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Short-term stability of the microbial community structure in a maritime Antarctic lake

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Abstract Spatial and temporal changes in the microbial community structure in a maritime Antarctic freshwater lake were investigated over a single day/night cycle in December 1999. The community structure of key microbial planktonic groups varied with depth and this was related to both physical and chemical stratification. However, in most cases, the community structure observed at specific depths did not change over the time period studied. These results suggested short-term stability in community structure, with only some minor effects of the diel changes in irradiance on the vertical distribution of planktonic organisms. This is in marked contrast to medium- and long-term studies, which show significant changes in microbial community structure with both time and depth.

Introduction

Earlier studies of plankton community structure in Antarctic freshwater lakes have relied upon samples taken at frequencies of between weeks and months (Ellis-Evans and Saunders 1988; Laybourn-Parry et al. 1991, 1992, 1995; Bayliss et al. 1997; Bell and Laybourn-Parry 1999; Butler 1999a, b; Butler et al. 2000; Izaguirre et al. 2001). More commonly, they have relied upon lower sampling frequencies where logistic and time constraints have prevented more regular sampling (Andreoli et al. 1992; Laybourn-Parry and Marchant 1992; Spalding et al. 1994; Andreoli et al. 1995; Laybourn-Parry et al. 1996; Unrein and Vinocur 1999; Laybourn-Parry et al. 2000; Roberts et al. 2000). However, results emerging from many of these studies have shown dramatic changes in both total population densities and in the direction of change between successive sampling dates. In addition, parameters such as specific growth rates, predation rates and sinking rates for different groups within the plankton, all suggest that significant changes in community structure may occur over a diurnal cycle (Laybourn-Parry et al. 1996, 2000; Butler 1999a, b). Yet, to date, the only studies to address this frequency of potential variation in Antarctic aquatic systems have been conducted on the marine zooplankton (Nishikawa and Tsuda 2001). It is clear, therefore, that whilst detailed consideration is often given to spatial sampling strategy, such as vertical depth in the water column, temporal sampling strategy might also have a considerable impact on the results obtained.

Changes in microbial population density and structure at different depths in these lakes could occur on a diel basis, since diurnal vertical migration has been observed for a number of groups of zooplankton (Gilbert and Hampton 2001) and phytoplankton (Figueroa et al. 1998) in a variety of different aquatic ecosystems. These groups include the Rotifera (Kuczynska-Kippen 2001), Cladocera (Horppila 1997; Ojaveer et al. 2001), Ostracoda (Macquart-Moulin 1999), Copepoda (DeMeester and Vyverman 1997; Santer 1998; Fortier et al. 2001), Cryptophyceae (Pithart 1997), Euglenophyta (Kingston 1999), daphnids (Angeli et al. 1995), Raphidophyceae (Salonen and Rosenberg 2000) and Cyanobacteria (Walace et al. 2000). In addition, Horppila (1997) observed that diurnal changes observed in the vertical distribution of cladocerans were not stable. So, whilst Antarctic freshwater communities are known to be of reduced complexity, diurnal vertical migration might occur in some groups, particularly the flagellated algae (Salonen and Rosenberg 2000), cyanobacteria (Howard

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2001) and autotrophic dinoflagellates (Kamykowski et al. 1999).

The potential for rapid community change in the microzooplankton has also been demonstrated in studies of marine bacterioplankton. Fuchs et al. (2000) showed that the community developing in dilution culture within 24 h of sampling could significantly differ from that in the original water samples. Molecular studies in temperate freshwater systems are also beginning to suggest similar frequencies of variation. Pernthaler et al. (1998) demonstrated that variability in individual bacterial populations clearly exceeded the fluctuations of the total microbial assemblage, suggesting that the apparent stability of total bacterioplankton abundances may mask highly dynamic community fluctuations.

