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Temporal changes of microbial assemblages in the ice and snow cover of a high mountain lake

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Abstract

It has recently been shown that a rich community of microorganisms inhabits the slush layers of the winter cover of high mountain lakes. In this study, temporal changes in species assemblages and environmental conditions in the ice and snow cover of Lake Redó in the Pyrenees (Spain) are presented. The winter cover was a highly dynamic environment, in which major changes occurred in physical structure and chemical characteristics. The biomass and species composition of the microbial groups present (autotrophic and heterotrophic flagellates, ciliates, and bacteria) reflected these physicochemical alterations. After an initial phase, during which the ice sheet formed and the first snows accumulated with little or no development of slush layers, there were two stages of microbial assemblages, which coincided with the two main phases in the physical change of the cover: the growth period (from January to mid-April) and the ablation period (from mid-April to ice-out in June). Initially, microbial biomass originated from inputs of phytoplankton-rich ($5.9 \mu\text{g}$ chlorophyll *a* liter⁻¹) surface lake water that flooded the cover as a result of the hydrostatic adjustment induced by the progressive accumulation of snow on top of the ice sheet. This was followed by a differential growth of mixotrophic or heterotrophic flagellated species, such as *Ochromonas* sp., *Cryptomonas* spp., *Monosiga ovata* and *Oikomonas termo*, which peaked at different times. Species depended primarily on bacterivory for their growth, since during this period, light did not reach the slush layers. By April, the transition from cover growth to ablation was marked by a reduction in biomass and in the number of species. During the ablation period, the rate of change of the cover was greater, and microbial assemblages were characterized by the vertical segregation and by the appearance of new species, some of which were typically nonplanktonic. During that time, the cover was highly influenced by the large amount of water from the melting of the snowpack within the catchment. This water likely provided chemicals and organisms, while increased light availability allowed for the growth of algae (*Pteromonas* sp. and other volvocales) and of the associated food web in the upper slush. In deeper layers, however, a bacteria-based food web provided prey for large ciliates (*Urosoma* sp., *Dileptus* sp., and *Lacrymaria* sp.), probably carried from littoral or watershed soils.

High mountain lakes are covered by ice and snow for several months of the year. Such covers are complex physical structures (Adams and Allan 1987). When the lake freezes, a sheet of clear ice, so-called "black ice" forms at the lake surface, and salts are efficiently exsolved, which, in turn, increases salt concentration in the water beneath the ice. Snow subsequently accumulates on top of the ice, and the stress produced causes the lake water to rise and to flood

the snowpack until a hydrostatic equilibrium is reached. The mixing of snow and water gives rise to slush layers, which, under certain circumstances, can freeze to produce white ice, which is much more opaque than black ice. Subsequent snowfalls with varying chemical compositions add layers with varying properties. Episodic flooding, melting, and freezing convert the cover into a complex stratigraphical structure (Catalan 1989). During spring, the snow cover is enriched by the dry deposition of particles, including pollen, small insects, organic debris, and dust (Barrie 1985). Temporally, there are two well-defined phases in temperate latitudes: (1) growth in the thickness of covers from the time the lake freezes to the end of April and (2) an ablation period, which lasts a few weeks and which consists of a progressive top-down melting of the different layers (Catalan 1989). During this period, melting of the snow in the watershed causes a large amount of water to circulate through

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Table 1. Sampling dates and physical characteristics of the cover. Hydrostatic water level and depth where slush layers were located are referenced to the top of the cover.

Survey number	Date	Cover thickness (cm)	Hydrostatic water level (cm)	Location depth of slush layers (cm)
1	19 Dec 91	30	—	10
2	8 Jan 92	40	—	—
3	22 Jan 92	75	33	40, 65
4	6 Feb 92	85	35	40, 80
5	18 Feb 92	98	30	40, 85
6	2 Mar 92	98	35	40, 85
7	17 Mar 92	98	20	25, 85
8	1 April 92	150	47	50, 100, 150
9	13 April 92	175	45	45, 120, 157
10	29 April 92	160	25	40, 100, 157
11	12 May 92	150	25	30, 75, 100
12	26 May 92	73	10	10

the surface of the lake near the ice sheet (Roberge and Jones 1991).

Microbial assemblages, composed of psychrophilic and psychrotrophic microalgae, bacteria, and protozoa, have been described in a variety of marine-ice habitats: in melt pools on the ice surface, in brine channels within the ice, attached to the bottom of the ice, and in the sub-ice platelet layer (Ackley et al. 1979; Palmisano and Sullivan 1985; Garrison 1991; Garrison and Close 1993). However, no equivalent assemblages have been reported in ice covers of freshwater lakes. Various chemical studies of the snow cover in high mountain lakes have indirectly identified microbial activity in the cover. Barica and Armstrong (1971) attributed NO_3^- decrease in the cover to denitrification. Jones and Ouellet (1983) measured adenosine 5' triphosphate concentration in slush and suggested that some bacteria or algae transported from the lake to the slush cover might survive for a short time. Catalan (1989) reported a carbon:nitrogen (C:N) ratio of particulate matter in the cover characteristic of living microbial organisms and an increase in NO_2^- , which is indicative of microbial activity in slush layers. Felip et al. (1995) demonstrated the presence of highly active microbial communities in the cover of high mountain lakes in the Pyrenees and the Alps. Bacteria and many taxa of autotrophic and heterotrophic flagellates and ciliates were found inhabiting slush layers and pools formed on top of the cover during spring. Microbial biomasses and bacterial and algal activities were often greater than in lake water. No organisms were found directly associated with solid phases, such as snow and ice layers, and in that sense, the biology of freshwater covers appears to be different from that of sea ice (Ackley and Sullivan 1994; Spindler 1994). It is still not known, however, what the colonization mechanisms are, whether there are successional trends, or what factors affect species assemblages during the cover period. In this paper, we present a detailed study of the temporal changes in microbial assemblages in the ice cover from ice formation through complete melting, and we discuss the main forces

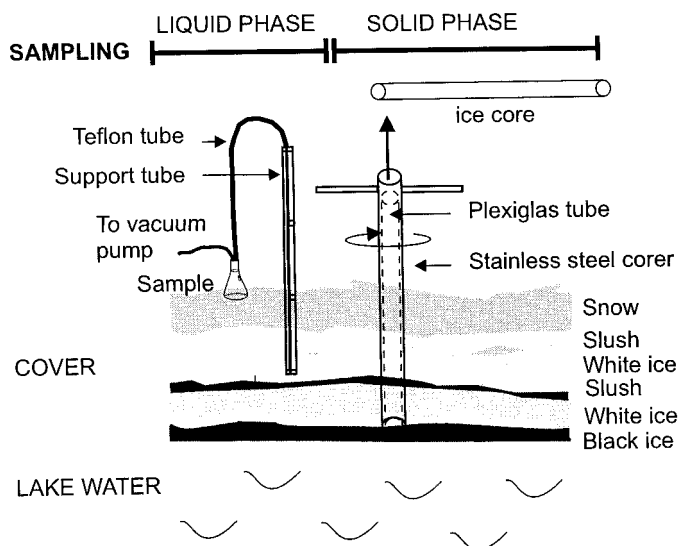


Fig. 1. Equipment for sampling the liquid phase (slush water) and the solid phase of the winter cover. Slush water was directly pumped from each layer. An integral ice core was obtained to characterize the solid phase. The stratigraphical structure of the lake cover is also shown.

driving the dynamics in this relatively unexplored low-temperature ecosystem.

