Microbial Ecology

Microbial Food Webs in Boreal Humic Lakes and Reservoirs: Ciliates as a Major Factor Related to the Dynamics of the Most Active Bacteria

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Abstract

In order to assess the factors that determine the dynamics of bacteria with high nucleic acid content in aquatic systems, we (i) conducted 24-h *in situ* dialysis experiments, involving different fractions of plankton and unfiltered water and (ii) examined empirical relationships between bacteria and both abiotic factors and protists, in boreal humic freshwaters (reservoir and lakes) in the James Bay region (Québec, Canada). Bacteria were subdivided into two subgroups on the basis of their nucleic acid content assessed by flow cytometry. The abundance of bacteria with the highest nucleic acid content and high light scatter (HNA-hs) was significantly correlated, across sites, to bacterial production, whereas bacteria with lower nucleic acid content (LNA) and total bacteria were not. In addition, HNA-hs growth was higher and more variable than LNA growth, indicating that HNA-hs were the most dynamic bacteria. Heterotrophic nanoflagellate and ciliate biomass represented, on average, 5 and 13% of bacterial biomass, respectively. Both in ambient waters and in experiments, ciliates were significantly and negatively correlated with bacteria, whereas heterotrophic nanoflagellates, likely under the grazing pressure from ciliates and metazooplankton, were not. Among ciliates, *Cyclidium glaucoma* appeared to play an important role. Its growth was significantly and negatively correlated to that of HNA-hs but not to that of LNA. In ambient waters, the abundance of this species explained 56% of the variations in HNA-hs abundance and only 27% of those for LNA. The abundances of total bacteria and LNA significantly increased with chlorophyll *a*, whereas those of HNA-hs did not. In addition, during the experiments, the estimated potential losses of HNA-hs significantly increased with the initial abundance of *C. glaucoma*. These results suggest selective removal of the most dynamic bacteria by *C. glaucoma* and indicate that ciliates may play an important role in the dynamics of active bacteria in natural waters. These findings suggest the existence, within the aquatic microbial food webs, of keystone species that are very important in regulating the activity structure of bacteria.

Introduction

Bacteria are significant components of the planktonic biomass [50] and are known to play an important role in biogeochemical processes in pelagic systems [3, 15]. Bacteria, as a whole community, are also the least variable component of the plankton [9, 11], although they are among the fast-growing microorganisms in natural waters. It is now well known that only a fraction of planktonic bacteria is metabolically highly active at a given time. It has also been shown that the abundance of these active bacteria is more variable, across systems, than total bacterial abundance [12, 51]. The development of new methodologies has allowed us to improve our understanding of bacterial communities. It is now known, for instance, that planktonic bacteria are very complex in terms of taxonomy, vulnerability to grazers, and physiological activity, among other factors, and that important temporal changes, with regard to these aspects, occur within these communities [13, 29, 49], despite the relative constancy in their total abundance. There is also evidence that active bacterial cells are responsible for a large portion of growth and production [34]. However, the factors that determine the dynamics of active bacteria in aquatic systems are still not well understood, although a relatively large number of studies have quantified their abundance and proportion (references in [46]). Addressing this question is important for microbial food web models (e.g., [22, 55]), since they still generally

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consider bacterial communities as homogeneous assemblages.

Laboratory experiments have shown that a significant number of bacteria that are apparently dormant may become active following nutrient and/or substrate addition (e.g., [2, 8]). Comparative studies over wide ranges of productivity have shown that variations in the abundance of active bacteria may be related to variations in water temperature and chlorophyll *a* [12, 51]. Selective removal of active cells by protists has also been suggested as a mechanism that may determine the abundance of active bacteria in aquatic systems [45]. In their *in situ* experimental study in the Mediterranean Sea, del Giorgio et al. [13] found, for instance, that the proportion of active bacteria decreased as the grazing rates of heterotrophic flagellates increased. Some other studies have indicated that grazing by protists and *Daphnia* may regulate the abundance of active bacteria in aquatic systems [2, 16, 46]. However, most of these studies have been conducted in laboratory and some have used relatively high concentrations of grazers (e.g., 3000 flagellates mL^{-1} and 50 *Daphnia galeata* L^{-1} [46]), which are not commonly found in oligotrophic systems. All the studies referred to above have used the CTC method to quantify the abundance of active bacteria. However, this method has been questioned because CTC may have toxic effects on bacterial metabolism (references in [44]).

Since the study of Li et al. [36], it has been suggested that the nucleic acid content of bacteria can be used as an index of their activity or that the percentage of bacteria with high nucleic acid content can be used as the number of active bacteria [e.g., 23, 34]. One way of examining this nucleic acid content is to stain bacteria with nucleic acid dye and analyze the sample by flow cytometry. This method, which allows the distinction of at least two bacterial subgroups characterized by high and low apparent DNA content [36], has increasingly been used in attempts to quantify the number of active bacteria in water systems. Several studies indicate that active cells tend to have higher nucleic acid content [5, 34]. To our knowledge, only two field studies, both from the Mediterranean Sea, have analyzed the relationships between bacterial physiological subgroups (as assessed by direct methods) and grazers. These studies have related heterotrophic flagellates to CTC-positive bacteria [13] or to bacteria with different DNA content [53].

The aim of this study was to explore the factors that determine the abundance of bacteria with high nucleic acid content (as determined by flow cytometry) in pelagic environments. This study was conducted in a boreal humic reservoir and four surrounding lakes. Data on microbial food webs in such environments are scarce. In humic aquatic systems, mixotrophic flagellates and, in some cases, heterotrophic nanoflagellates (HNF) are considered key in determining the fate of bacterial production [24, 30]. In boreal humic aquatic systems, the qualitative and functional importance of ciliates has received little attention [30, 56]. Given the existing evidence that protist grazers may selectively remove active bacteria, we hypothesized that the dynamics (abundance and growth) of bacteria with high nucleic acid content would be controlled by their dominant protist group. To test this hypothesis, we (i) carried out *in situ* experiments involving size fractionation of plankton, in order to examine the relationships between the growth of bacterial subgroups (with high and low nucleic acid content) and that of protists, and (ii) analyzed the empirical relationships between the abundance of the subgroups of bacteria and both the abiotic factors and the abundance of protists (HNF, mixotrophic flagellates, and ciliates) in ambient waters.

Materials and Methods

Study Sites. The present study was conducted in the Reservoir Lagrande 2 (LG 2, now known as Reservoir Robert Bourassa) and four surrounding lakes (Lakes Desaulnier, Patukami, Ukaw, and Yasinski) located in the eastern part of the James Bay region (Québec, Canada) (Fig. 1). LG2 is a large hydroelectric reservoir that belongs to the hydroelectric complex of the Lagrande River (52°N, 54°N). The relief of this region comprises, from west to east, a coastal plain (150 km wide with scattered peat bogs and clay deposits), a hilly central plateau with numerous lakes, and, at the eastern end, an area of rougher terrain. The vegetation is a coniferous forest mainly composed of black spruce or jack pine. The entire area is underlain by granitic bedrock (Canadian Shield). The morphometric characteristics of the study sites are given in Fig. 1 and Table 1. Both the reservoir and lakes were sampled because this work is part of a comprehensive study intended to compare reservoirs with natural reference lakes, in terms of carbon flow within the food web.

Sampling. Samples were collected in mid spring (~2 weeks after snow melt) and midsummer in 2001, at two stations in each lake (near the inlet and at the deepest station) and 10 stations in the reservoir (Fig. 1). Experimental samples were taken and incubated at two additional stations (station Marina in LG2 and station E in lake Desaulnier). The experimental stations were chosen for their accessibility and location near the field laboratory. Samples were taken using a seaplane for stations with a depth >10 m and a boat for the others (Table 1). Prior to sampling at each station, thermal and light profiles were determined. Temperature was measured *in situ* using either a temperature/O_2-meter (YSI 5718 DO probe) or a multiparameter probe (YSI 6600). Light

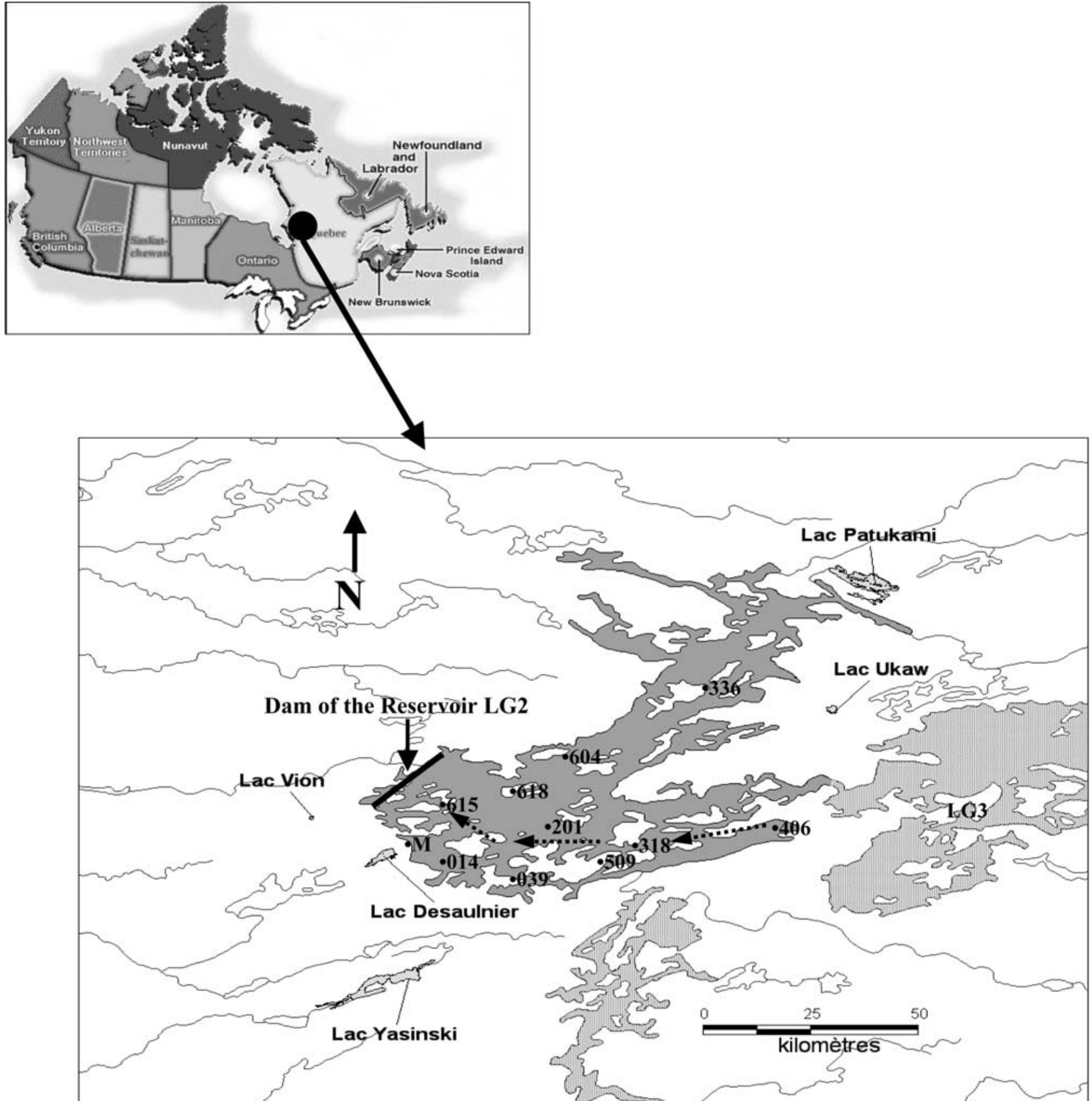

Figure 1. Spatial location of the study sites. The numbers in the reservoir indicate the sampled stations. The dotted arrows indicate the main flow of waters. The letter M indicates the experimental station in the reservoir (Marina station).

profiles in the water column were measured using a Li-1400 apparatus equipped with a submersible probe (Li-193 SA), and the limit of the euphotic zone was determined as the depth at which 1% of the subsurface photosynthetically active radiation (PAR, 400 to 700 nm) penetrated.