The lakes of Signy Island (60°43′S, 45°38′W) in the maritime Antarctic have been the focus of a number of seasonal studies of microbial plankton dynamics over the last three decades (e.g. Light et al. 1981; Hawes 1985; Ellis-Evans 1996; Butler 1999a). These studies have been facilitated by the characteristically short food chains of Antarctic lakes, which are dominated by the microbial community. The Signy Island lakes contain no higher predators and only three species of planktonic microcrustacean (Heywood 1970). An abundant protozoan population is, however, present (Butler 1999a, b; Butler et al. 2000). This population is dominated by heterotrophic nanoflagellates, as observed in many Antarctic lakes (e.g. Vincent and Vincent 1982; Laybourn-Parry 1997). Previously, substantial changes in the abundance and composition of the microbial plankton in Signy Island lakes have been observed on temporal scales of weeks or months (e.g. Butler 1999b), with population densities varying by several orders of magnitude. The present study examined the community structure of the microbial plankton at a range of depths in mesotrophic Sombre Lake on Signy Island over a 24-h period.

Materials and methods

Study area

Sombre Lake is situated in Paternoster Valley, on the east side of Signy Island. It has an area of 2.66×10^4 m^2, a maximum depth of 11.2 m and is ice-covered for approximately 9 months each year. Details of long-term seasonal changes in plankton abundance, primary productivity and physical and chemical conditions are given by Butler (1999b).

Sampling regime and physical measurements

The study was conducted from 1400 hours on 21 December 1999 to 1400 hours on 22 December 1999 (during the austral summer period). Sampling equipment was deployed through a hole previously drilled through the 49 cm of lake ice with a motorised ice auger. Water samples (1.5 l per depth) for chlorophyll-*a* analysis, determination of bacterial abundance and molecular analysis were obtained every 3 h, using a hand-operated diaphragm pump. Samples (1 l) for determination of phyto- and protozooplankton numbers were collected twice, with a 12-h interval, using an NIO sampling bottle. Water for chemical analyses was collected separately on 13 and 27 December, using the hand-operated diaphragm pump, as part of the long-term Signy lake-monitoring programme. All samples were taken above the deepest part of the lake from depths of 0.3, 1.5, 3, 5, 7 and 9 m and just above the bottom (at approximately 10 m).

Light penetration was measured using a Grant 1000 series squirrel logger (UK) connected to 3 SKP 215 quantum sensors (Skye Instruments, Llandridnod Wells, UK). A cantilever arm, described by Light (1977), was used to position the sensor under the ice away from the sampling hole. Vertical profiles of temperature, oxygen concentration and conductivity were not measured on the day of sampling, but results were available from the regular Signy lake-monitoring programme for 13 and 27 December 1999. Measurements were taken at 1-m intervals with a YSI model 57 dissolved oxygen meter and polarographic oxygen probe (model 5739) with submersible stirrer and, for conductivity, with a Solomat WP4007 water-quality meter connected to an 803PS Sonde (Zellweger Analytics, Dorset, UK).

Chemical analyses

Immediately after collection, sub-samples (500 ml) were filtered through Whatman GF/C filters in a hut next to the lake, using a hand vacuum pump (= 100 mm Hg). Filters were returned to the laboratory for fluorometric analysis of chlorophyll-*a* concentrations (Welschmeyer 1994). Measurements of concentrations of ammonium-N, nitrate-N, dissolved reactive phosphate (DRP), total dissolved phosphate (TDP) and chloride were measured separately (on 13 and 27 December 1999) as part of the lake-monitoring programme, using GF/C-filtered water and an Alpkem FS 3000 autoanalyser (Alpkem, Wilsonville, Ore.) with attached 5027 autosampler. Total dissolved nitrogen (TDN) and dissolved reactive silicate were determined spectrophotometrically using the methods of D'Elia et al. (1977) and Mackereth et al. (1989), respectively. An Orion 250A pH meter was used to measure pH and alkalinity by the Gran titration technique (Mackereth et al. 1989).

Plankton counts

Immediately after collection, 2×10 ml of water from each depth was fixed with phosphate-buffered glutaraldehyde, to a final concentration of 2%, for enumeration of flagellates and bacteria. The next day the fixed samples were filtered onto black polycarbonate membranes (0.2 μm for bacteria and 1 μm for flagellates, both with 8 μm backing filter) under a low vacuum (< 50 mm Hg). Filters were stained with the fluorochrome DAPI (4′, 6-diamidino-2-phenylindole), mounted onto glass slides and stored frozen at –20°C, prior to enumeration by epifluorescence microscopy at ×1250 (Porter and Feig 1980). Heterotrophic nanoflagellates (HNF) were distinguished from phototrophic nanoflagellates (PNF) by use of separate UV and blue filters.