The lake cover is a transient habitat that undergoes constant physical and chemical change. These changes determine the physical habitat that is available for the microorganisms, modify resource availability (light, nutrients, and organic matter), and may provide inocula for new organisms. The description of events that we considered in this paper seeks to establish the environmental framework in which biological changes occur. Other studies have already emphasized the physical and chemical aspects per se (Adams and Lasenby 1985; Catalan 1989).

Methods

The study was conducted in Lake Redó (42°N, 0°E), an oligotrophic high mountain lake in the Central Pyrenees (Spain), a lake that is located at 2,240 m above mean sea level. Lake Redó has a surface area of 24 ha, a maximum depth of 73 m, and a mean depth of 32 m. A complete description of its physical and chemical features can be found in Catalan (1988, 1989, 1992). The lake is usually covered by ice and snow for 6–7 months of the year.

The lake cover and surface lake water were sampled in the central area at 12–20-d intervals in 1991–1992, from the time the lake froze (mid-December) until the cover had completely melted (mid-June) (Table 1). The solid phase of the cover (snow and ice) was sampled separately from the liquid phase (slush water). The solid phase was sampled using a 220-cm-long stainless-steel corer, which was manually driven and fitted with a clean Plexiglas inner tube with 7-cm diameter (Fig. 1). After drilling, the inner tube was removed, and the thicknesses of different cover layers were measured. The lake-cover core was then extracted, carefully sectioned,

and stored in clean polyethylene bags. In the laboratory, ice and snow samples were melted and stored in polyethylene bottles at -20°C until chemical analysis. Water from the slush layers was pumped through small holes drilled in the lake cover, as shown in Fig. 1, and progressively deeper slush layers were sampled until lake water was reached. Immediately after sampling, a polyethylene bottle was filled with a subsample for chemical analysis (stored at -20°C); in order to determine microbial abundance, subsamples were fixed, while those collected for particulate and pigment analyses were filtered and then stored frozen. On the basis of mean density (calculated using a buoyancy equation; Adams 1976) and the volume of the cover, the water equivalent mass was estimated. A stratigraphical sampling of the watershed snowpack was carried out at the same time (Camarero 1994a). Precipitation was estimated with data obtained from the Baserca Reservoir station, located in the same valley, 4 km from Lake Redó. Flooding water was calculated by difference between cover-water equivalent and precipitation. The percentage of incident light transmitted through the lake cover was estimated with reflection and extinction coefficients found in the literature for different types of snow, ice, and slush (Catalan 1988) and based on the thickness of the layers.

All samples (solid and liquid phase of the cover and lake water) were analyzed for chemical composition (standard accuracy of the method is indicated between brackets). Conductivity ($\pm 0.2 \mu\text{S cm}^{-1}$) was measured with an Instran10 conductimeter with automatic temperature correction, and pH (± 0.02) was measured using electrodes for low-ionic-strength solutions. Na^+ and K^+ ($\pm 1 \mu\text{atg liter}^{-1}$) were determined by atomic absorption spectrometry. Ca^{2+} and Mg^{2+} ($\pm 0.5 \mu\text{atg liter}^{-1}$) were quantified by inductively coupled plasma spectrometry. Cl^- ($\pm 1 \mu\text{atg liter}^{-1}$) and SO_4^{2-} ($\pm 0.5 \mu\text{atg S liter}^{-1}$) were measured by ion chromatography. NO_3^- ($\pm 1 \mu\text{atg N liter}^{-1}$) was determined by ultraviolet spectrophotometry. NO_2^- ($\pm 0.01 \mu\text{atg N liter}^{-1}$), NH_4^+ ($\pm 0.1 \mu\text{atg N liter}^{-1}$), and total phosphorus (TP) ($\pm 0.01 \mu\text{atg P liter}^{-1}$) were quantified by colorimetric methods (Grasshoff et al. 1983). Soluble reactive phosphorus (SRP) ($\pm 0.003 \mu\text{atg P liter}^{-1}$) was determined by the malachite-green method in concentrated samples (Camarero 1994b). NRP (non-reactive phosphorus) was calculated as TP-SRP. Dissolved organic carbon (DOC) was measured with a Shimadzu TOC-5000 analyzer ($\pm 1 \mu\text{atg C liter}^{-1}$). Subsamples (500–1,500 ml) of the liquid phase of the cover and lake water were filtered on previously ignited Whatman GF/F glass-fiber filters, and particulate carbon (PC) and particulate nitrogen (PN) were measured in a Carlo-Erba Nitrogen Analyzer 1500 ($\pm 1 \mu\text{atg liter}^{-1}$). Subsamples were also processed to determine microbial abundance and biomass.

Bacterial numbers were determined by epifluorescence microscopy using 4',6-diamidino-2-phenylindole (DAPI) staining on black Nucleopore filters (pore size $0.2 \mu\text{m}$) following Porter and Feig (1980). We followed the recommendations of Kirchman (1993) in order to minimize the error of the count. Bacterial biomass, size, and shape were determined by automated image analysis, as described in Felip et al. (1995). Six length classes were considered ($0.2\text{--}0.4 \mu\text{m}$, $0.4\text{--}0.8 \mu\text{m}$, $0.8\text{--}2 \mu\text{m}$, $2\text{--}10 \mu\text{m}$, $10\text{--}20 \mu\text{m}$, and $>20 \mu\text{m}$).

Abundance of algae, heterotrophic flagellates, and ciliates was estimated following the Utermöhl method after fixation with Lugols solution (Sournia 1978). The error associated with abundance calculations was $\pm 10\%$. Biovolume was estimated by shape approximation to known geometric forms and from direct measurement of the main cell dimensions. In many cases, especially for soft flagellates and for some ciliates, genus assignment was not possible. If size differences were observed within a species, the individuals of that species were divided into several cell classes so that we could better estimate biovolume and in order to take into account the ecological significance of size (Rodríguez and Li 1994). Only those species that constituted more than 1% of the total biovolume in at least one sample were used in the statistical analysis.

Absorbance spectra of photosynthetic pigment extracts in 90% acetone were used for chlorophyll (Chl) determinations (Jeffrey and Humphrey 1975). The ratio between absorbances at 430- and 410-nm wavelengths (A430:A410) was used as pheopigment indicator (Moss 1967). The A480:A665 ratio was used as an indicator of the relative abundance of carotenoids (Strickland and Parsons 1968).

In order to explore species–environment relationships, we performed a canonical correspondence analysis (CCA) (ter Braak 1986). We included in the analysis environmental variables that define the chemical setting (i.e., pH and conductivity), those that are potential growth resources (i.e., light, SRP, NO_3^- , NO_2^- , NH_4^+ , NRP, and DOC), and, given that many of the species found inhabiting the cover are potentially bacteriovores, we also included bacterial biovolume, distinguishing size classes. Species biovolumes and all environmental variables except pH and light were log transformed because of their skewed nature. The CCA extracts the axes of main variability of the species assemblages, but with the restriction that this axis must be a linear combination of environmental variables measured, and assuming a unimodal distribution of the species along the environmental gradients (ter Braak 1995). The resulting diagrams provide a simultaneous ordination of species, sites, and environmental variables. The statistical significance of each environmental variable was tested by a Monte Carlo permutation test with 999 unrestricted permutations (Besag and Clifford 1989).