All samples were collected with a 8-L Van Dorn sampler. Samples for examination of empirical relationships between microbial compartments in ambient waters were taken every 0.5 m in the entire water column for stations with depths <4 m and in the epilimnion or the euphotic zone (when the water column was not thermally stratified) for the other stations. The samples taken at each station were then pooled, because they were also used for concurrent measurements of chlorophyll *a* and plankton photosynthesis and respiration. Samples for the

Table 1. Location and characteristics of the sampled stations in Reservoir LG2 and lakes

Ecosystem	*Volume* ($\times 10^6 m^3$)	*Maximum area* (km^2)	*Sampled station*	*Location*	*Water depth at the sampled station (m)*	*DOC* ($\mu M\ L^{-1}$)
Lake Desaulnier	86	10.6	A	53°34′10″N, 77°34′ 07″W	14	749
			B	53°38′ 40″N, 77°35′13″W	1	744
			E	nd	7	741
Lake Patukami	247.1	42.4	A	54°14′ 28″N, 75°53′ 43″W	40.7	452
			B	54°14′ 30″N, 75°51′46″W	1.6	349
Lake Ukaw	16.3	3.3	A	53°55′35″N, 76°00′44″W	17	550
			B	53°55′52″N, 76°59′43″W	3.18	623
Lake Yasinski	198	41	A	53°17′10″N, 77°29′07″W	21	904
			B	53°17′59″N, 77°26′ 50″W	1	889
Reservoir LG2	19365	2835	318	53°41′13″N, 76° 45′ 39″W	26	408
			406	53°43′06″N, 76°10′05'W	26	390
			615	53°44′14″N, 77°32′17″W	59	525
			610	53°45′09″N, 77°11′50″W	21	479
			039	53°40′58″N, 77°14′50″W	23	508
			014	53°36′28″N, 77°27′17″W	44.5	528
			336	54°01′35″N, 76°30′ 32″W	36.5	424
			604	53°47′20″N, 76°59′02″W	18.5	421
			Marina	nd	9.4	nd
			509	53°32′14″N, 76°38′ 57″W	43.3	511
			D24-B	nd	nd	591

DOC (Dissolved organic carbon) values are average for the two seasons, except for station D24-B, which was sampled only once (in summer). Details on DOC measurements will be presented elsewhere (Tadonléké et al., in prep.).
Station A: deep station; Station B: shallow station; Station E: experimental site in Lake Desaulnier; station Marina: experimental site in the Reservoir LG2; nd: not determined.

experiments were taken at the 1-m depth. All the samples taken for microbial community counts were preserved after collection, using formaldehyde (final concentration 2 % v/v) for bacteria, glutaraldehyde (final concentration 1% v/v) for plastidic nanoflagellates (ANF) and heterotrophic nanoflagellates (HNF), and acidic Lugol's solution for ciliates. These samples were stored at ~4°C until analysis. Bacteria were analyzed within 2 weeks after the end of each sampling interval. All protists were counted within 2 months after collection.

Experimental Setup. Five experiments were conducted during our investigation. Two of them were performed at station E in Lake Desaulnier (one in spring and one in summer: experiments 1 and 5, respectively) and the other three at the station Marina of LG2 (two in spring and one in summer: experiments 2, 3, and 4, respectively). These experiments were done with size-fractionated and unfiltered plankton samples, in order to (i) examine trophic dynamics within the planktonic communities, especially the grazer effects on bacteria and (ii) check possible limitation of bacteria by resources. Lake water was passed through screens of varying pore size by gentle vacuum (on 1 μm filters and 5 μm Nitex net) or by gravity (20 μm Nitex net) in order to produce treatments with varying grazer concentrations. Our treatments were thus <1 μm (grazer-free), <5 μm, <20 μm, and unfiltered lake water. In most of these filtrates, organisms with sizes larger than the filter pore size were not found. In the other, <1% of these organisms were found. Three replicate dialysis bags were filled with water from each treatment. The dialysis bags (Spectra/por 1) had a cutoff size of 6000–8000 Da and a flat width of 80 mm. They were cut in lengths of ~60 cm to hold 1100–1200 mL, washed in distilled water, rinsed overnight, and then soaked for 5 h in Milli-Q water prior to the start of each experiment. Before the dialysis bags were clamped, subsamples were collected from the three replicates of each treatment and preserved, as indicated above, in order to determine initial concentrations of bacteria, ANF, HNF, and ciliates. The dialysis bags were tied randomly on four incubation systems, each designed to support three bags. Incubations were performed *in situ* at the 1 m depth (i.e., where water samples were collected) for 24 h, and then all dialysis bags were retrieved and resampled to examine changes in the abundance of the above-cited microorganisms. The incubations last only 24 h because of the extremely windy conditions, which did not allow collection of samples by boat at many stations and also destroyed some of our experiments before 36 h or even before 24 h of incubation (e.g., in Lake Yasinski).

Sample Analyses. Samples for chlorophyll *a* (Chl *a*) determination were passed through GF/C glass fiber filters and the latter were frozen until analysis. Chl *a* collected on filters was extracted in hot ethanol in the dark and concentrations were measured spectrophotometrically.

A few minutes before bacterial analyses, a working solution of the nucleic acid dye was prepared by adding

10 μL of a stock solution of the dye SYBR Green II (Molecular Probes) to 90 μL of 0.2-μm-filtered Milli-Q water. The affinity of SYBR Green II for RNA, which is considered as an indicator of cellular activity, is higher than for DNA [33]. To determine the abundance and relative size of bacteria (by flow cytometry), subsamples of 0.5–1 mL of the formaldehyde-preserved samples were spiked with 2.5–5 μL of the working solution of dye and 50 μL of a solution of yellow-green 0.92-μm fluorescent latex beads as an internal standard. The prepared subsamples were incubated in the dark at room temperature for 15 min and then analyzed using a Becton-Dickinson flow cytometer equipped with a laser emitting at 488 nm. Samples were run at low speed ($\sim$12 μL min^{-1}). For each subsample $\sim$10,000 events were recorded in log mode. A plot of green fluorescence measured at 530 ± 30 mn (FL1) versus 90° light scatter (SSC, a parameter related to cell size) was used to discriminate and count stained bacteria (i.e., the total and those delimited as subgroups based on their fluorescence). To refer to bacterial subgroups, we have followed the terminology of Lebaron et al. [34], i.e., we have used the terms bacteria with high and low nucleic acid, instead of bacteria with high and low DNA.

Bacterial biovolumes were estimated using an equation that links bacterial cell volume to bacterial average SSC obtained from flow cytometry analyses. This relationship was determined using 26 samples collected mainly from ambient oligotrophic waters in spring and summer of 2002, because we did not prepare samples for counts of bacteria by microscopy in 2001 (i.e., this study). For each of the 26 samples, we carried out concurrent measurements of bacterial cell dimensions under an epifluorescence microscope and bacterial average SSC normalized to beads, using flow cytometry after staining of samples with SYBR Green II. These cell dimensions were taken on bacteria stained with DAPI (4′6 diamidino-2-phenylindole, [39]) and converted into cell biovolume assuming that the forms of bacteria corresponded to geometric shapes. Filament morphotypes were very scarce. The obtained cell volumes were then plotted against the normalized SSC values. This yielded a relationship (see Results) that was then used to estimate bacterial cell volume in 2001 (i.e., this study), from bacterial average SSC (normalized to beads) obtained with samples of the same year. Bacterial carbon biomass was estimated according to the carbon-volume relationship described by Norland [37].

Bacterial production in ambient waters was estimated from ^{3}H-leucine (Leu) incorporation. One formaldehyde-killed control and three replicate subsamples (5 mL) in black glass vials were spiked with 40 nM Leu (specific activity = 167 Ci mmol^{-1}) and incubated for 60 to 90 min. Incubations were done in the dark in a box containing water from the sampled station, in order to maintain the temperatures of the incubated samples close to those of the original samples. Leu incorporation was stopped using formaldehyde (2% v/v final concentration). Labeled bacteria were collected on 0.2-μm polycarbonate filters, incubated for 10 min, and rinsed twice with 5 mL of cold 5% trichloroacetic acid. Filters were stored at 4°C and analyzed later by liquid scintillation. Rates of Leu incorporation were converted to carbon production using the conversion factor of 3.1 kgC mol Leu^{-1} [31].

For heterotrophic nanoflagellate (HNF) counts, 20 to 30 mL of glutaraldehyde-preserved sample was stained with DAPI (3 mg L^{-1} final concentration) and filtered through 0.8-μm pore size polycarbonate filters. Counts were carried out under both blue and UV light at magnification × 1000, using a Leitz DMR epifluorescence microscope equipped with a 50-W HBO mercury lamp and the appropriate exciter/barrier filter set. Plastidic nanoflagellates (ANF) were counted on the slides prepared for HNF counts. The autofluorescence of Chl a was used to distinguish ANF and HNF. Analyses were done in triplicate, and at least 100 cells for ANF (when there were enough individuals) and 200 cells for HNF were counted for each replicate.