Following collection, further 500-ml water samples were fixed with acid Lugol's iodine (final concentration 10%) for the determination of ciliate and algal abundances. These were concentrated by sedimentation and enumerated using an inverted microscope at ×200 (Utermöhl 1958).

Molecular analysis of the bacterioplankton

Sub-samples of water (1 l) were filtered through 0.2-μm cellulose nitrate filters. Cells were re-suspended from the filter surface and DNA was extracted by freeze-thaw cycling. 16S rRNA gene fragments were amplified using the polymerase chain reaction (PCR) with primers designed by Muyzer et al. (1993) for amplification from bacteria. The Muyzer primer pair produced a fragment of 233 bp equivalent to position 341–534 in the 16S rDNA of *Escherichia coli*.

Cell lysate (5 μl) was added to 50 pmol of Muyzer primers M2 and M3, 200 μmol l^{-1} of each of the deoxyribonucleotide

triphosphates (HT Biotechnologies, Cambridge, UK) and 10 μl of ×10 PCR buffer (HT Biotechnologies, Cambridge, UK) in a 0.5-ml Eppendorf tube and made up to a volume of 100 μl with DNase- and RNase-free 0.2 μm-filtered sterilised water (Sigma, St Louis). The reaction mixture was covered with 70 μl mineral oil and placed in a PHC-3 temperature cycler (Techne, Cambridge, UK). Amplification was conducted by hotstart, touchdown PCR: first denaturing the template DNA at 94°C for 5 min and then reducing the temperature to 80°C for 5 min while 10 μl of a solution containing 0.05 U μl^{-1} *Taq* polymerase was added (Super *Taq*, HT Biotechnology, Cambridge, UK). The annealing temperature was then lowered to 65°C for 1 min and decreased by 1°C every second cycle until 55°C, at which temperature an additional 8 cycles were carried out. Primer extension was carried out for 3 min at 72°C after each annealing step before further denaturation at 94°C for 1 min (adapted from Muyzer et al. 1993). All DNA extraction procedures and manipulations were carried out in a laminar flow hood to minimise aerial contamination, and all plasticware and equipment were exposed to 254-nm UV radiation for 15 min in a UV crosslinker (UVTech, Cambridge, UK).

Amplification products were separated by DGGE, based on the method described by Helms (1990). A 0–50% denaturing gradient, 20 cm in length, was established (where 0% denaturant consisted of 6.5% 37.5:1 acrylamide:bisacrylamide mix in 1×TAE and 100% denaturant consisted of 6.5% 37.5:1 acrylamide:bisacrylamide mix, 40% formamide and 7 M urea in 1×TAE). The gel was run for 80 min at 60°C and 10 V cm^{-1} before staining for 45 min in 0.5 μg ml^{-1}ethidium bromide solution. This was visualised on a UV transilluminator (UVP, Cambridge, UK). Photographs were taken with a Gelcam (Polaroid, Cambridge) using Polaroid 665 professional positive/negative instant pack film and exposure times of 20, 35 and 60 s.

Results

Physical analyses

Water samples were taken on 21 and 22 December during a period of transition (between 13 and 27 December 1999) in which significant warming occurred in the upper layers of the lake. This resulted in a change from a steep thermal gradient, where temperature increased with depth, to a uniform temperature with depth throughout the lake, altering the degree of thermal stratification. Over the same period, dissolved oxygen levels changed from a steady decrease in concentration with depth down to 8 m, to a uniform oxygen concentration between 2 and 8 m. There was a marked hypoxic sump below 8 m seen on both sampling days. Physical parameters determined in Sombre Lake on 13 and 27 December are given in Table 1.