Results

Physical and chemical changes in the lake cover—For the sake of simplicity, the temporal sequence of physical changes in the lake cover can be divided into three main periods, following conventional snowpack descriptions (Loth et al. 1993): formation, from December to January; growth, from February to mid-April; and ablation, from mid-April to May. The lake froze during the second week of December, and a 10-cm ice sheet quickly formed. Episodic snowfalls and concurrent flooding as a result of hydrostatic adjustment increased cover thickness until mid-April (Table 1), when melting started. During May, pools up to 40-cm deep extended across most of the cover surface. Different layers of slush were identified from January to mid-May, all of which

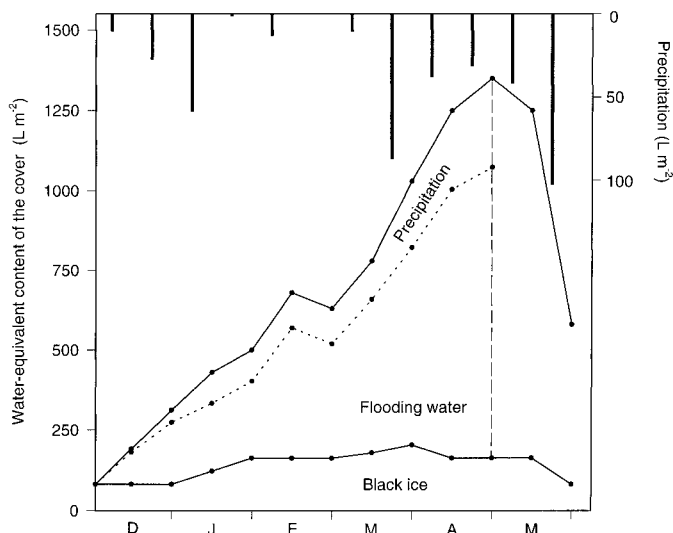


Fig. 2. Changes in the cover water-equivalent content during the sampling period. Water origin is indicated for the growth period. Vertical black bars indicate the amount of snow deposited between surveys. Note the low precipitation from mid-January to mid-March. In May, some precipitation events were rainfalls that contributed to the cover melting.

were located below the hydrostatic water level; thus, they were susceptible to the influence of lake water. At the beginning of June, the cover consisted of only a thin layer of melting ice, which occupied one-half of the lake surface and which finally broke up into many floes.

Most of the water equivalent volume (average of 78%) of the cover was accounted for by flooding water (Fig. 2) during the growth phase. Detailed analysis of the sequence of events showed that snow fell heavily during a few episodes but that hydrostatic adjustment and the consequent flooding of the snow cover occurred progressively after these events.

From December to April, the amount of light reaching the most shallow slush layer was low ($<1 \mu\text{E m}^{-2} \text{s}^{-1}$). Only from May onward did higher light levels reach some of the slush layers. In such cases, more than $10 \mu\text{E m}^{-2} \text{s}^{-1}$ were available for several hours. By this time of the year, surface pools were exposed to high radiation ($1,500\text{--}2,000 \mu\text{E m}^{-2} \text{s}^{-1}$).

The chemical composition of the deposition varied temporally (Table 2). Some snowfalls presented a high content of dissolved salts, whereas others were highly diluted. A general feature of the snow was its high NH_4^+ (10 times greater than in lake water) and SRP and NRP content (about three times greater than in lake water). A clear seasonal trend was evident in the chemical composition of the slush layers. During the formation period, slush was enriched, with higher conductivity and a concomitant increase in all substances except SRP and NRP (Table 2). During the whole growth phase, the chemical composition of the slush was similar to that of lake water. At the end of April, the slush composition started to change, becoming chemically more similar to that of snow and melting water. The main trends at this time were a general dilution, a fall in pH, and higher NH_4^+ content.

Changes in microbial biomass—The Chl concentration in the slush layers decreased from 5 to $0.2 \mu\text{g Chl } a \text{ liter}^{-1}$ (Fig. 3) between January (well-developed slush layers) and the end of April (cover ablation and onset of the watershed thaw). The initial high values were concomitant with those in lake surface water ($5.9 \mu\text{g Chl } a \text{ liter}^{-1}$). The decrease in Chl during the growth phase was accompanied by an increase in the proportion of pheopigments. The A430:A410 ratio decreased to values of 0.77 in mid-April, indicating that about 70% of the total Chl could be present in degraded forms, according to a high-performance liquid chromatography calibration carried out in a previous study of plankton during winter in Lake Redó (Catalan 1992). During the ablation phase, there was a slight increase in Chl levels (Fig. 3). This increase was accompanied by a rise in the A480:A665 ratio, from typical lake values of 1–1.5 up to 6.3 in the cover surface pools.

Slush layers were generally richer in PC and PN than was the lake water (Table 2). In about one-half of the slush samples, the proportion of PC to Chl was similar to lake water proportions, yet the remainder of the samples were significantly enriched in PC. Microscopic observations revealed that this enrichment was due to a large amount of organic debris, mainly from atmospheric deposition, and was not the result of a higher proportion of nonpigmented microorganisms. Only mid-April slush layers showed a large proportion of damaged cryptophytes and chlorophytes instead of debris, which was consistent with the results given by the pheopigment indicator.

The various microbial groups present exhibited different temporal patterns from that of Chl. Biomass maxima occurred at distinct times and at different depths within the cover (Fig. 4). Nonflagellated phytoplanktonic groups, such as desmids and chlorococcales, were found at the beginning of cover formation but quickly disappeared. Moreover, other groups, such as volvocales, chrysophytes, cryptophytes, and heterotrophic flagellates, showed higher densities in the cover than in the lake water. A discontinuity in April in all major groups clearly marked the boundary between sequences. Cryptophytes, chrysophytes, and heterotrophic flagellates dominated the microbial biomass during the growth phase, whereas volvocales, dinoflagellates, and ciliates were more abundant during the cover formation (which is most likely due to their abundance in lake water) and during the ablation period. Some planktonic rotifers, such as *Kellicottia longispina*, *Polyarthra* sp., and *Asplanchna priodonta*, were scarce, appearing only sporadically during the cover growth phase.

Bacterial abundance ranged between 0.5×10^5 and 1.5×10^6 bacteria ml^{-1} and was highly correlated with bacterial biomass ($r = 0.86$, $P < 0.001$), which ranged from 5,200 to 300,000 $\mu\text{m}^3 \text{ml}^{-1}$. Bacteria were a significant part of total microbial biovolume during the whole cover period (32% on average). In mid-April, bacteria even became the dominant group in the slush layers (88% of total biomass).