Duplicate subsamples (200 to 250 mL) of Lugol-preserved samples were placed in settling chambers for 48 to 60 h for ciliate counts. These counts were done in duplicate because during the processing of samples after incubation, water from some replicates was lost. Counts were done under an inverted microscope with phase contrast, at magnifications × 200 and × 800. The whole settling chamber was scanned at low magnification, for large ciliates. At least 300 ciliates were counted in each replicate, and species or genera were identified using several book guides [10, 19, 20]. To confirm our determinations, we also examined samples brought to the laboratory at Montreal, and stained using the Fernandez-Galiano's silver carbonate technique [18, 52]. HNF and ciliate cell volumes were estimated from the linear dimensions of cells in preserved samples, assuming their form corresponded to the nearest geometric shapes. Carbon content was estimated using the volume to carbon conversion factors of 0.22 pgCμm^{-3} for HNF [7] and 0.2 pgCμm^{-3} for Lugol's preserved ciliates [41]. For ANF, we were not able to estimate cell dimensions in all samples during counting, as we did for HNF and ciliates. ANF cell dimensions were taken in only 39% of samples at the time we were counting. Unfortunately, cell damage that occurred due to problems in the storage of slides did not allow us to complete the measurements and to calculate ANF biomass.

Growth Rates of Microorganisms. Net growth rates of bacteria, HNF, and ciliates in the experiments were calculated as changes in their respective abundances

in the dialysis bags after the 24 h incubation, assuming exponential growth: $\mu = \ln(N_t/N_0)/T$, where μ is the net growth rates (day^{-1}), T is the time (in days), and N_0 and N_t the initial and final abundances of each of these communities. The exponential model was used on the basis of field-laboratory tests that consisted in examining changes in the microbial abundance every 6 h for 60 h incubations (not shown). ANF growth rates were not calculated because these cells were found in low abundance (<12 cells per slide) in the Marina samples, which did not allow reliable counts.

Statistical Analyses. Seasonal mean values of the studied variables were compared using the nonparametric Wilcoxon rank sum test. With the exception of temperature, percentages, and growth rates, all variables were $\log_{10}$-transformed for regression analyses, in order to stabilize the variance. The slopes of the regressions were compared using analysis of covariance (ANCOVA). The probability level at which statistical analyses were accepted as significant was ≤ 0.05.

Results

Temperature and Chlorophyll a Standing Stock. The temperature of surface waters ranged between 4.1 and 19.7°C, the mean value in summer (15.2°C) being significantly higher than that in spring (12°C, $P = 0.0053$). All stations with depth >4 m (Table 1) were weakly to strongly stratified, except for those in lakes Ukaw (both in spring and summer), Desaulnier (in spring) and Yasinski (in summer). For stratified stations, the limit of the epilimnion was >6 m, 13 times out of 21.

Chl *a* concentrations were low and varied across sites from 0.55 to 2.19 mg m^{-3}. The mean value in summer (1.44 mg m^{-3}) was significantly higher than that in spring (1.19 mg m^{-3}) ($P = 0.042$), as for water temperature.

Microbial Community Standing Stocks—Bacterial Production. With respect to bacteria, three subgroups were found in our samples (Fig. 2A). Here we followed the terminology of authors who have also reported three bacterial subgroups in natural samples (e.g., [57]. The three subgroups were (i) bacteria with the highest nucleic acid content and high light scatter (HNA-hs); (ii) bacteria with high nucleic acid content and low light scatter (HNA-ls); and (iii) bacteria with low nucleic acid content (INA). Because clear and ecologically relevant patterns were found only for HNA-hs among these three subgroups, we summed the abundance of HNA-ls and INA and presented these two bacterial subgroups as one (LNA, for bacteria with lower nucleic acid content) in this paper, for the purpose of simplification (however, see Discussion).

The relationship used to estimate bacterial average cell volume in this study is shown in Fig 2B. Values of these estimated volumes ranged between 0.031 and 0.044 μm^3 $cell^{-1}$ (mean = 0.037) for the whole community. The average cell volumes were higher for HNA-hs (0.052–0.08 μm^3 $cell^{-1}$, mean = 0.067) than for LNA (0.029–0.04 μm^3 $cell^{-1}$, mean = 0.035). Because the abundance and the biomass of each of the two bacterial subgroups (HNA-hs, LNA) exhibited similar relationships with the potential controlling factors under study (Chl a, temperature, and protists), we will present only the abundance of these subgroups in the rest of the article.

Total bacterial abundance varied among sites by a factor of 4.7, ranging from 0.8 to 3.65 (mean = 1.97) × 10^6 cells mL^{-1} (Fig. 3A). About 6% (range 3–11%) of this total bacterial abundance was from HNA-hs, whose abundance ranged from 0.35 to 2.5 x 10^5 cells mL^{-1}. Total bacterial carbon biomass averaged 22 μgCL^{-1} and ranged across sites from 9 to 42.5 μgCL^{-1} (Fig. 3B).

Rates of bacterial production, as assessed by leucine incorporation, were low (0.3 to 9.5 $\mu gC\ L^{-1}d^{-1}$) averaging 1.62 and 2.1 $\mu gCL^{-1}\ d^{-1}$ in spring and summer, respectively. None of these bacterial variables showed significantly different seasonal means ($P > 0.18$).

HNF abundances in this study were at the low end of values (20–>20,000 HNF mL^{-1}) generally reported for pelagic waters [6]. Our values ranged from 37 to 601 (mean = 145) cells mL^{-1}, all samples included, and from 93 to 278 cells mL^{-1} in initial experimental samples (i.e., before incubations). HNF carbon biomass varied between 0.24 and 2.31 (mean = 0.97) μgCL^{-1} and represented, on average, only 5% (1 to 14%) of bacterial carbon biomass. Both HNF abundance and biomass mean values significantly decreased from spring to summer (Fig. 3 C, D). The HNF assemblages were numerically dominated by <5 µm cells, whose contribution to total HNF abundance ranged from 55 to 96% (mean = 81%) in all ambient samples, and from 71 to 83% in experimental samples at the start of incubations. In terms of biomass, HNF with size < 5 µm contributed, on average, to 38% (4–79%) of total HNF. In terms of both abundance and biomass, no significant difference was found between spring and summer in the relative contributions of these small HNF to total HNF ($P > 0.31$). Most HNF cells resembled Bicosoecids and Kinetoplastids.

Plastidic nanoflagellates (ANF) were considered in this study as potential mixotrophs since it is difficult to distinguish strict autotrophs from mixotrophs by only visual inspection under an epifluorescence microscope. ANF abundance was low, ranging from 3.3 to 246 (mean = 89, $n = 33$) cells mL^{-1}. In contrast to HNF, ANF did not show significant seasonal difference in abundance (100 and 92 cells mL^{-1} in spring and summer, respectively, $P = 0.36$). The community comprised

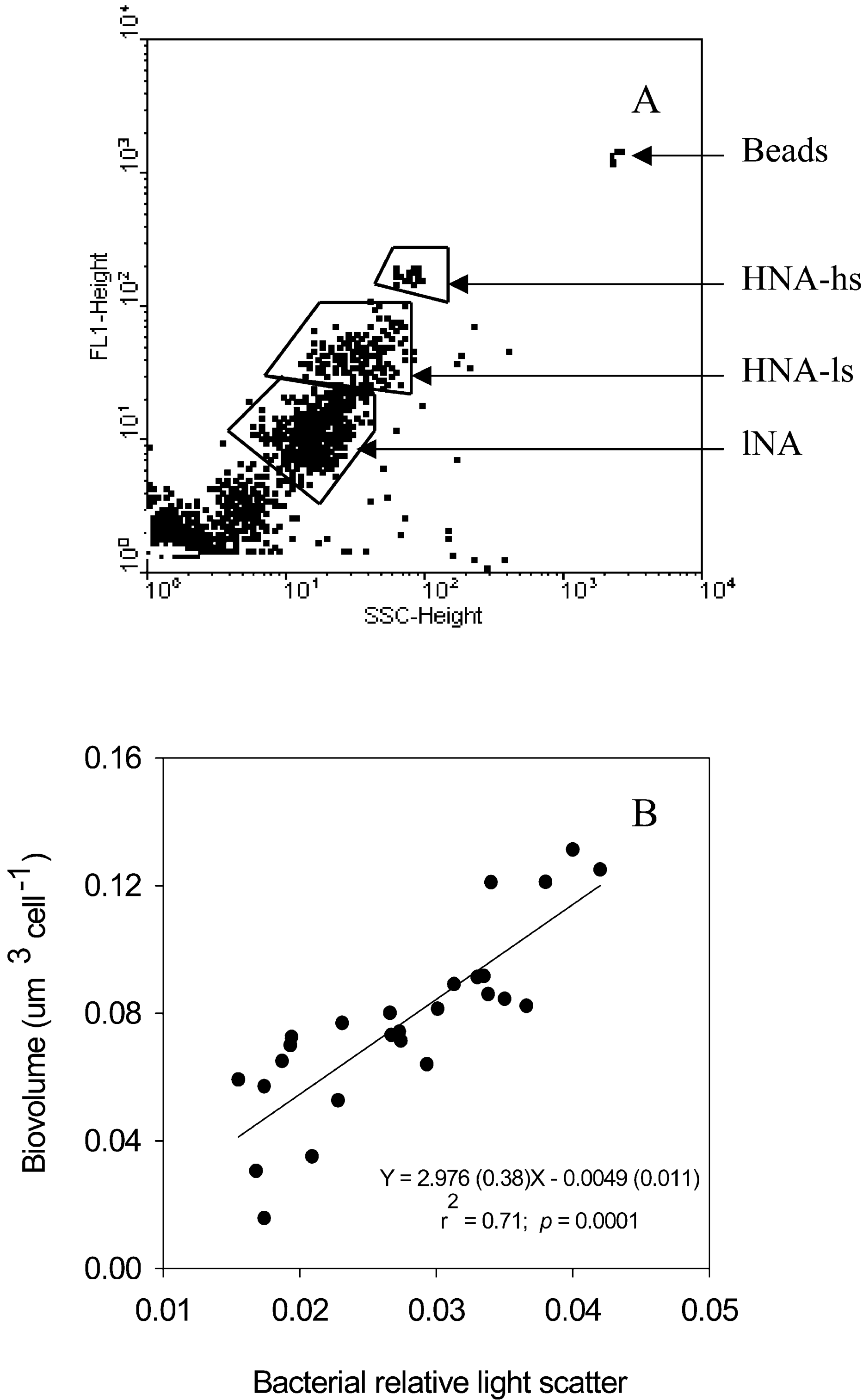

Figure 2. (A) Example of cytograms found during our study (sample from Lake Ukaw 1 July 2001). FL1: green fluorescence; SSC: 90° light scatter. HNA-hs, HNA-ls, and lNA represent bacteria with high nucleic acid content and high light scatter, bacteria with high nucleic acid content and low light scatter, and bacteria with low nucleic acid content, respectively. The particles in the spot just below lNA were not considered as bacteria, based on their very small relative size. (B) Illustration of the relationship used to estimate bacterial cell volume. The numbers in brackets are standard error of estimates (see text for details).

mostly Rhodomonas-like and spherical cells. Larger plastidic flagellates were scarce.

