteria from nanoflagellates predation. They demonstrated that direct-interception-feeding nanoflagellates (*Ochromonas* sp.) successfully consumed different tested filamentous strains (among which were four Beta-proteobacteria) [81]. According to those authors, the investigated filamentous bacteria are not protected from nanoflagellate predation but have a selective advantage due to the decrease in the flagellates' ingestion efficiency with increasing length of bacteria.

Regulation of Protists. Heterotrophic nanoflagellates are usually considered to be the main bacterivores [13, 40, 55], but some studies have highlighted the lack of coupling between heterotrophic bacteria and HNF [25, 80]. We did not find any significant correlation between these two compartments. Wieltschnig et al. [80] suggested that this weak or absent coupling could be explained, on the one hand, by the relatively low impact of HNF grazing on bacterial abundance and, on the other hand, by the fact that HNF abundance is regulated to a greater extent by predator abundance (top-down) than prey abundance (bottom-up). Our results on the relative grazing impact of heterotrophic, mixotrophic flagellates and ciliates actually showed that HNF were not always the main bacterivores—for example during the clear water phase. To assess the top-down control of HNF, Gasol [24] proposed a model that estimates the importance of predation in the regulation of HNF communities in a given system. He defined the relationship between the abundance of bacteria and HNF as the "Maximal Attainable Abundance" (MAA) line. This line corresponds to the highest attainable HNF abundance when bacteria constitute the only available prey. Empirical data suggest that this maximum attainable HNF abundance is rarely reached, and this is why Gasol computed a second line, known as the "Mean Realized Abundance" (MRA), based on large series of data