Microbial assemblage diversity—Bacteria in the slush layers were morphologically diverse; short rods and cocci between $0.4\text{--}0.8\text{-}\mu\text{m}$ long were the most abundant forms, constituting up to 76% of the total number of bacteria in

Table 2. Chemical composition for the different periods sampled. Units are $\mu\text{atg liter}^{-1}$, except pH and conductivity ($\mu\text{S cm}^{-1}$).*

	pH	Conduc- tivity	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	Cl ⁻	NO ₂ ⁻	NO ₃ ⁻	NH ₄ ⁺	SO ₄ ²⁻	DOC	SRP	NRP	PC	PN
Fresh Snow																
Mean	5.4	7.8	6	1	11	4	8	0.1	9	10.8	18	84	0.096	0.541	—	—
SD	0.4	7.4	5	1	11	5	7	0.1	13	11.8	21	65	0.083	0.689	—	—
Minimum	4.3	1.5	0	0	0	0	0	0.0	0	0.8	0	26	0.012	0.003	—	—
Maximum	6.1	52.1	26	7	43	19	29	0.9	93	80.4	100	360	0.401	3.440	—	—
Lake Water																
Formation																
Mean	6.6	13.5	10	2	93	17	12	0.4	17	1.4	30	100	0.049	0.350	17	2
SD	0.06	1.2	<1	<1	9	2	10	0.3	<1	0.5	8	12	0.015	0.116	2	<1
Minimum	6.6	12.1	10	2	85	15	5	0.2	17	0.9	22	91	0.034	0.219	16	2
Maximum	6.7	14.3	11	3	104	18	24	0.7	17	2.0	38	114	0.064	0.440	18	3
Growth																
Mean	6.6	11.7	8	2	66	10	5	0.1	13	2.4	27	92	0.039	0.257	9	1
SD	0.04	0.9	<1	<1	24	5	4	0.02	2	0.8	10	49	0.039	0.129	4	<1
Minimum	6.6	10.3	7	1	18	1	0	0.10	10	1.7	12	42	0.016	0.107	5	2
Maximum	6.7	12.6	9	2	81	15	9	0.15	15	3.4	38	167	0.117	0.463	13	1
Ablation																
Mean	6.5	12.4	6	1	51	4	6	0.08	15	3.4	25	72	0.044	0.221	5	1
SD	0.3	6.0	2	<1	25	3	3	0.003	4	1.3	4	5	0.027	0.178	<0	<1
Minimum	6.3	8.8	5	1	23	0	4	0.08	11	2.1	22	67	0.013	0.110	5	1
Maximum	6.8	19.3	8	2	72	6	10	0.09	19	4.7	29	76	0.062	0.426	6	1
Slush layers																
Formation																
Mean	6.6	34.1	27	7	209	44	21	0.6	53	12.1	123	177	0.053	0.358	21	2
SD	0.2	23.1	20	6	114	33	18	0.3	44	16.5	118	64	0.016	0.104	2	<1
Minimum	6.5	13.6	11	2	101	19	8	0.2	20	1.4	30	127	0.035	0.295	19	2
Maximum	6.9	59.2	49	14	328	82	42	0.9	103	31.2	255	250	0.063	0.478	23	3
Growth																
Mean	6.6	12.3	9	2	66	10	5	0.1	14	3.8	30	95	0.059	0.283	17	2
SD	0.1	2.7	4	2	30	6	5	0.03	6	2.8	14	38	0.028	0.123	8	<1
Minimum	6.5	6.7	3	1	14	0	0	0.08	4	1.9	0	46	0.027	0.132	3	1
Maximum	6.9	16.6	19	9	94	18	14	0.2	28	13.3	53	185	0.128	0.507	30	3
Ablation																
Mean	5.8	6.5	4	1	26	3	8	0.07	13	10.2	20	70	0.028	0.302	15	2
SD	0.5	4.3	4	<1	31	3	5	0.07	9	6.3	11	15	0.022	0.340	18	2
Minimum	5.3	1.6	0	0	2	0	2	0.01	3	2.1	3	52	0.009	0.100	4	1
Maximum	6.6	13.6	11	3	81	9	17	0.2	26	19.1	35	102	0.077	1.098	57	7

* PC, particulate carbon; PN, particulate nitrogen.

some samples. Long (up to 95- μm), thin ($\sim 1\text{-}\mu\text{m}$) filaments were found mostly in slush layers in March and especially during the end of the ablation phase. Although cell size variability was considerable and changed with depth, no clear direct relationship was established between the size distribution of bacteria and eukaryotic groups.

About 100 different eukaryotic species were counted, although the abundance and frequency of most of these species was low. Table 3 lists those species that constituted more than 1% of the total biovolume in at least one sample. Figure 5 illustrates the distribution of the most abundant species over time and at different cover depths. All these species were flagellated forms capable of bacterivory, although they were quite diverse in phylogenetic origin and included some pigmented species. Some of these species showed clear pat-

terns. For instance, the choanoflagellate *Monosiga ovata* developed exclusively during the growth phase of the cover, peaking in the middle of the period. However, the temporal patterns of most species were not so apparent, and changes in assemblages could not be identified. Nevertheless, it was clear that the rate of change was not constant throughout the winter and that the assemblages differed considerably in samples collected a few months apart.

Environmental variables and species distribution—Figure 6 illustrates the ordination results of the analysis performed with the whole data set. The axis 1 eigenvalue is 0.242, and it explains 17.9% of species variability, while the axis 2 eigenvalue is 0.126, and it explains 9.6% of species variability. The main gradient (axis 1) was defined by pH, con-

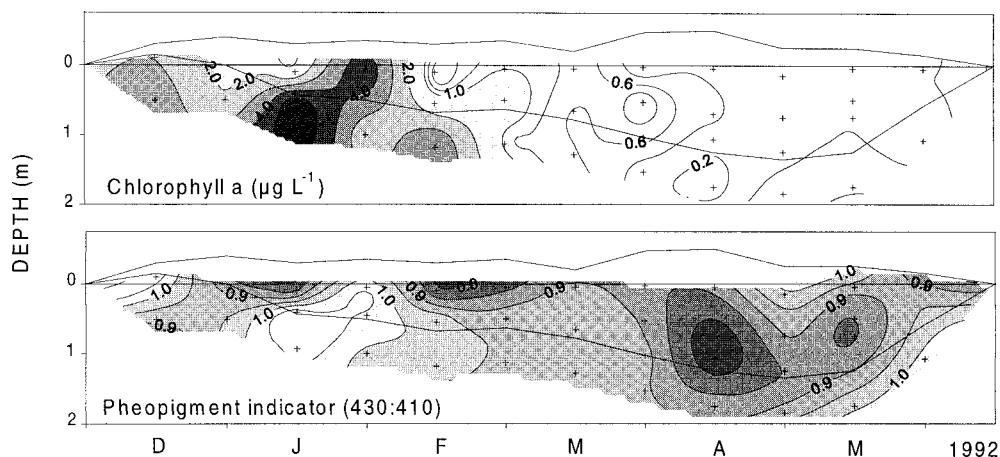


Fig. 3. Temporal changes of chlorophyll *a* concentration and pheopigment indicator in the slush layers of the lake cover and surface lake water. Snow and ice cover is represented by the base map, which shows thickness progress throughout the period. 0-m depth corresponds to the hydrostatic water level; all slush layers were located below that level, which conforms to the painted border. The sampling points, several slush layers, and surface lake water are indicated by small crosses.