During the 24-h study, light (measured as photosynthetically active radiation, PAR) was never detected below 8 m, even when the levels of recorded surface irradiance were at their highest (1430 hours). Light penetration was detected to 2 m at 2330 hours but was not detected again until 0530 hours, giving a maximum period of irradiance to the surface layers of approximately 18 h. The deepest recorded light penetration was 0.49% at 7 m (during the period of maximum surface irradiance at 1430 hours). The light levels determined at different depths in Sombre Lake over the 24-h period are given in Fig. 1.

Table 1. Physical parameters in Sombre Lake determined on 13th and 27th December 1999

Depth (m)	Temperature (°C)		Dissolved O_2 (mg l^{-1})		Conductivity (μS cm^{-1})	
	13	27	13	27	13	27
1	0.3	1.7	13.07	11.26	113.9	149.7
2	0.3	1.6	11.77	9.77	184.6	174.6
3	0.7	1.6	7.53	9.50	225.9	230.6
4	0.9	1.7	6.20	9.52	230.3	229.0
5	1.0	1.7	6.01	9.49	230.5	226.0
6	1.0	1.7	4.18	9.59	230.9	234.4
7	1.0	1.7	3.37	9.69	235.8	221.3
8	1.0	1.7	2.82	9.03	248.2	225.4
9	1.0	1.4	2.43	3.44	275.3	242.6
10	1.1	1.2	0.32	0.47	324.7	305.1
10.5	1.2	1.2	0.14	0.31	514.5	494.2

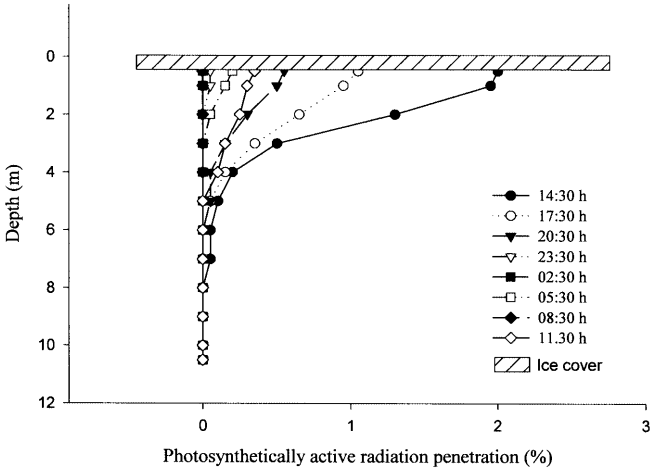

Fig. 1. Photosynthetically active radiation (*PAR*) penetration during the 24-h study period

Chemical analyses

The chemical composition of the water column in Sombre Lake on both 13 and 27 December is given in Table 2.

Nitrogen

A significant increase in total dissolved nitrogen was observed with depth on both 13 and 27 December, and this increase was approximately the same order of magnitude on both sample dates (Table 2). On 13 December, nitrate ion concentration increased slightly (by approximately 5%) with depth from 1.5 to 7 m, but below this decreased by >50%. This roughly corresponded to the transition from oxygenated to deoxygenated water, observed at about 8 m. On the 27th, there was a greater increase in nitrate ion concentration with depth between 1.5 and 7 m (of approximately 33%), and the marked drop in concentration (>50%) then occurred below 9 m. Both of these changes were

Table 2. Chemical concentrations at different depths in Sombre Lake on 13th and 27th December 1999