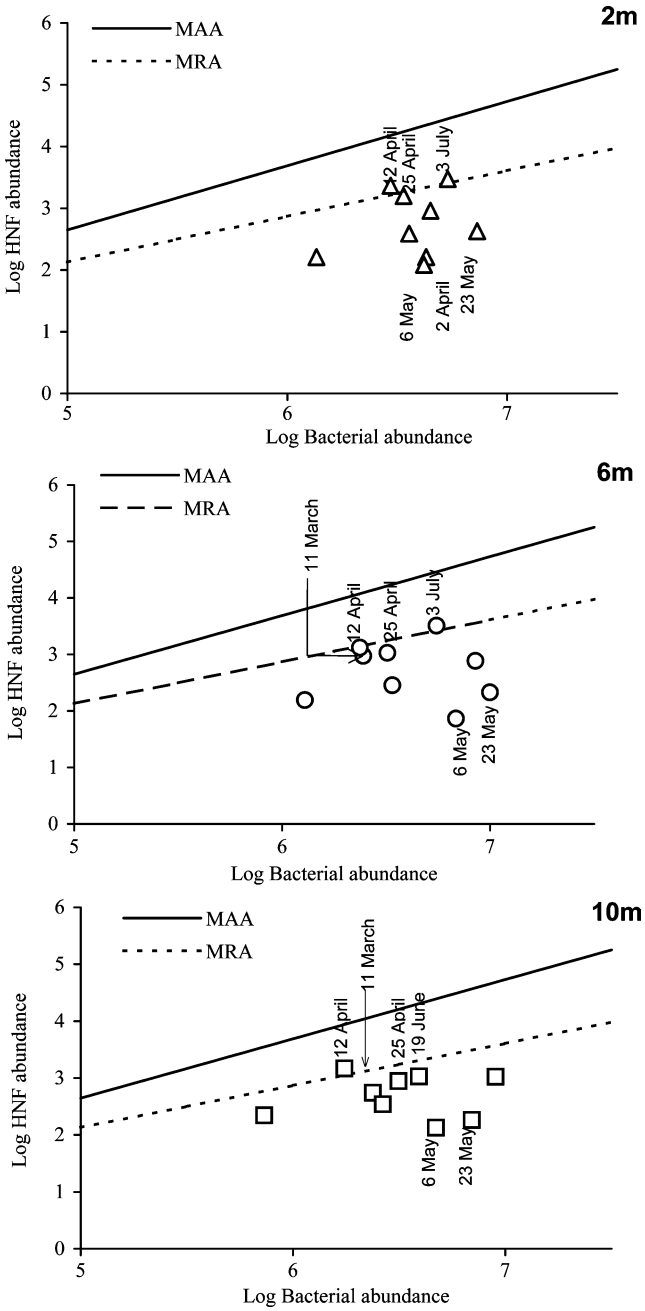

Figure 9. Simultaneous observations of bacterial and HNF abundances (Log) from March to July 2002 in Lake Bourget plotted on the framework devised by Gasol (1994). MAA: "maximum attainable abundance" line; MRA: "mean realized abundance."

from marine and freshwater ecosystems. The simultaneous observations of bacterial and HNF abundance can be plotted in a bivariate space, which includes the MRA and MAA lines. Points near the MAA line would indicate a bottom-up control of HNF abundance, whereas points situated below the MRA line would indicate a top-down form of control. We applied this model to our data (Fig. 9), and found a stronger top-down control of HNF abundance. In addition, this impact was more strongly

marked during the clear water phase (6 and 23 May) than at other sampling dates. The high predation impact during May was clearly linked to the presence of large cladocerans (especially *Daphnia*), particularly at the 2 m, 6 m, and 10 m depths. The top-down control was unclear in the deeper layers (data not shown), where concentration of zooplankton was low (Avois-Jacquet, unpublished). During early spring, the dates for which HNF seemed to be strongly regulated by the availability of their prey were 12 April and 25 April; the points corresponding to this period were near to the MAA line, indicating bottom-up control at 2 m, 6 m, and 10 m. A similar level of control appeared on 3 July, but only at 2 m, and 6 m depth; we assume that, at 10 m depth, ciliates may significantly participate to predation pressure on small flagellates, leading to a higher predation control.

Ciliates seemed to be mostly dependent on resource availability (i.e., prey abundance) throughout the period of interest, except in May, when the metazooplankton were responsible for a large proportion of the microbial mortality. The high ciliate abundance was associated with the development of a deep chlorophyll maximum (DCM) (6–10 m), which usually occurs in lakes of medium productivity such as the mesotrophic Lake Bourget. As reported by Weisse et al. [77] in Lake Constance during a spring phytoplankton bloom, bacteria and ciliates responded rapidly to increasing phytoplankton biomass, whereas the heterophic flagellate increase was restrained by grazing feeding impacts of ciliates and metazooplankton. The main ciliate species identified during this study were not mixotrophic, but were feeding on algae and HNF. Hence the higher densities of ciliates in the epilimnion and DCM layers were consistent with the higher densities of resources in these layers (HNF, algae, and bacteria). Simek et al. [69] reported the important role of *Halteria*, which displayed a high uptake and clearance rate on picoplankton, a large spectrum of prey according to their omnivorous nature, a high growth rate, and relatively low vulnerability to zooplanktonic predation in the reservoirs of řĺmov (Czech Republic) and Sau (Spain). Ciliates, and particularly the small Oligotrichs, are also known to have high grazing rates on HNF [1, 16]. In Lake Bourget, *Halteria* was particularly abundant during early summer, when total ciliate abundance was at its greatest concentration.

A detailed analysis of zooplankton, notably of the copepods, should provide very useful additional information to our data, especially for improving our interpretation of top-down effects on ciliates and flagellates. Yoshida et al. [82] recently pointed out the contrasting affects of cladocerans and copepods on microbial plankton in Lake Biwa. They showed that the relationships between macrozooplankton and microbial plankton cannot be fully understood without taking into account both the feeding characteristics of the macrozooplankton, and the food web structure, the subsidized algal resource, and nutrient regeneration from the macrozooplankton.