ductivity, DOC, and SRP on one edge and by light and NH_4^+ on the other, reflecting the seasonal changes in the chemical composition of the cover (Fig. 6A). The partition of samples between those with negative and those with positive values on this axis generally coincided with the initial samples (cover formation and growth) and the ablation samples, respectively (Fig. 6B). If we compare Figs. 3, 4, and 5, it appears that the main partition was related to a general decline in biomass in most of the groups and main species. Furthermore, bacteria were segregated according to size, with smaller classes (0.2–0.4 and 0.4–0.8 μm) associated with environmental variables that were higher during the growth phase and large classes (10–20 and $>20 \mu\text{m}$) associated with light and NH_4^+ . Bacteria smaller than 0.8 μm accounted for a high percentage of the species matrix variability, similar to the variability explained by some of the chemical factors (Table 4). A second axis, defined by NO_2^- and bacteria (0.8–2 μm , and secondarily, $>20 \mu\text{m}$), reflected the initial conditions in the lake according to the sample scores (Fig. 6B). The lake samples' seasonal trajectory moved along axis 2, from the time at which the lake froze to the beginning of March, and then showed little change until the beginning of May, when it displaced along axis 1. During the growth and most of the ablation phase, lake samples appeared as a boundary for slush samples on the ordination axes, indicating a departure inside the cover from lake communities. Only the last lake-water sample mixed with the cover samples, indicating that there was a higher influence of the melting of the cover on the surface lake water. Figure 6C shows the species associated with this trajectory. Growth assemblages were more diverse than the formation or ablation assemblages. The ablation phase was characterized by the appearance of new species, some of them typical non-planktonic species, such as the large ciliates *Urosoma* sp., *Lacrymaria* sp., and *Dileptus* sp., and algae known to grow on snow beds (for instance, *Chlamydomonas nivalis*). Overall, the distribution of species reflected the tremendous physicochemical modifications of the lake cover rather than dif-

ferential resource use or bacterivory effects. Because the relationships between the species and the potential growth resources may have been obscured, we applied CCA separately to the growth and ablation samples. In this way, we reduced the strong influence of the drastic changes in the physicochemical environment.

In considering the growth phase, total species variability decreased, and variables defining the main gradients changed radically (Table 4, Fig. 7A): bacteria and, to a lesser extent, nutrients, became more relevant than environmental factors, such as pH and conductivity. Light also lost its importance because during this period, all slush layers received low levels. Many variables had significant loadings on both main axes, with the exception of SRP, which was associated with the first axis, and the longest bacteria ($>20 \mu\text{m}$), which were associated with the second axis. The first axis discriminated between the initial part of the period until mid-February, and the rest of the growth phase (Fig. 7B), showing a gradient of decreasing SRP and total bacteria, particularly of the small ones. The second axis indicated the high proportion of very long bacteria in the slush layers on 17 March. The loadings of the various bacterial size classes on the axis defined a partition of the ordination space according to bacterial abundance and size distribution. The negative values on the first axis indicated low bacterial numbers, accompanied by a large proportion of long filaments, in the case of concomitance with negative values on the second axis. These samples were depleted in bacteria in the more edible size classes, which is consistent with the higher proportion of bacterivore species (ciliates and colorless flagellates, Fig. 7C) present.

The total species variance in the ablation period samples was almost as high as the variance in all the samples (formation, growth, and ablation) collected (Table 4). This indicates that changes were more significant during the ablation period than during the formation and growth phases. The first axis still reflected the temporal variability in pH and water dilution (Fig. 7D), while the second axis reflected

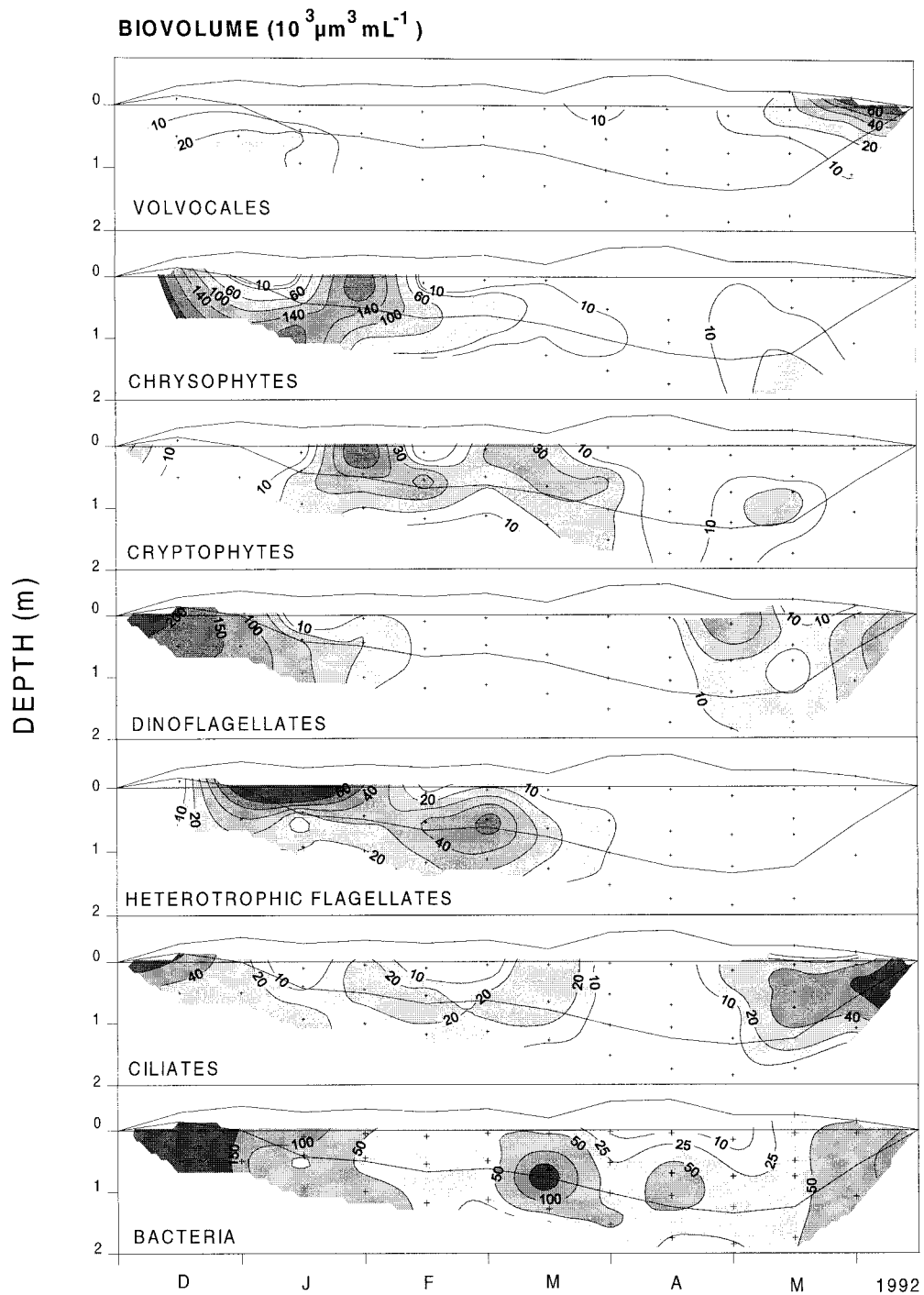


Fig. 4. Temporal change of biovolume of diverse microbial groups in the slush layers and lake water. Map interpretation as in Fig. 3.

the vertical light gradient within the cover, particularly differentiating the samples from the surface pools (Fig. 7E). It also shows the vertical distribution of species that appear for the first time (Fig. 7F). The abundance of rather large bacteria (2–10 μm) and light availability explained a high percentage of the species variability (Table 4). Species associated with the upper layers included *Pteromonas* sp., which

dominates pool assemblages, and other volvocales (*Chlamydomonas* and *Chloromonas*), whereas deeper slush layers were mainly characterized by the presence of ciliates (Figs. 4, 7F). Subtle relationships between species and resources did not emerge from the analysis, perhaps because of the speed and magnitude of changes in the physical and chemical characteristics of the cover during this period.