Ciliate abundance fluctuated between 1500 and 10,600 (mean = 5394) cells L^{-1}, whereas ciliate carbon biomass varied from 0.48 to 5.47 (mean = 2.38) μgCL^{-1} (Fig. 3 E, F). Ciliate biomass averaged 13% (2–43%) of total bacterial biomass and exceeded HNF biomass in all but two samples. These two samples excluded, HNF biomass represented from 8 to 93% (mean = 40%) of ciliate biomass. As for HNF, the mean values of both the abundance and the biomass of ciliates significantly decreased from spring to summer (Fig. 3 E, F). Ciliate

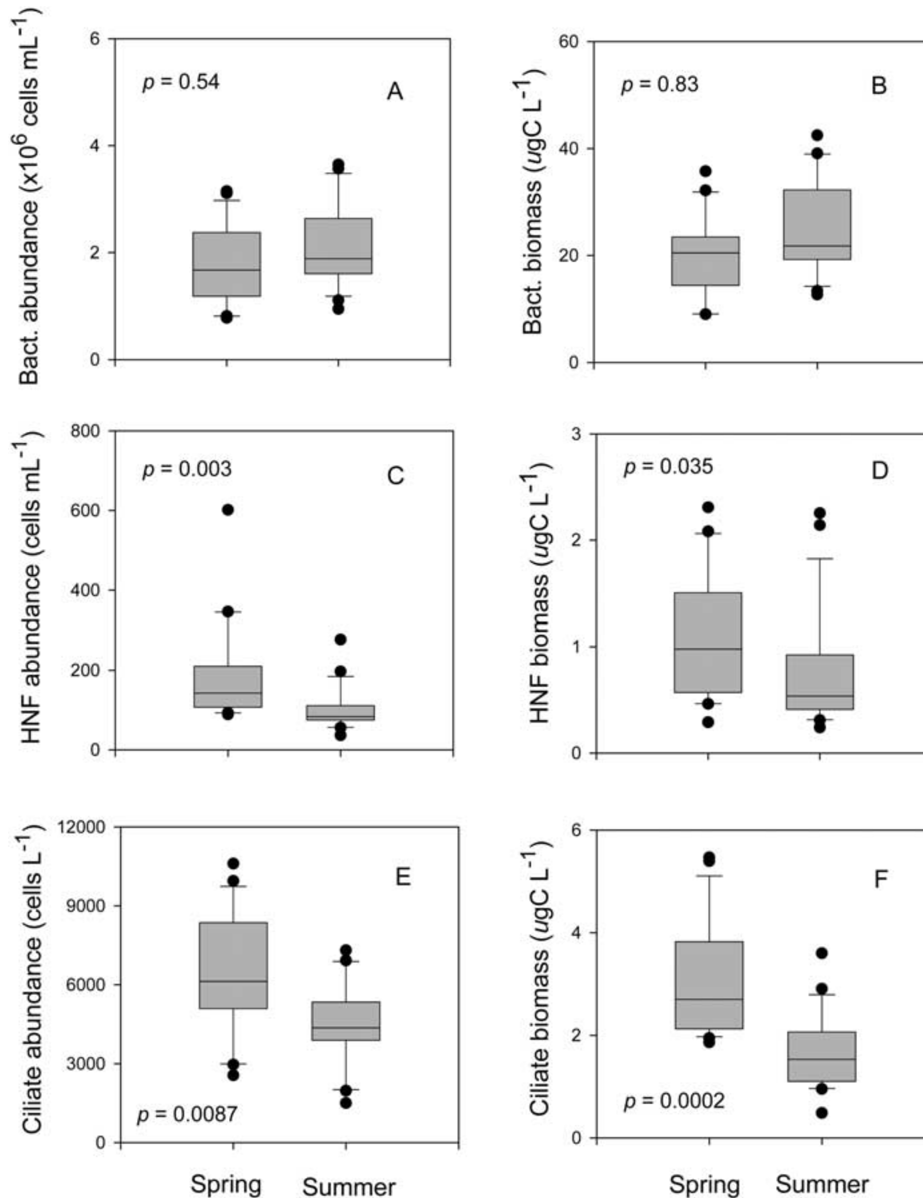

Figure 3. Seasonal variations in bacterial (A,B), heterotrophic nanoflagellate (C,D), and ciliate (E,F) standing stocks. The lower limit of bars indicates the 10th percentile whereas the upper limit indicates the 90th percentile. The points indicate outliers. *P* is the probability for the seasonal mean comparisons.

communities comprised mostly cells with sizes <20 µm, whose contributions to total ciliate abundance and biomass were 82% (46–98%) and 52% (12–91%), respectively.

Eight of the encountered ciliate species accounted, at least once, for 10% of either the abundance or the biomass of total ciliates (Table 2). *Mesodinium acarus* was, on average, the most abundant species. In terms of biomass, the large oligotrich *Strombidium viride* had the highest mean contribution. The species composition of <20-µm ciliates (which were dominant) in the experimental samples prior to incubations is shown in Fig 4. *Mesodinium acarus* and *Urotricha globosa* were numerically dominant during the first experiment, whereas *Cyclidium glaucoma*, *Cyclidium heptatricum*, and *Strobilidium humile* were the major contributors to the abundance of ciliates <20 µm, in the four other experiments (Fig. 4).

Microbial Growth in the Dialysis Bags. Throughout the experiments, bacterial net growth rates were higher for HNA-hs than for LNA in almost all treatments (Fig. 5A–E). In unfiltered treatments for example, HNA-hs net growth ranged from 0.13 to 0.24 d^{-1} while LNA net growth rates varied from 0.032 to 0.065 d^{-1}. The responses of HNA-hs and LNA to removal of grazers were clearly different. LNA net growth rates varied little across treatments, in contrast to HNA-hs net growth rates, which were highest in the <1-µm treatment and strongly decreased as the size of the prescreening mesh

Table 2. Dimensions, abundance, and importance (relative, to total ciliate abundance and biomass) of the most abundant ciliate species found in the study sites

Taxonomic group	*Species*	*Range of the maximum length (µm)*	*Abundance (cells L^{-1})*	*% of total ciliate abundance*	*% of total ciliate biomass*
Haptorid	*Mesodinium acarus*	12–20	1122	22	10
			(97–5483)	(3–96)	(1–50)
Oligotrich	*Strobilidium humile*	10–14	845	14	4
			(97–1883)	(3–25)	(3–25)
	Strobilidium sp. (spherical)	14–20	574	13	10
			(80–2440)	(2–41)	(1–33)
	Strombidium viride[a]	35–52	356	7	20
			(4–2386)	(<1–17)	(1–80)
Scuticociliate	*Cyclidium hepatatrichum*[b]	14–18	720	14	14
			(44–2413)	(1–70)	(1–68)
	Cyclidium glaucoma[c]	11–14	488	8	9
			(29–2308)	(1–39)	(1–50)
Prostomatid	*Urotricha furcata*	9–14	343	7	5
			(36–1281)	(<1–24)	(1–21)
	Urotricha globosa	16–18	338	6	5
			(49–1145)	(1–19)	(1–16)

For the absolute abundance and relative contribution, mean values are given on the first line, whereas the range of values is given on the second.
[a,b,c]Not found at all sampled sites.

used increased, the minimum value being found in the <20-µm treatment. Compared to those in the <20-µm treatments, bacterial net growth rates in the unfiltered treatments tended to increase.

HNF net growth rates varied from 0.12 to 0.6 d^{-1} across treatments and from 0.19 to 0.25 d^{-1} in unfiltered treatments. HNF net growth rates were highest in treatments where organisms with size >5 µm were removed, and minimal in the unfiltered treatments (the first three experiments) or in the <20 µm treatment (the last two experiments) (Fig. 6).

Table 3 shows the responses of five ciliates, in terms of growth, after the removal of > 20 µm organisms during the experiments. *C. glaucoma* and, to a lesser extent, *C. heptatrichum* were less affected by the removal of > 20-µm organisms, since their growth rates in the <20-µm treatments were most of the time comparable to those in the unfiltered treatments. Removal of >20-µm grazers resulted, in most cases, in a strong increase in the growth rates of the three other ciliates in the < 20 µm treatments, in comparison to the unfiltered treatments. This increase was more marked for *Strobilidium* sp., which was found in low number in initial samples (Fig. 4, Table 3).

Relationships between Variables. To test our hypothesis (see Introduction) we examined the rela-

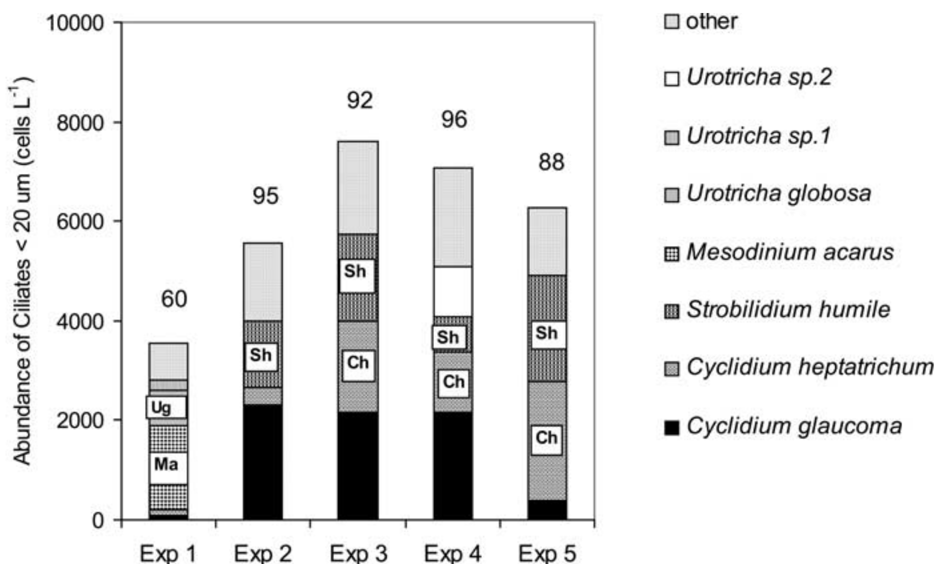

Figure 4. Species composition of the <20 µm ciliates in the experimental samples prior to the 24-h *in situ* incubations. The numbers on top of histograms indicate the relative contribution of ciliates <20 µm to total ciliate abundance.