Depth (m)		0.3	1.5	5.0	7.0	9.0	10.0
pH	13	7.10	7.10	7.02	6.90	6.87	6.85
	27	6.67	6.85	6.88	6.95	6.76	6.81
Alkalinity	13	0.08	0.13	0.31	0.34	0.38	0.42
(mEq l^{-1})	27	0.01	0.17	0.28	0.28	0.35	0.52
Chloride	13	6.03	39.88	39.30	41.26	50.18	78.94
(mg ml^{-1})	27	7.56	27.15	39.65	37.95	49.13	84.51
Nitrate	13	57.57	415.77	421.35	438.05	174.55	176.72
(μg ml^{-1})	27	54.64	311.55	428.97	430.45	668.73	253.91
Ammonium	13	29.70	23.49	29.58	55.35	176.15	523.69
(μg ml^{-1})	27	22.17	0	0	0	83.56	560.86
TDN	13	195.56	335.55	966.88	880.19	916.55	1464.38
(μg ml^{-1})	27	201.10	213.79	431.24	386.21	500.81	1082.77
DRP	13	3.72	3.31	33.64	48.25	52.62	37.97
(μg ml^{-1})	27	6.81	6.81	5.57	7.88	48.32	53.54
TDP	13	14.56	7.96	38.81	58.53	61.87	55.38
(μg ml^{-1})	27	8.94	7.10	8.92	8.00	42.21	50.01
TP	13	14.96	15.57	41.90	70.55	63.87	68.62
(μg ml^{-1})	27	11.27	14.27	29.97	29.78	44.44	53.01
Silicate	13	23.35	261.67	614.56	792.84	872.04	1150.52
(μg ml^{-1})	27	110.86	333.25	524.77	515.55	831.21	1423.51

associated with a corresponding increase in the concentration of ammonium nitrogen below 9 m. Above 9 m, ammonium nitrogen was either at a very low concentration (13th) or below the limits of detection (27th).

Phosphate

Total dissolved phosphate concentration increased with depth on the 13th, but was generally low throughout the water column on the 27th. In both cases, concentrations peaked at 9 m. A similar pattern was observed for total phosphate concentration (TP), which peaked at 7 m on the 13 and between 9 and 10 m on the 27th. Dissolved reactive phosphate concentration was highest between 7 and 9 m on the 13th and between 9 and 10 m on the 27th (when concentrations were very low).

pH showed regular changes with depth, decreasing by a total of 0.25 units over the entire water column on the 13th and increasing by 0.14 units with depth on the 27th. Results of the other chemical analyses showed no differences between the two sample dates. For example, chloride, silicate and alkalinity all increased regularly with increasing depth on both sample dates and to approximately the same orders of magnitude. Differences in the salinity profiles between the two sampling dates might have occurred as a result of heavy precipitation into the lake (or catchment) during or before the first sample period, or a temporary increase in the rate of ice melt.

Microbial community structure

Chlorophyll-*a* concentrations were greatest between 5 and 7 m during the whole of the 24-h study period (Fig. 2), and occurred at the chemocline. Concentrations at these depths, however, were relatively high during the darkest hours and lower during the day, when chlorophyll-*a* was more evenly distributed with depth.

The separate algal groups generally had greatest abundances between 5 and 7 m (corresponding with the chlorophyll-*a* maximum) and very low numbers of cells were found under the ice and at the bottom of the lake (Fig. 3). *Ankistrodesmus* sp. population density peaked at 7 m but showed little temporal change. *Gymnodium* sp. population density changed slightly at some depths between the different sample times, but numbers were generally high between 5 and 7 m. *Chlorella* sp., however, showed a more pronounced change with time, particularly at the key depths of 5 and 7 m. The number