Table 3. List of the main microbial species and bacteria classes inhabiting slush layers classified according to their taxonomic position and cell size. Some dinoflagellate species with large variability in cell size are divided into small (s), medium (m), and large (l). Only species that comprised more than 1% of total biovolume in at least one sample are listed.

Group	Cell size			
	≤3 μm	>3–5 μm	>5–10 μm	>10–30 μm
Bacteria	0.2–0.4 μm long (B0.2) 0.4–0.8 μm long (B0.4) 0.8–2 μm long (B0.8) <i>Synechocystis</i> sp. (Syne)	2–10 μm long (B2.0)	10–20 μm long (B10.)	>20 μm long (B20.)
Cyanobacteria		<i>Chroococcus</i> sp. 1 (Chr1)	<i>Chroococcus</i> sp. 2 (Chr2)	
Chlorophyta				
Volvocales	<i>Chloromonas</i> sp. (Clo1)	<i>Chloromonas grovei</i> (Clog) <i>Chloromonas</i> sp. 2 (Clo2)	<i>Chlamydomonas</i> sp. 1 (Cla1) <i>Dysmorphococcus variabilis</i> (Dysv) <i>Provasoliella</i> sp. (Prov)	<i>Chlamydomonas</i> sp. 2 (Cla2) <i>Chlamydomonas</i> sp. 3 (Cla3) <i>Chlamydomonas nivalis</i> nonmotile (Cln) <i>Chloromonas infirma</i> (Cloi) <i>Chloromonas modesta</i> (Clom) <i>Pteromonas</i> sp. (Pter) <i>Botryococcus braunii</i> (Botb)
Chlorococcales		<i>Dictyosphaerium</i> cf. <i>sublittarium</i> (Dics)	<i>Oocystis parva</i> (Oocp) <i>Sphaerocystis schroeteri</i> (Sphs) <i>Cosmarium</i> sp. (Cosm)	<i>Ankistrodesmus fusiformis</i> (Ankf)
Desmidiaceae		<i>Monallantus</i> sp. (Mona)		
Xantophyceae		<i>Chromulina</i> sp. (Cro1)		
Chrysophyceae	<i>Chromulina parvula</i> (Crop)		<i>Chrysooccus</i> cf. <i>rufescens</i> (Chyr) <i>Chrysolynos skujae</i> f. <i>tattirica</i> (Chrs) <i>Kephyrion planctonicum</i> (Kepp) <i>Ochromonas</i> sp. 1 (Och1) <i>Ochromonas</i> sp. 2 (Och2) <i>Pseudokephyrion inflatum</i> (Psei)	<i>Dinobryon cylindricum</i> (Dinc) <i>Ochromonas</i> cf. <i>globosa</i> (Ochg) <i>Ochromonas</i> sp. 3 (Och3) <i>Ochromonas</i> sp. 4 (Och4)
Heterotrophic forms		<i>Spumella</i> -like sp. 1 (Spu1)	<i>Oikomonas termo</i> (Oikt) <i>Spumella</i> -like sp. 2 (Spu2) <i>Cryptomonas marsonii</i> (Crym) <i>Rhodomonas minuta</i> (Rhom)	<i>Cryptomonas ovata</i> (Cryo)
Cryptophyta				
Dinophyta			<i>Gymnodinium</i> sp. 1 (Gy1s) <i>Gymnodinium</i> sp. 2 (Gy2s) <i>Gymnodinium cnecooides</i> (Gycs) <i>Gymnodinium</i> cf. <i>lantzschii</i> (Gyml)	<i>Gymnodinium austriacum</i> (Gyma) <i>Gymnodinium</i> sp. 1 (Gy1l) <i>Gymnodinium</i> sp. 2 (Gy2l) <i>Gymnodinium cnecooides</i> (Gycm)(Gycl) <i>Peridinium incospicuum</i> (Pteri)

Table 3. Continued.

Group	Cell size			
	≤3 μm	>3–5 μm	>5–10 μm	>10–30 μm
Choanoflagellata			<i>Desmarella</i> sp. (Desm)	
Flagellates of uncertain taxonomic position		Bodonidlike sp. 1 (Bod1)	<i>Monosiga ovata</i> (Mono)	
		Nonidentified specie 1 (Fla1)	Bodonidlike sp. 2 (Bod2)	
			Nonidentified specie 2 (Fla2)	Amoeboid flagellate (Ambf)
				Nonidentified specie 3 (Fla3)
				Nonidentified specie 4 (Fla4)
			Nonidentified specie 5 (Fla5)	
Ciliophora				
Colpodids				Nonidentified specie 1 (Col1)
				Nonidentified specie 2 (Col2)
Heterotrichs				Nonidentified specie 1 (Het1)
Holotrichs				<i>Dileptus</i> sp. (Dile)
				<i>Holophrya</i> sp. 1 (Hol1)
				<i>Lacrymaria</i> sp. (Lacr)
				<i>Rhopalophrya</i> sp. (Rhop)
				<i>Urotricha pelagica</i> (Urop)
				<i>Urosoma</i> sp. (Uros)
				<i>Pelagostrombidium fallax</i> (Pelf)
Hypotrichs				
Oligotrichs				
				<i>Askenasia</i> spp. (Askn)
				<i>Holophrya</i> sp. 2 (Hol2)
				<i>Mesodinium pulex</i> (Mesp)
				<i>Urotricha furcata</i> (Urof)
				<i>Rimostrombidium</i> cf. <i>humile</i> (Rimh)
				<i>Strombidium</i> sp. (Strom)
				<i>Uronema</i> sp. (Uron)
				<i>Sphaerophrya</i> sp. (Spha)
				Nonidentified ciliate (Cil1)
				Chrysophyte cysts (Cscy)
				Cyst (Cyst)
Scuticociliates				
Suctorina				
Others				
Others		Nonidentified specie 1 (Spe1)		