Distinct Periods in Microbial Loop Functioning. Our results allow us to distinguish an unambiguous seasonal succession in the microbial community structure and, more especially, a seasonal succession of the main bacterivores (Fig. 10). This succession was particularly clear in the upper layers (2 m, 6 m, and 10 m) whereas in the hypolimnion, seasonal variations were lower and did not allow us to draw a similar scenario. During spring, the relatively high proportion of flagellates compared to ciliates, as well as the assessment of taxon-specific grazing rates, suggests that flagellates play a major role as bacterial grazers during this period. Although HNF are generally recognized as the primary consumers of bacterioplankton and picoplankton in both marine and freshwater ecosystems, recent studies have shown the importance of mixotrophs as bacterial consumers [23, 30, 65]. In the oligotrophic Lake Annecy, it was demonstrated that mixotrophic flagellates (especially *Dinobryon* and *Cryptomonas*) could represent an important link in the flux of material through planktonic food webs throughout a rather long period [23]. Unlike in Lake Bourget, the potential bacterial activity of mixotrophs occurs in a more occasional way. *Cryptomonas*, and *Ochromonas*, which are known bacterivores [23, 30, 34] were particularly abundant during April, whereas *Dinobryon* peaked later (mainly in two sampling dates in May and June). Heterotrophic flagellates were also abundant during April and dominated bacterial grazers at several times. During April, both mixotrophic and heterotrophic flagellates, each in its turn, were responsible of high bacterial grazing rates. Nevertheless, this springtime predation pressure did not affect bacterial abundance, which increased during this period following the phytoplanktonic bloom. Bacteria were probably primarily bottom-up regulated as previously discussed. The high concentrations of bacterial filaments (especially in the *Cytophaga-Flavobacterium* cluster) during spring suggested strong resistance to grazing by flagellates [29, 59, 71]. During the clear water phase, the presence of large zooplankton produced severe predation pressure on bacteria and protists, and their abundance decreased. Several authors have reported similar observations, and have shown that large filtering feeders, such as cladocerans (*Daphnia*), can modify the bacterial community structure through both direct grazing and indirect cascade effects due to grazing on protist bacterivores [1, 21, 35]. At the beginning of the clear water phase (6 May) only mixotrophic flagellates were still responsible for quite considerable bacterivorous behavior among protists. Finally, during early summer, the lower abundance of large filters and the occurrence of the second phytoplankton peak allowed a significant increase in bacterial