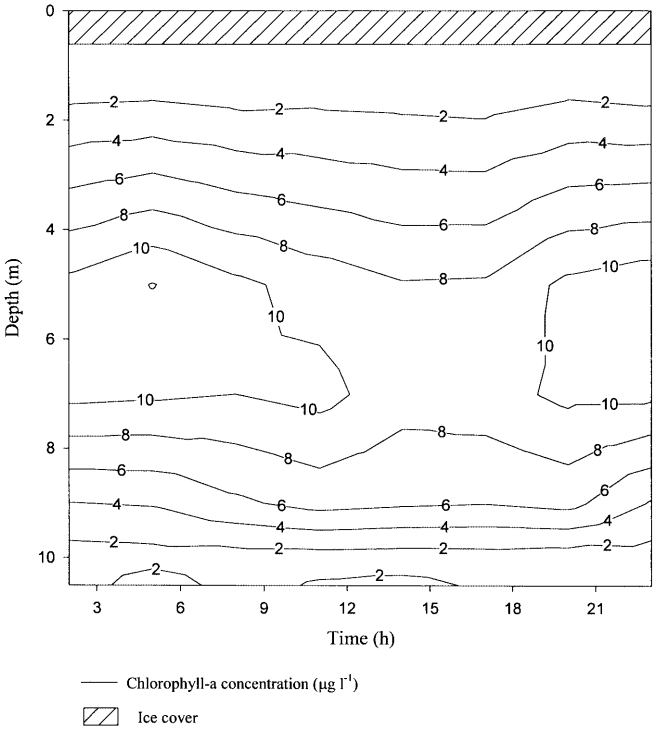

Fig. 2. Chlorophyll-*a* concentrations during the 24-h study period

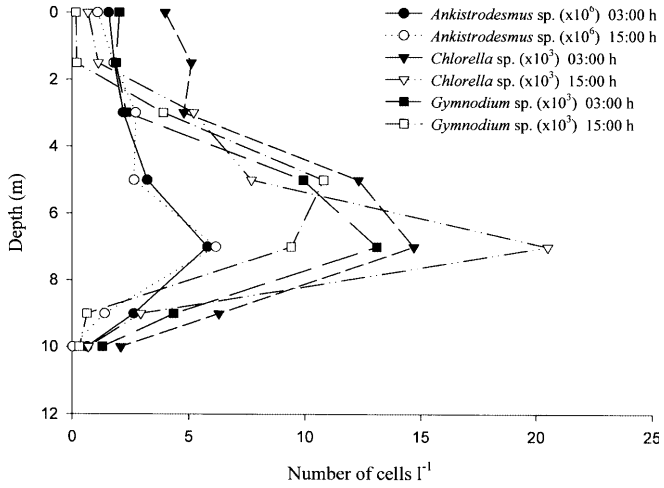

Fig. 3. The abundances of different algal groups at 0300 hours and 1500 hours

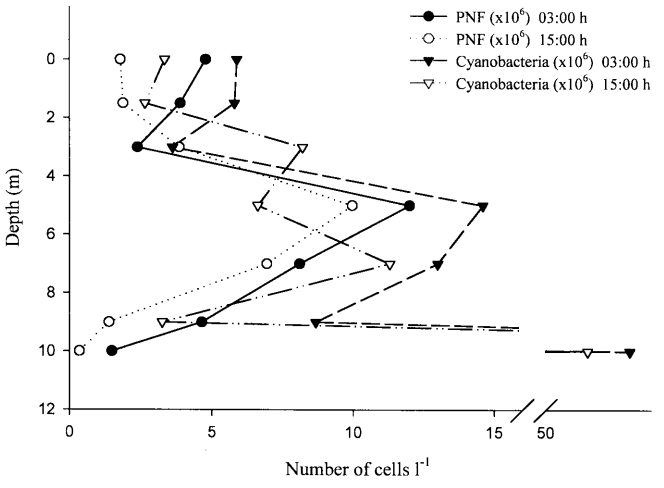

Fig. 4. The abundances of PNF and cyanobacteria at 0300 hours and 1500 hours

of PNF was highest at 5 m, and did not change significantly between sampling times, although there appeared to be a decrease just below the ice surface at 1500 hours (Fig. 4). The maximum number of HNF was found at the surface at 0300 hours, but the counts fluctuated with depth at 1500 hours (Fig. 5). As might be expected, anaerobic species were only found in significant numbers in the low oxygen environment below 8 m, and there appeared to be no difference in population size between sample times. At 1500 hours, ciliate numbers were high between 3 and 5 m and low at 7 m, whilst at 0300 hours they were low between 3 and 5 m and high at 7 m (Fig. 5). Maximum numbers of cyanobacteria occurred between 5 and 7 m (Fig. 4), with minimum numbers in the first 3 m. There was a significant increase in the number of cyanobacteria at the bottom, and this could have resulted from the inclusion of cyanobacterial mat fragments in the sample.