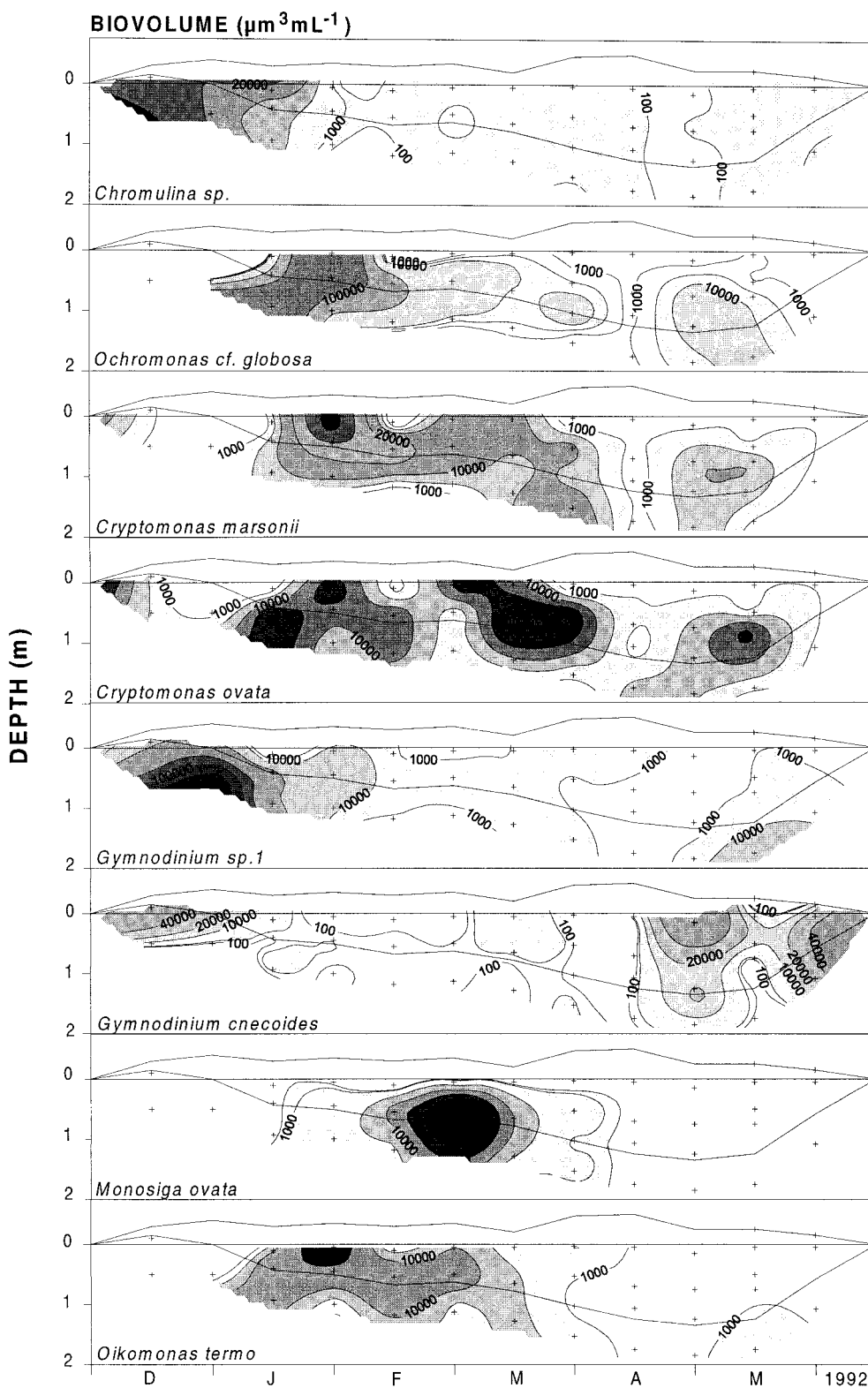


Fig. 5. Temporal change of biovolume of diverse species in the slush layers and lake water. Map interpretation as in Fig. 3.

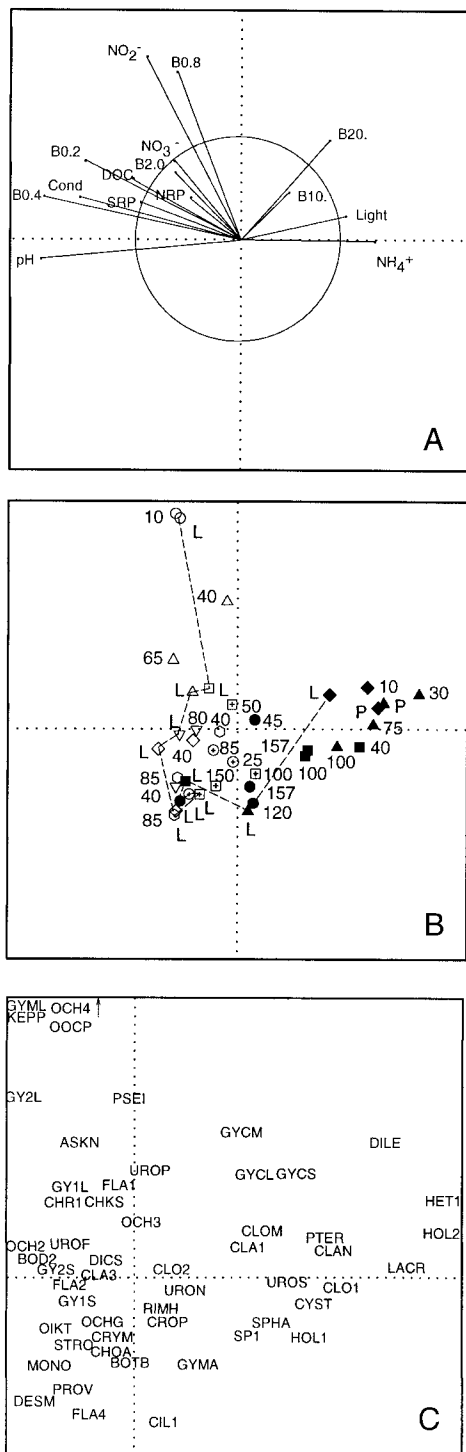


Fig. 6. Results of the CCA analysis performed with species biovolumes and environmental conditions (factors and resources). (A) Ordination of environmental variables. The circle delimits the significance at $P < 0.05$. Bacteria are represented by the lowest length value of the class interval. (B) Ordination of samples. Light symbols correspond to formation and growth samples (○, 19 December; □, 8 January; △, 22 January; ▽, 6 February; ◇, 18 February; ◊, 2 March; ⊕, 17 March; and ⊞, 1 April); dark symbols represent ablation samples (●, 13 April; ■, 29 April; ▲, 12 May; and ◆, 26 May); numbers indicate the slush-layer depth in centimeters; L indicates lake water; and P indicates surface pool. Lake-

Table 4. Total variance in the species data and percentage of the variance explained independently by each factor and resource (environmental variables) in the CCA analysis performed with all samples (formation, growth, and ablation) and with growth and ablation only samples. Only those variables that are significant ($P < 0.05$ in Monte Carlo permutation test) in each case has been included (NS, not significant; B, bacterial biovolume of the size class).

	All*	Growth*	Ablation*
% species variance explained by			
pH	19	NS	14
Conductivity	14	NS	15
Light	11	NS	16
SRP	9	7	NS
NO ₃ ⁻	NS	7	NS
NO ₂ ⁻	13	7	14
NH ₄ ⁺	11	NS	NS
NRP	NS	7	NS
DOC	8	NS	NS
B 0.2–0.4 μm	14	NS	NS
B 0.4–0.8 μm	19	9	14
B 0.8–2 μm	9	8	NS
B 2–10 μm	NS	7	18
B 10–20 μm	NS	NS	NS
B >20 μm	8	7	NS

* Total species variance for the samples was as follows: for all samples, 1.348; for growth samples, 0.885; and for ablation samples, 1.339.

Discussion

This study documents a sequential change in microbial assemblages that is highly affected by the physical transformation of the lake cover and watershed snowpack, and it confirms the presence of a rich community of microorganisms in the slush layers of the cover of lakes that freeze during winter in snowy regions. Figure 8 summarizes the main trends and the sequence of events we found in Lake Redó. From cover formation to complete ice-out, two periods can be clearly distinguished (growth and ablation); these periods show differences in both physicochemical variables and species composition.