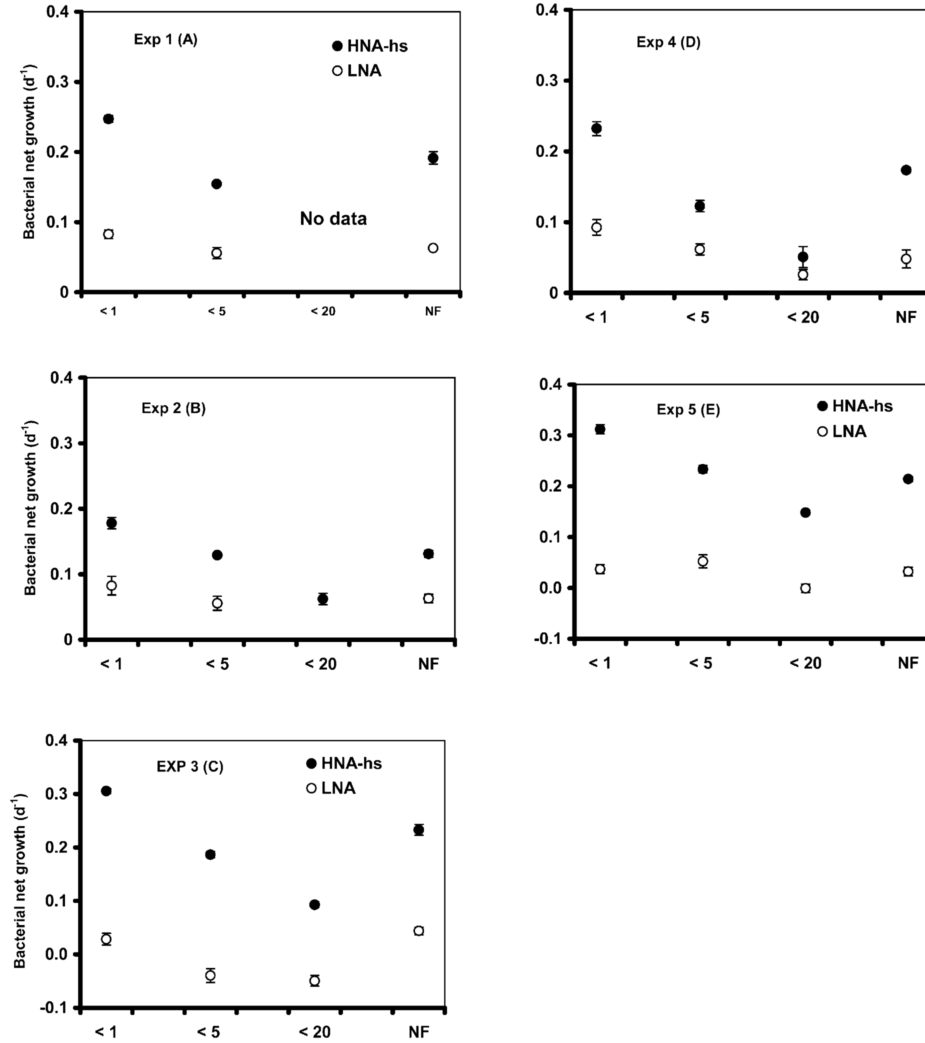

Figure 5. Across-treatment variations in the growth rates of bacteria during the experiments. The bars on points are standard errors for the three replicates. The error bar is not visible when smaller than the diameter of the point.

tionships of bacterial abundances with bacterial production and with the potential controlling factors under study (i.e., temperature, Chl *a*, ANF, HNF, and ciliates). Additional regression analyses were performed with ambient water data to test empirical relationships between protists and both temperature and Chl *a*, and

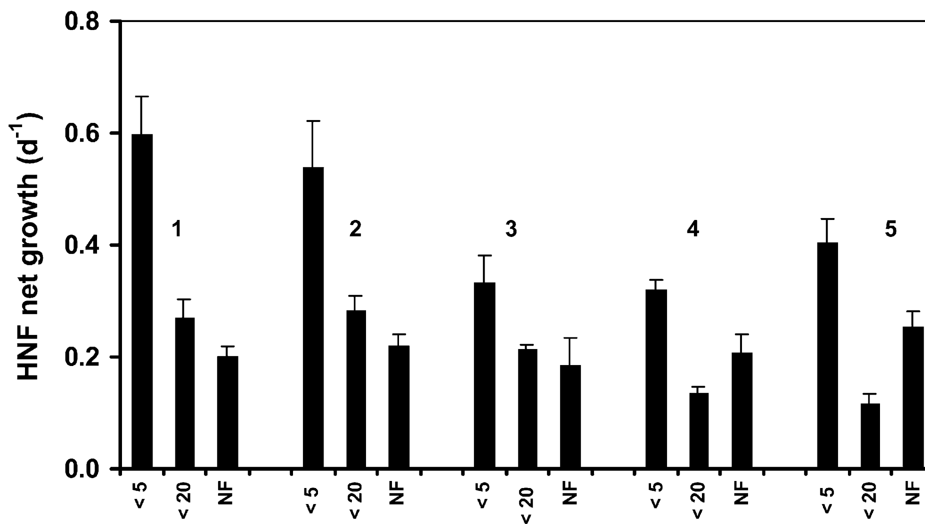

Figure 6. Across-treatment variations in the growth rates of heterotrophic nanoflagellates. The numbers on top of histograms indicate the number of the experiment. Error bars as in Fig. 5.

Table 3. Growth rates (d^{-1}, mean of two replicates) of selected ciliate species during the experiments in the < 20-μm and the nonfiltered (NF) treatments

Species	Exp. 1 <20 μm	NF	Exp. 2 <20 μm	NF	Exp. 3 <20 μm	NF	Exp. 4 <20 μm	NF	Exp. 5 <20 μm	NF
Cycidium glaucoma	0.11	0.11	0.3	0.26	0.41	0.23	0.42	0.35	0.2	0.15
Cycidium heptatrichum	0.13	0.12	0.11	0.15	0.32	0.17	0.34	0.26	0.35	0.15
Strobilidium humile	nd	nd	−0.13	0.39	0.31	0.17	0.51	0.15	0.43	0.16
Strobilidium sp.	nd	nd	nd	nd	0.36	nc	0.35	nc	0.61	nc
Mesodinium acarus	0.5	0.21	0.2	0.18	nd	nd	−0.22	nd	0.3	0.11

nd: Not determined or species found in low number in the samples; nc: no obvious change.

within protists for evidence of potential interactions (Table 4).

Temperature was significantly and positively correlated to bacteria in ambient waters, the slopes and the regression coefficients for HNA-hs being similar to those of total bacteria and LNA (ANCOVA, $F < 3$; $P > 0.24$). Chl *a* was not correlated to HNA-hs, but was significantly and positively correlated to LNA and total bacteria. Interestingly, the proportion of HNA-hs varied inversely with Chl *a* (Fig. 7A, Table 4). These results indicate that there were differential increases in HNA-hs versus LNA abundance with increase in Chl *a*, and suggest that the influence of potential controlling factors other than temperature and substrates from phytoplankton was greater on HNA-hs than on LNA. BP was significantly and positively correlated to HNA-hs but not to LNA ($P = 0.08$) and total bacteria ($P = 0.1$) (Fig. 7B, C, Table 4).

Protist (i.e., ANF, HNF, and ciliates) abundances in ambient waters were not correlated to either temperature or Chl *a* ($P > 0.19$). Similarly, neither ANF nor HNF abundance was significantly correlated to bacterial abundance ($P > 0.15$). Likewise, HNF growth was not correlated to bacterial growth during the experiments ($P > 0.74$), thus confirming the lack of coupling between these two compartments observed in ambient waters.

In contrast to ANF and HNF, total ciliate abundance was significantly and negatively correlated, across sites, to HNA-hs, LNA, and total bacterial abundance; no significant difference was found between the slopes of these three regressions (ANCOVA, $F < 1$; $P > 0.89$) (Table 4). When individual ciliate species were considered, *Cyclidium glaucoma* showed significant negative relationships with bacterial abundances across sites (Fig. 8A, B). Although the slopes of the regressions of the abundance of this species against HNA-hs, LNA, and total bacteria were similar, the r^2 for HNA-hs (0.56) was approximately double those found for LNA and total bacteria (<0.32). Interestingly, the growth rates of HNA-hs in the experiments also decreased significantly as the growth rates of *C. glaucoma* increased, whereas there was no relationship at all with LNA (Fig. 8C, D, Table 4). No other ciliate

Table 4. Relationship between variables; SEE (in brackets) is the standard error of estimates

Type of data	Y	X	Slope (SEE)	Intercept (SEE)	r^2	p	n
Ambient water	HNA-hs	Temperature	0.023 (0.007)	4.72 (0.098)	0.252	0.0025	34
	LNA		0.019 (0.006)	5.97 (0.087)	0.241	0.003	34
	BactA		0.019 (0.006)	6.01 (0.086)	0.234	0.0038	34
	HNA-hs	Chl *a*	0.32 (0.23)	5 (0.037)	0.057	0.172	34
	LNA		0.609 (0.181)	6.18 (0.028)	0.26	0.0023	34
	Bact A		0.585 (0.17)	6.22 (0.028)	0.25	0.0026	34
	% HNA-hs		−2.1 (0.795)	8.88 (1.09)	0.183	0.012	34
	BP (all data)	HNA-hs	1.09 (0.365)	−5.47 (1.84)	0.219	0.0052	34
	BP (Lakes)		1.89 (0.44)	−9.76 (2.28)	0.62	0.0013	13
	BP(LG2)		1.81 (0.54)	−8.9 (2.76)	0.378	0.0039	20
	HNA-hs	Ciliate abundance	−0.521 (0.130)	6.94 (0.47)	0.363	0.0004	30[a]
	LNA		−0.42 (0.121)	7.76 (0.44)	0.303	0.002	30[a]
	Bact A		−0.467 (0.127)	7.94 (0.469)	0.323	0.001	30[a]
	HNF Abundance		0.355 (0.134)	2.95 (0.28)	0.19	0.013	33
	HNA-hs	*C. glaucoma*	−0.234 (0.042)	5.59 (0.105)	0.564	0.001	25
	LNA		−0.166 (0.057)	6.63 (0.139)	0.27	0.008	25
	Bact A		−0.195 (0.057)	6.71 (0.14)	0.31	0.003	25
Experimental	HNA-hs	*C. glaucoma*	−0.449 (0.106)	0.26 (0.029)	0.554	0.0009	16
	LNA		−0.173 (0.09)	0.074 (0.024)	0.207	0.076	16

Note that the relationship between Chl *a* and the percentage of HNA-hs (% HNA-hs) was tested with nontransformed data.
Bact A: total bacterial abundance; BP: bacterial production; other acronyms as in the text; *n*: number of samples.
[a]Three outliers were not included in the regressions.