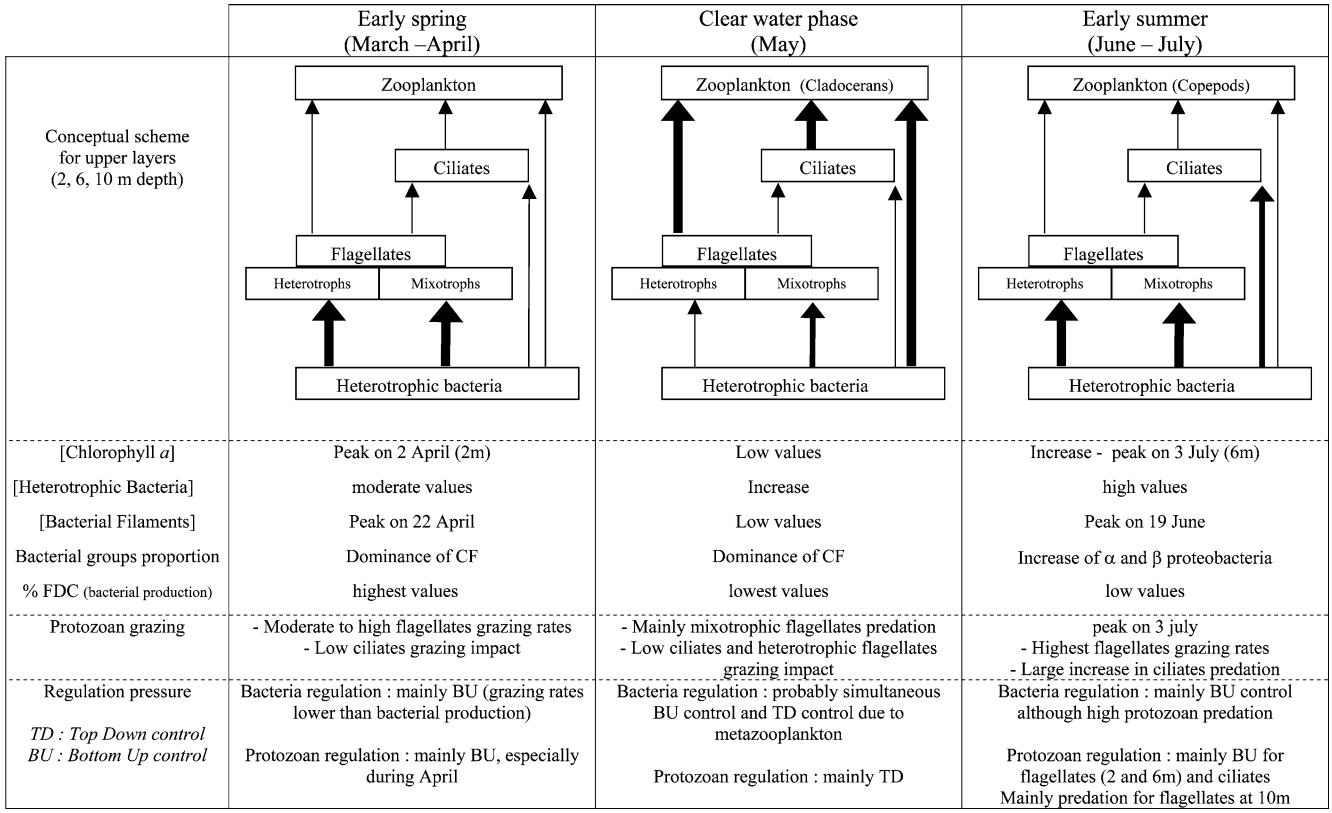

Figure 10. Conceptual scenario for the seasonal succession of bacterivores and of the microbial food web structure in upper layers (0–10m) of Lake Bourget between March and July 2002. Cf: *cytophaga-Flavobacterium* cluster.

abundance. This bacterial increase continued, although flagellates and ciliates, in turn, reached their maximum abundance. This period was characterized by a rapid shift in the carbon pathway transfer within the microbial food web, with a fast succession of the dominant bacterivores. The mixotrophic flagellate *Dinobryon* was the most abundant bacterial grazer on June 19, On July 3, ciliates had increased massively and consumed bacteria as well as HNF at 10 m depth, while in the upper layers (2 m, and 6 m) heterotrophic picoflagellates (undetermined uniflagellates) and *Katablepharis* were the main bacterivores. Bacteria, flagellates, and ciliates, which increased concomitantly, seem to be mainly bottom-up regulated. However, the predation impact on heterotrophic flagellates varied according to depth and seemed to be of greater importance at 10 m depth. During early summer, we observed a change in the bacterial community structure, characterized by an increase in the Alpha and Beta groups of proteobacteria in the upper layers, whereas the CF group declined. This may suggest a preferential predation of CF by protist grazers, which presented the highest grazing impact. The quantity and/or quality of nutrient may also be a determinant in the structure of the phylogenetic bacterial groups, which could highlight more favorable environmental conditions for Alpha- and Beta-proteobacteria groups than for the CF cluster. It is probable that combined effects of resources and predation could be considered, determinants of bacterial structure as recently showed by Jardillier et al. [33]. Last, it is noteworthy that the seasonal succession observed at the reference station of the Lake (north basin) was the same as that at another pelagic point (the central point in the south basin—maximum depth: 112 m), where similar data were collected. Indeed, no significant differences in the microbial structure or dynamics were recorded at the two basins (data not shown).

Conclusions and Research Perspectives

Our study highlights the importance of taking each individual protist species or at least genus into account, and not considering flagellates and ciliates as a uniform group in pelagic ecosystems. The seasonal succession of the main bacterivores observed in this study, and their impact on bacterial structure and dynamics is probably not only relevant for the investigated ecosystem Lake Bourget. Moreover these results provide a basis for future experimental field studies to test the impact of planktonic predation on the structure and dynamics of microbial communities and the way their regulation proceeds. This is of particular interest in light of how little is known about the biogeochemical consequences of bacterial grazing.

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