Initial stages: seeding of the slush—After a short initial period of cover formation, the first slush layers developed rapidly. These layers were enriched by the exclusion of salts during ice freezing and by falling snow. However, during the growth phase, the water content of the slush layers appeared to originate mainly from the lake water that was flooding the cover (Fig. 2), as is indicated by their composition in conservative ions (Table 2). The similarity in the surface lake water and slush Chl content suggests that flooding is an efficient mechanism for transporting planktonic organisms that seed the slush and stimulate the development of

← water samples were successively joined by a dashed line in order to show temporal sequence. (C) Distribution of the species with more than 50% of variance explained by the analysis. Abbreviations as designated in Table 3.

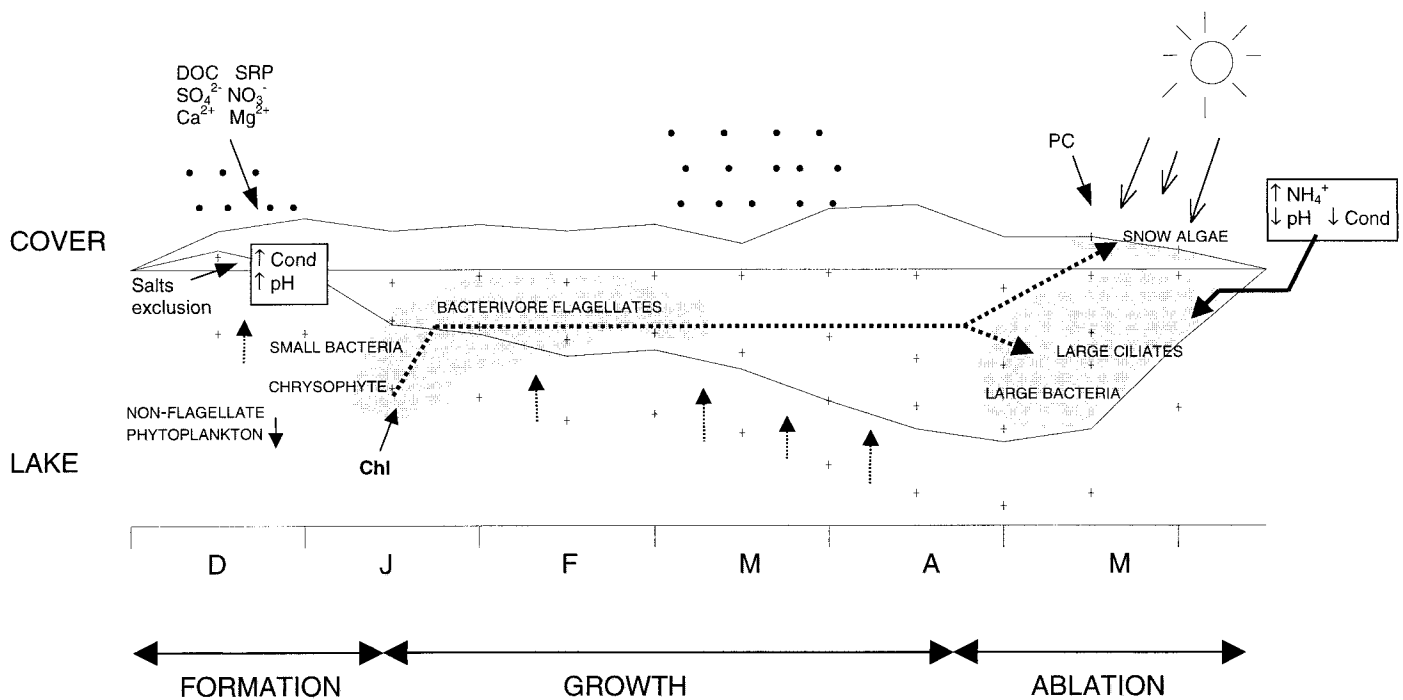


Fig. 8. Summary of the temporal change of the ice and snow cover of Lake Redó in winter 1991-1992.

vious, but the consistent appearance of the decline during several sampling dates suggests it was not a random event but rather the result of a collapse within the microbial community as a result of some general limitation. Macroscopically, snowfalls do not compensate for melting, and, hence, there is no growth in cover thickness. Meanwhile, the flooding of the cover with lake water probably stops. Subsequent to biomass decline, new assemblages appeared, while some species recovered with the increased flow of water from the watershed, which modified the chemistry of the cover. The water apparently brings with it new organisms, including various volvocales (*Chlamydomonas*, *Chloromonas*, and *Pteromonas*), as well as other species that do not appear in the lake assemblage, such as the large ciliates *Urosoma*, *Dileptus*, and *Lacrymaria* (predaceous species typical of benthic environments). These species may come from the snow-pack or from littoral or watershed soil water. During ablation, the rate of change of microbial assemblages is high and is caused by rapid variations in the physicochemical nature of the melting cover. At this time, the vertical segregation of assemblages was accentuated, from those receiving full irradiance in surface pools (dominated by red-pigmented algae) to deeper slush layers that were not receiving sufficient radiation to support photosynthesis and that were distinguished by the presence of large ciliates. To understand the rapid biological changes during the ablation period would require a higher sampling frequency than the one used in our study, and a parallel study of the microbial assemblages growing in the snow and transported by the melt waters running above and through the soil. A quantitative analysis of these microbial assemblages would indicate whether this transport represented a colonization mechanism followed by growth in the slush or whether the cover simply

acts as a trap, retaining some of the microorganisms washed out from the snow or from the soils during the watershed thaw. Previous measurements of primary production during ablation showed values of up to $1.2 \text{ g C g}^{-1} \text{ Chl h}^{-1}$ (Felip et al. 1995), which, regardless of the colonizing mechanisms, indicates the possibility of sustaining a microbial food web based on in situ production during this phase of the cover.

The present study was carried out in a year of low snow deposition in Pyrenean lakes. It is common to find snow covers more than 3-m thick in Lake Redó during the April maximum, and in some years, we have recorded thicknesses of more than 5 m. Slush layers are isolated one from another by much thicker and, usually, harder layers of white ice than those found in the present study. In such years, the cover lasts longer, and, therefore, the patterns observed in this study may be more accentuated and more clearly defined. Evaluation of such interannual variability, spatial heterogeneity, and common features and discrepancies between sites will provide us with a better understanding of this relatively unexplored microbial habitat. Nevertheless, we believe that the temporal patterns observed in this study will be of general application to lakes that freeze in snowy regions. Preliminary observations in lakes from the Alps seem to confirm this pattern (Psenner pers. comm.)

Some of the assemblages described here are similar to planktonic or spring-snow surface assemblages (Kol 1968) but are different from marine-ice assemblages. Microbial communities that inhabit sea ice are highly diverse, and there are marked differences between the communities that develop in physically distinct ice environments. There is a strong interaction between the physical processes that form, shape, transform, and degrade the ice and the microbial assemblages within it (Palmisano and Garrison 1993; Ackley

and Sullivan 1994). Our study is consistent with these marine studies in that we also demonstrate the importance of the physical transformation of the lake cover in shaping microbial assemblages. Yet, on the other hand, our study confirms the lack of microbial assemblages directly associated with the solid phase of the ice as it occurs in sea ice. Perhaps the specific properties of freshwater ice (for instance, a smooth face) or the ephemeral nature of the lake cover prevent ice microbial community development. Nevertheless, the reason why no benthic life develops directly on freshwater ice is still in need of a convincing explanation.

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