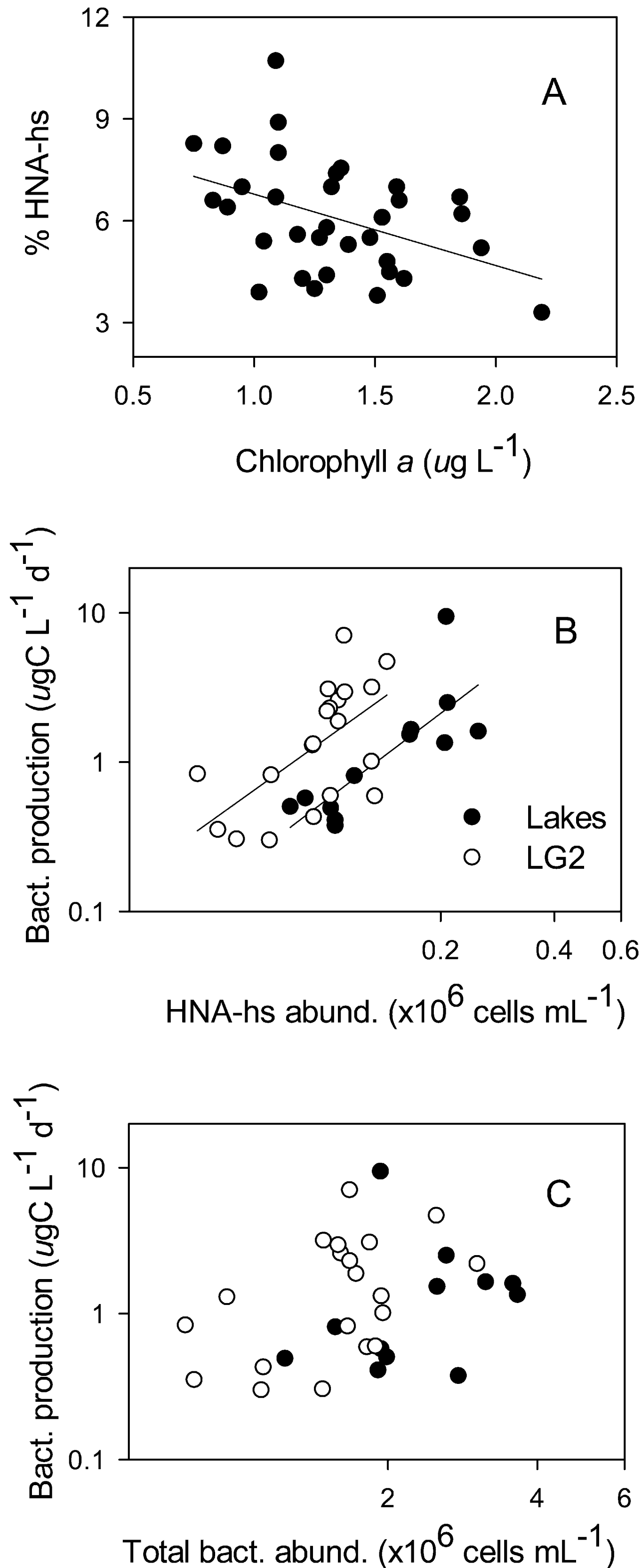

Figure 7. Across-site relationships between chlorophyll *a* and the proportion of HNA-hs (A) and between bacterial production and HNA-hs (B) and total bacterial (C) abundance. Note that for figure (A), no log transformation was done. See Table 4 for equation parameters.

exhibited such significant relationships (in both ambient and experimental samples) with bacteria. Within the protist communities under study, ciliate abundance significantly increased, across sites, as HNF abundance increased (Table 4), whereas ciliate and HNF biomasses were not correlated at all ($P = 0.16$). Neither HNF nor ciliates were significantly correlated to ANF ($P > 0.21$).

Discussion

The aim of this study was to investigate factors that determine the dynamics of heterotrophic bacteria, especially bacteria with high nucleic acid content, in pelagic systems. Most studies that have assessed factors controlling bacterial communities have considered the latter and their main grazers (protists) as homogenous assemblages. In this study, we related the different bacterial subgroups (based on their nucleic acid content, an index of their activity) to the potential controlling factors (temperature, Chl *a*, and protists) and took into account the diversity within protist groups when possible. Our results showed that ciliates rather than heterotrophic flagellates played a major role in the dynamics of bacteria in the studied sites. Among ciliates, *Cyclidium glaucoma* appeared to be very important, as it controlled the abundance of bacteria with the highest nucleic acid content, likely the most active. To our knowledge, this is the first study showing that an individual species within natural protist communities may play an important role in regulating the activity structure of bacterial communities in aquatic systems.

Abundance and Proportion of Bacteria with High Nucleic Acid Content. On the basis of their nucleic acid content, three bacterial subgroups were found in our samples. Flow cytometry analyses of raw and 0.7 μm (GF/F)-filtered samples in red and orange versus green fluorescence during our study (data not shown), as well as correlations between the subgroups and bacterial production (Table 4) confirmed that neither autotrophic picoeucaryotes nor picocyanobacteria were among the three subgroups that we observed. Other studies, mainly in marine waters, have reported the presence of more than two bacterial subgroups (e.g., [57]. The delimitation of the regions separating bacterial subgroups is sometimes subjective. Other authors who found three subgroups of bacteria have considered the two groups with higher green fluorescence as one group of bacteria (i.e., those with high nucleic acid content). When we did that in the present study (i.e., HNA-hs + HNA-ls, Fig. 2A), we obtained percentage (22 to 48%) of bacteria with high nucleic acid content comparable to those found for what most authors consider as high-DNA bacteria [14, 34, 53]. However, we did not find any significant relationship

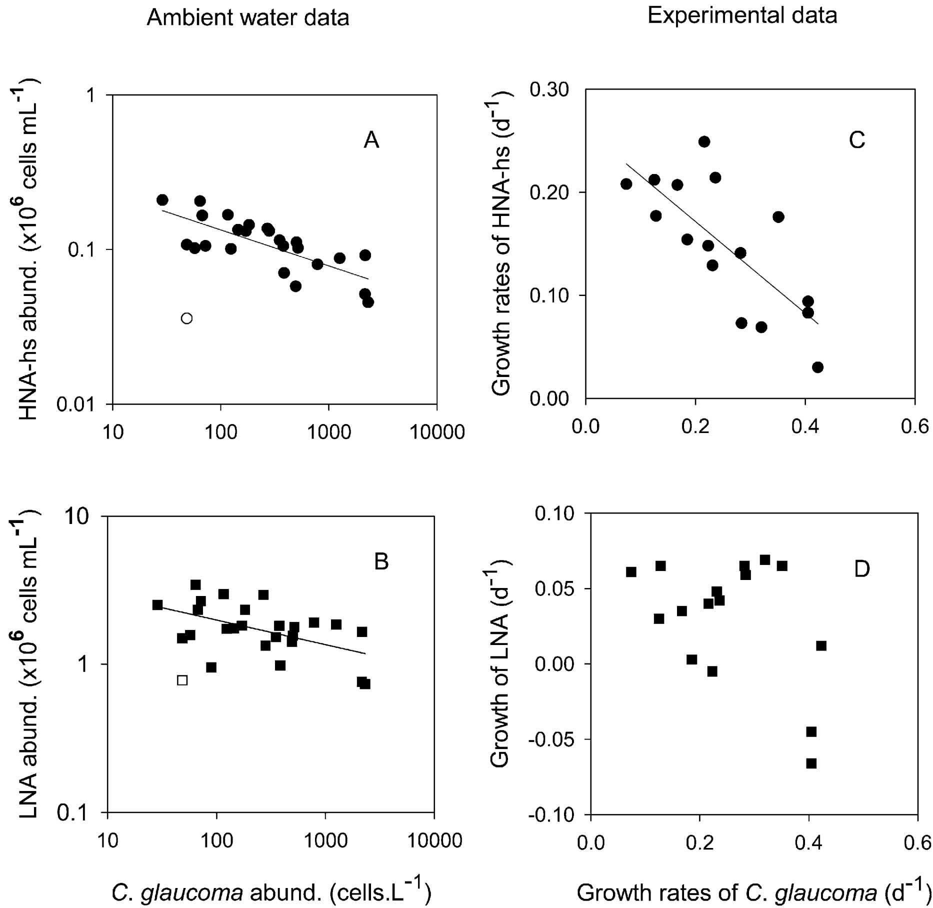

Figure 8. Relationships between *Cyclidium glaucoma* and bacterial abundance across sites (A,B) and growth rates across experimental replicates (C,D). See Table 4 for equation parameters. The outlier in (A) (open circle) and the corresponding point in (B) (open square) were not included in the regressions.

between this group and bacterial production, total ciliate, and *C. glaucoma* abundances or growth, as we did find for HNA-hs alone. Thus, including HNA-ls in the LNA group in the present study does not mean that they really belong to this group. As stated earlier, this was done for the purpose of simplification, because clear patterns were observed only for HNA-hs.

Our results indicated that HNA-hs were the most dynamic members of bacterial communities. HNA-hs net growth rates were, indeed, higher and more variable in the experiments than LNA net growth rates (Fig. 5A–E). In addition, HNA-hs were significantly correlated with bacterial production in ambient waters, whereas LNA and total bacteria were not (Table 4). These results are consistent with other reports, mainly from marine waters, indicating that bacteria with high nucleic acid content are those that are actively growing [5, 23, 36]. In the Mediterranean Sea, Vaqué et al. [53] also found that bacteria with high DNA content were significantly correlated to bacterial production whereas bacteria with low DNA content were not. Using radioactive labeling followed by flow cytometry sorting of planktonic bacterial cells, Lebaron et al. [34] showed that bacteria with high nucleic acid content were responsible for most of the bacterial production. On the basis of the above results, we consider that HNA-hs were likely the most active bacteria in our samples.

Although HNA-hs appeared to be the most active bacteria in our samples, their growth rates were low, even after removal of bacterial grazers (i.e., in the < 1-μm treatment, Fig. 5 A–E). The measured bacterial growth rates were comparable to those often reported in the most unproductive water systems (e.g., [42]). These results indicate that bacterial communities were resource-limited during our study. Rates of leucine incorporation by bacteria support this contention, as they were low and similar to those reported for other humic lakes where P-limitation of bacteria has been demonstrated (e.g., [24]).

Major Role of Ciliates in the Dynamics of Bacteria in the Study Sites. Phytoplankton is known to generally fuel bacterioplankton with organic matter through exudates [1]. Positive relationships have therefore been found between these two compartments [4, 9]. Since bacteria with the highest nucleic acid content and high light scatter (HNA-hs) seemed to be the most active bacteria in this study, a significant correlation to Chl *a* was expected, as was found for LNA and total bacteria

Table 5. Comparison of various estimates of bacterial production during the experiments

Experiment and site	*Estimated bacterial production (μgCL^{-1} d^{-1}) Leucine incorporation*	*Change in abundance*[a]	*Growth rate × initial biomass*
Exp 1 (Desaulnier)	0.776 (A) 0.494 (B)	1.217	0.701
Exp 2 (Marina)	0.834	0.916	0.533
Exp 3 (Marina)	0.353	0.949	0.551
Exp 4 (Marina)	1.303	1.184	0.687
Exp 5 (Desaulnier)	0.41 (A) 0.81 (B)	1.647	0.961

Note that leucine incorporation measurements were not carried out at the experimental site (E) at Lake Desaulnier; values are from stations A and B, where measurements were done the day of the experiment.
[a]Calculated using the cell to carbon conversion factor of 20 fgC cell^{-1} [35].
Stations A and B as in Table 1.

(Table 4). However, this was not the case, and the % HNA-hs even varied inversely with Chl *a* (Fig. 7A, Table 4). The question is, what determined these patterns observed between HNA-hs and Chl *a*? Factors such as allochthonous organic carbon of terrestrial origin (especially in unproductive lakes), cell lysis, and grazing may play a significant role in the uncoupling of bacteria with phytoplankton. In this study, dissolved organic carbon (DOC) concentrations were positively correlated with Chl *a* ($P = 0.019$), but not with HNA-hs ($P = 0.9$). These results suggest that allochthonous DOC was not a major factor responsible for the uncoupling between HNA-hs and Chl *a*. The rates of infection of bacterial cells by viruses are affected by bacterial production [21]. As HNA-hs seemed to be the most active bacteria in our study, it is conceivable that they were more affected by viral lysis than less active bacteria. However, we do not know to what extent such a process might have affected the HNA-hs–Chl *a* relationship in our study. The impacts of viral infection on active versus less active components of bacterial communities are still poorly known and need to be investigated.

Our results strongly suggest that grazing by ciliates was a major factor responsible for the observed lack of coupling between HNA-hs and Chl *a*, and that grazing losses due to ciliate bacterivory were higher for HNA-hs than for LNA. The lowest growth of HNA-hs were always found in the <20-μm treatment, which comprised, in many cases, an important proportion of the bacterivorous scuticociliates (Figs. 4, 5, Table 2). More importantly, the growth of *C. glaucoma* was significantly and negatively related to the growth of HNA-hs but not to that of LNA. These experimental results support the data from ambient waters (across sites), which showed that variations in the abundance of *C. glaucoma* explained 56% of the variations in HNA-hs abundance and only 27% of the variations in LNA abundance (Fig. 8 A–D, Table 4). Whereas in the field many other organisms might have reduced, through interactions, the grazing pressure from *C. glaucoma* or also have cropped on bacteria as suggested by the significant and negative relationships found between total ciliate abundance and bacteria in ambient waters, removal of >20 μm in the experiments might have enhanced the grazing pressure from this ciliate species. This may help explain why in ambient waters, the slope of the regression of *C. glaucoma* with HNA-hs was similar to that with LNA (Table 4). The uncoupling between Chl *a* and HNA-hs in this study, therefore, seems to be a consequence of selective removal of these active bacteria. These results and interpretation are consistent with the model of Pace and Cole [38], which indicates that enhanced grazing on bacteria uncouples them from resources.

The values of bacterial production as estimated from leucine incorporation in ambient waters and those estimated during the experiments from changes in bacterial abundance or from bacterial growth rates and initial biomass (i.e., prior to incubations) were in the same order of magnitude (Table 5), suggesting that confinement in dialysis bags did not strongly affect plankton during our experiments. This has allowed us to further explore the role of *C. glaucoma* in the dynamics of HNA-hs in the studied sites. We estimated the potential grazing losses (PGL) of HNA-hs as the difference between the net production of this subgroup in the <l μm treatment and that in the unfiltered treatment (based on its growth and change in abundance, and calculated as in Table 5), and tested their relationship with *C. glaucoma* (Fig. 9). The reason why a negative PGL value was found for experiment 3 is unclear, even though few small HNF (8–10 per slide) were observed in the <l μm treatment during this experiment. That PGL values tended to increase with increase in the initial abundance (prior to incubation) of *C. glaucoma* (Fig. 9) supports the idea that this ciliate was very important in determining the grazing losses of the most dynamic bacteria during this study, at least at the experimental sites.

Laboratory studies have indicated that *Cyclidium* species selectively ingest medium-to-larger sized particles or natural bacterioplankton [17, 43, 47]. Species from

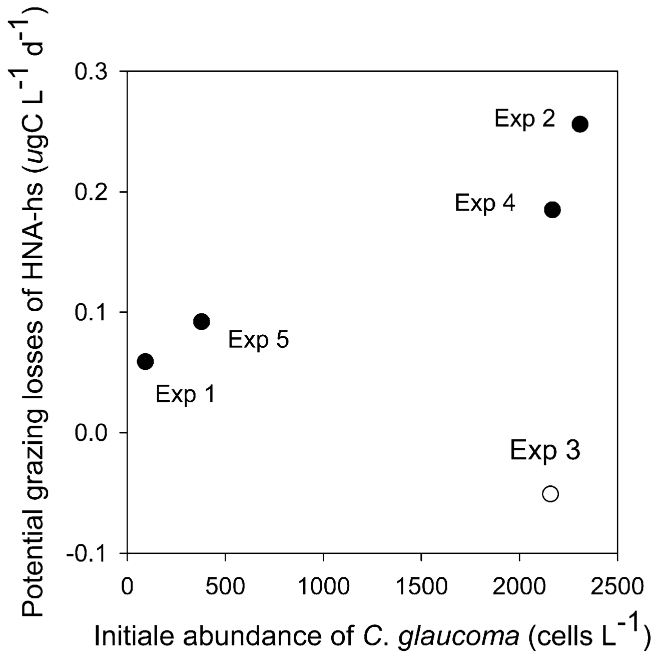

Figure 9. Relationship between the estimated potential grazing losses of HNA-hs and the abundance of *Cyclidium glaucoma* in initial experimental samples (i.e., prior to the *in situ* incubations).

this genus have also been found to discriminate between particles of similar sizes, on the basis of their surface chemistry [17], or to affect mainly bacteria with volume between 0.04 and 0.1 μm^3 and members of the beta-Proteobacteria and *Cytophaga–Flavobacterium* groups in a continuous cultivation system [40]. In this study, bacteria were natural (i.e., no stained or heat-killed bacteria were added to samples; such processes may affect surface chemical properties of prey [16]), and the estimated average volumes of the most dynamic HNA-hs were within the range given above and larger than those of LNA. Likely, both bacterial size and activity were important in determining the observed significant relationships between *C. glaucoma* and HNA-hs. Size-selective grazing by protists (especially HNF) has been used to explain selective removal of dividing or CTC positive marine bacterioplankton [13, 45]. Using a growth efficiency of 0.4 and assuming that Lugol fixation reduced the live cell volume by a maximum value of 30% [26], we calculated that the carbon demand for the observed growth of *C. glaucoma* during the experiments (Table 4) represented 21%, 98%, 119% and 40% of the estimated losses of HNA-hs in experiments 1, 2, 4, and 5, respectively. This suggests that *C. glaucoma* could meet most of its carbon requirements if it consumed only the lost HNA-hs. If the different subgroups of bacteria found during this study were dominated by different phylotypes, has been found in the Celtic Sea [57], this would imply that *C. glaucoma* also had a strong impact on bacterial taxonomic composition in our study sites. We are not aware of any other field and across-site study that has demonstrated that an individual species within natural protist communities may regulate the abundance of the most dynamic (likely the most active) bacteria in aquatic systems. In most studies of the microbial food webs, total bacteria are related to the total abundance or biomass of HNF and/or ciliates. Our data showed that taking into account the physiological heterogeneity of bacterial communities and the diversity of their grazers may provide information that can help improve the microbial food web models. In the planktonic classical food chain in freshwaters, the cladocera *Daphnia* is considered a keystone species [27]. By analogy, we suggest for the first time that keystone species, which play a significant role in regulating the structure of bacterial communities, also exist within the pelagic microbial food webs.

Minor Role of HNF and ANF in the Bacterial Dynamics. Ciliates likely outweighed HNF and ANF in determining bacterial dynamics during this study, as neither HNF nor ANF were related to bacteria. Although ciliates have been found to be the main consumers of bacteria in other pelagic waters (e.g., [48]), HNF are known as the major bacterivores in most aquatic environments [32]. In this study, top-down control and/or competitive exclusion of HNF by ciliates and possibly metazooplankton may help explain why HNF apparently had a minor role in bacterial dynamics. Indeed, HNF and ciliate were positively correlated both across sites and on the seasonal scale (Fig. 3 C-F), which suggests the existence of a prey–predator relationship between these compartments. The decrease in their standing stocks from spring to summer (Fig. 3 C–F) coincided with an overall increase in metazooplankton average biomass from 13.9 to 24.9 mg m^{-3} (dominated by cladocerans; J. Marty, UQAM, pers. comm.). Finally, our experimental data showed that HNF growth rates were highest when organisms > 5 μm were removed, and strongly depressed, compared to those in the <5 μm treatments, when >20 μm organisms were removed (Fig. 6). These results are consistent with the well-known negative impacts of cladocerans and small oligotrichs (present in this study) on HNF [27, 28, 54]. On the other hand, recent studies suggest that thick epilimnion are not favorable for mixotrophic flagellates [25]. If mixotrophic cells were present among ANF in this study, their role in the dynamics of bacteria was perhaps reduced, as most sampled stations were not stratified or had epilimnion > 6 m.

In summary, our results indicated that the studied humic lakes and reservoirs were oligotrophic and suggest that bacteria with the highest nucleic acid content were the most dynamic and the most active within the bacterial communities. These most dynamic members rep-

resented only a small fraction of the whole bacterial communities and seemed to be controlled by ciliates, particularly the bacterivorous scuticociliate *Cyclidium glaucoma*. Our results confirm that protists play a role in the dynamics of active bacteria and suggest the existence, within the pelagic microbial food web, of keystone species that are very important in regulating the structure of bacteria.

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References

1. Baines, SB, Pace, ML (1991) The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. Limnol Oceanogr 36: 1078–1090
2. Berman, T, Kaplan, B, Chava, S, Viner, Y, Sherr, BF, Sherr, EB (2001) Metabolically active bacteria in Lake Kinneret. Aquat Microb Ecol 23: 213–224
3. Biddanda, B, Ogdahl, M, Cotner, J (2001) Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. Limnol Oceanogr 46: 730–739
4. Bird, DF, Kalff, J (1984) Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters. Can J Fish Aquat Sci 41: 1015–1023
5. Bird, DF, Kalff, J (1993) Protozoan grazing and the size–activity structure of limnetic bacterial communities. Can J Fish Aquat Sci 50: 370–380
6. Boenigk, J, Arndt, H (2002) Bacterivory by heterotrophic flagellates: community structure and feeding strategies. Ant van Leeuwen 81: 465–480
7. Borsheim, KY, Bratbak, G (1987) Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. Mar Ecol Progr Ser 36: 171–174
8. Choi, JW, Sherr, BF, Sherr, BE (1999) Dead or alive? A large fraction of ETS-inactive marine bacterioplankton cells, as assessed by reduction of CTC, can become ETS-active with incubation and substrate addition. Aquat Microb Ecol 18: 105–115
9. Cole, JJ, Caraco, NF (1993) The pelagic food web of oligotrophic lakes. In: Ford, TE (Ed.) Aquatic Microbiology. Blackwell Scientific, London, pp 101–110
10. Corliss, JO (1979) The Ciliate Protozoa: Characterization, Classification and Guide to the Literature, 2nd ed. Pergamon Press, Elmsford, NY
11. Del Giorgio, PA, Gasol, JM (1995) Biomass distribution in freshwater plankton communities. Am Naturalist 146: 135–152
12. Del Giorgio, PA, Scarborough, G (1995) Increase in the proportion of metabolically active bacteria along gradient of enrichment in freshwater and marine plankton: implications for estimates of bacterial growth and production rates. J Plankton Res 17: 1905–1924
13. Del Giorgio, PA, Gasol, JM, Vaqué, D, Mura, P, Agusti, S, Duarte, CM (1996) Bacterioplankton community structure: protists control net production and the proportion of active bacteria in a coastal marine community. Limnol Oceanogr 41: 1169–1179
14. Del Giorgio, PA, Bouvier, TC (2002) Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. Limnol Oceanogr 47: 471–486
15. Ducklow, HW, Carlson, CA (1992) Oceanic bacterial production. Adv Microb Ecol 12: 113–181
16. Epstein, SS, Rossel, J (1995) Methodology of *in situ* grazing experiments: evaluation of new vital dye for preparation of fluorescently labeled bacteria. Mar Ecol Progr Ser 128: 143–150
17. Fenchel, T (1980) Suspension feeding in ciliated protozoa: functional response and particle size selection. Microb Ecol 6: 1–11
18. Fernadez-Galiano, D (1976) Une nouvelle methode pour la mise én evidence de l'infraciliature des ciliés. Prostistologica 2: 35–38
19. Finlay, BJ, Rogerson, A, Cowling, JA (1988) Collection, isolation, cultivation and identification of frehwater protozoa. Freshwater Biological Association, Ambleside
20. Foissner, W, Berger, H (1996) A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes and waste waters, with notes on their ecology. Freshwat Biol 35: 375–482
21. Fuhrman, JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399: 541–548
22. Gasol, JM (1994) A framework for the assessment of top-down vs bottom-up control of heterotrophic flagellate abundance. Mar Ecol Progr Ser 113: 291–300
23. Gasol, JM, Zweifel, UL, Peters, F, Fuhrman, JA, Hagstrom, A (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl Environ Microbiol 65: 4475–4483
24. Jansson, M, Blomqvist, P, Jonsson, A, Bergstrom, A-K (1996) Nutrient limitation of bacterioplankton, autotrophic and mixotrophic phytoplankton and heterotrophic nanoflagellates in the Lake Ortrasket. Limnol Oceanogr 41: 1552–1559
25. Jansson, M, Bergstrom, A-K, Blomqvist, P, Isaksson, A, Jonsson, A (1999) Impact of allochthonous organic carbon on microbial food web carbon dynamics and structure in Lake Ortrasket. Arch Hydrobiol 144: 409–428
26. Jerome, CA, Montagnes, DJS, Taylor, FJR (1993) The effects of the quantitative protargol stain and Lugol's and Bouin's fixatives on cell size: a more accurate estimate of ciliate species biomass. J Eucaryot Microbiol 40: 254–259
27. Jürgens, K (1994) Impact of *Daphnia* on planktonic microbial food webs. A review. Mar Microb Food Webs 8: 295–324
28. Jürgens, K, Wickham, SA, Rothhaupt, KO, Santer, B (1996) Feeding rates of macro- and microzooplankton on heterotrophic nanoflagellates. Limnol Oceanogr 41: 1833–1839
29. Jürgens, K, Pernthaler, J, Schalla, S, Amann, R (1999) Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. Appl Environ Microbiol 65: 1241–1250
30. Kankaala, P, Arvola, L, Tulonen, T, Ojala, A (1996) Carbon budget for the pelagic food web of the euphotic zone in a boreal lake (Lake Paajarvi). Can J Fish Aquat Sci 53: 1663–1674
31. Kirchman, DL (1993) Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: Kemp, PF, Sherr, EB, Sherr, EB, Cole, JJ (Eds.) Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Ratung, FL, pp 509–512

32. Laybourn-Parry, J, Parry, J (2000) Flagellates and the microbial loop. In: Leadbeater, BSC, Green, JC (Eds.) The Flagellates. Taylor & Francis, London, pp 216–239
33. Lebaron, P, Pertuisot, N, Catala, P (1998) Comparison of blue nucleic acid dyes for the flow cytometry enumeration of bacteria in aquatic systems. Appl Envrion Microbiol 64: 1724–1730
34. Lebaron, P, Servais, P, Agogué, H, Courties, C, Joux, F (2001) Does the high nucleic acid content of individual bacterial cells allow us to discriminate between active cells and inactive cells in aquatic systems? Appl Environ Microbiol 67: 1775–1782
35. Lee, S, Fuhrman, JA (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl Environ Microbiol 53: 1298–1303
36. Li, WKW, Jellett, JF, Dickie, PM (1995) DNA distribution in planktonic bacteria stained with TOTO or TO-PRO. Limnol Oceanogr 40: 1485–1495
37. Norland, S (1993) The relationship between biomass and volume of bacteria. In: Kemp, PF, Sherr, BF, Sherr, EB, Cole, JJ (Eds.) Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, FL, pp 303–307
38. Pace, ML, Cole, JJ (1994) Comparative and experimental approaches to top-down and bottom-up regulation of bacteria. Microb Ecol 28: 181–193
39. Porter, KJ, Feig, YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25: 943–948
40. Posch, T, Jezbera, J, Vrba, J, Simek, K, Pernthaler, J, Andreatta, S, Sonntag, B (2001) Size selective feeding in *Cyclidium glaucoma* (Ciliophora, Scuticociliatida) and its effects on bacterial community structure: a study from a continuous cultivation system. Microb Ecol 42: 217–227
41. Putt, M, Stoecker, DK (1989) An experimentally determined carbon: volume ratio for marine oligotrichous ciliates from estuarine and coastal waters. Limnol Oceanogr 34: 1097–1104
42. Rivkin, RB, Anderson, MR (1997) Inorganic nutrient limitation of oceanic bacterioplankton. Limnol Oceanogr 42: 730–740
43. Sanders, RW (1988) Feeding by *Cyclidium* sp. (Ciliophora, Scuticociliatida) on particles of different sizes and surface properties. Bull Mar Sci 43: 446–457
44. Servais, P, Agogué, H, Courties, C, Joux, F, Lebaron, P (2001) Are the actively respiring cells (CTC+) those responsible for bacterial production in aquatic environments? FEMS Microbiol Ecol 35: 171–179
45. Sherr, BF, Sherr, EB, McDaniel, J (1992) Effects of protistan grazing in the frequency of dividing cells in bacterioplankton. Appl Environ Microbiol 58: 2381–2385
46. Sherr, BF, Del Giorgio, PA, Sherr, EB (1999) Estimating abundance and single-cell characteristics of respiring bacteria via the redox dye CTC. Aquat Microb Ecol 18: 117–131
47. Šimek, K, Vrba, J, Hartman, P (1994) Size-selective feeding by *Cyclidium* sp. on bacterioplankton and various sizes of cultured bacteria. FEMS Microbiol Ecol 14: 157–168
48. Šimek, K, Babenzien, D, Bittl, T, Koschel, R, Macek, M, Nedoma, J, Vrba, J (1998) Microbial food webs in an artificial divided acidic bog lake. Int Rev Hydrobiol 83: 3–18
49. Šimek, K, Kojecká, P, Nedoma, J, Hartman, P, Vrba, J, Dolan, J (1999) Shifts in bacterial community composition associated with different microzooplankton size fraction in a eutrophic reservoir. Limnol Oceanogr 44: 1634–1644
50. Simon, M, Cho, BC, Azam, F (1992) Significance of bacterial biomass in lakes and the ocean: comparison to phytoplankton and biogeochemical implications. Mar Ecol Progr Ser 86: 103–110
51. Sondergaard, M, Danielsen, M (2001) Active bacteria (CTC+) in temperate lakes: temporal and cross-system variations. J Plankton Res 23: 1195–1206
52. Tadonléké, DR (1991) Etude des infraciliatures corticales et buccales de *Puyteraciella dibryophrys* et *Tillina magma*, deux ciliés de l'ordre des colpodida. BSc dissertation, University of Yaoundé
53. Vaqué, D, Casamayor, EO, Gasol, JM (2001) Dynamics of whole community bacterial production and grazing losses in seawater incubations as related to the changes in the proportions of bacteria with different DNA content. Aquat Microb Ecol 25: 163–177
54. Weisse, T (1991) The annual cycle of heterotrophic freshwater nanoflagellates: role of bottom-up versus top-down control. J Plankton Res 13: 167–185
55. Wright, RT, Coffin, RB (1984) Measuring microzooplankton grazing on planktonic marine bacteria by its impact on bacterial production. Microb Ecol 10: 137–149
56. Zingel, P, Huitu, E, Makela, S, Arvola, L (2002) The abundance and diversity of planktonic ciliates in 12 boreal lakes of varying trophic state. Arch Hydrobiol 155: 315–332
57. Zubkov, MV, Fuchs, BM, Burkill, PH, Amann, R (2001) Comparison of cellular and biomass specific activities of dominant bacterioplankton groups in stratified waters of the Celtic Sea. Appl Environ Microbiol 67: 5210–5218