Excluding the surface and bottom samples, total DAPI bacteria counts varied between 0.55 ($\pm$0.2)$\times 10^8$ and1.89 ($\pm$0.3)$\times 10^8$ l^{-1} (Fig. 6). At 7 m, maximum counts were obtained between 2000 hours and 0500 hours. However, no marked differences between depths were found between 0800 hours and 1700 hours. Generally, minimum numbers of bacteria occurred during the hours of daylight and maximum numbers at night. DGGE band patterns for depth profiles during the study period are presented in Fig. 7. Marked differences in community structure were observed with depth, but this structure did not change with time even when population density fluctuated.

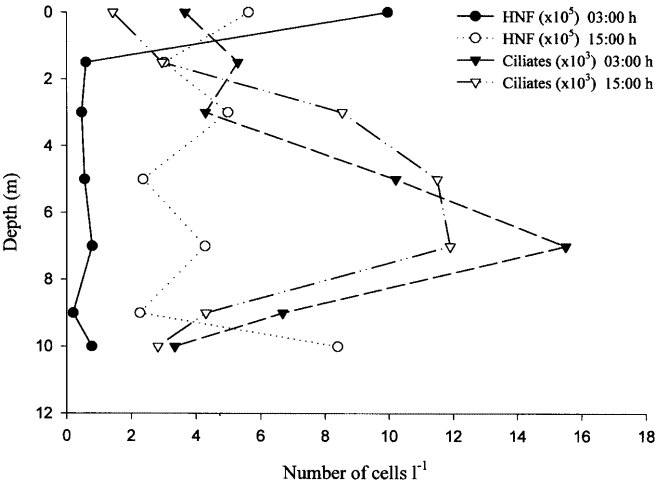

Fig. 5. The abundances of HNF and ciliates at 0300 hours and 1500 hours

Discussion

Physical analyses

Under ice cover, the water column was stratified into three zones: a sub-ice zone of well-oxygenated, low-conductivity water to a depth of 2–3 m, a mid-water zone from 3 to 9 m of near-constant oxygen concentration, and an hypoxic sump below 9 m with increased conductivity. This hypoxic sump in the bottom 2 m of Sombre Lake occurs each winter (Gallagher 1985) and has been shown to contain a distinct community of anaerobic flagellates (Butler 1999b). Ice cover is responsible for the observed physical stratification by preventing wind-induced mixing of the water column. It also reduces the amount of PAR reaching the water column (Belzile et al. 2001; Hawes and Schwartz 2001). In this study, light never penetrated to 9 m, but was detected in the surface layers over a period of 18 h during the day.

Chemical analyses

Winter ice cover and the consequent lack of wind-induced mixing of the water column resulted in gradients

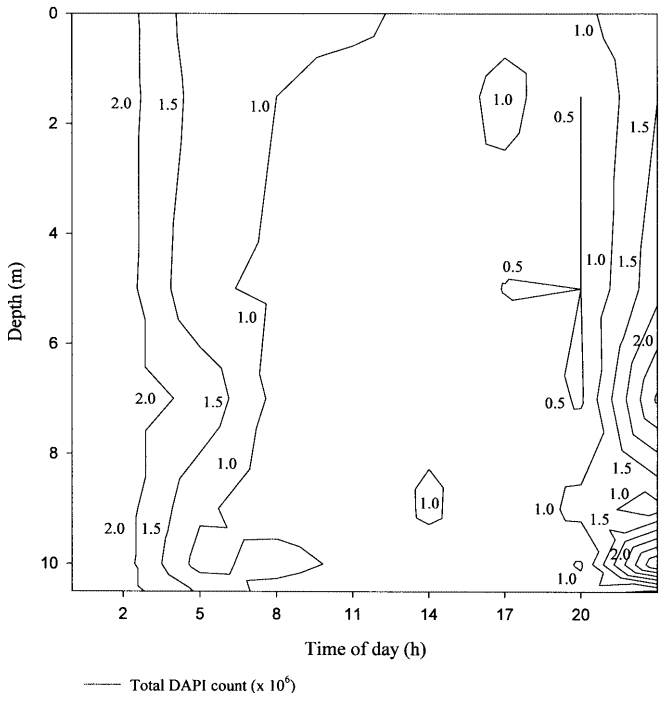

Fig. 6. Total DAPI stained bacteria counts over the 24-h study period

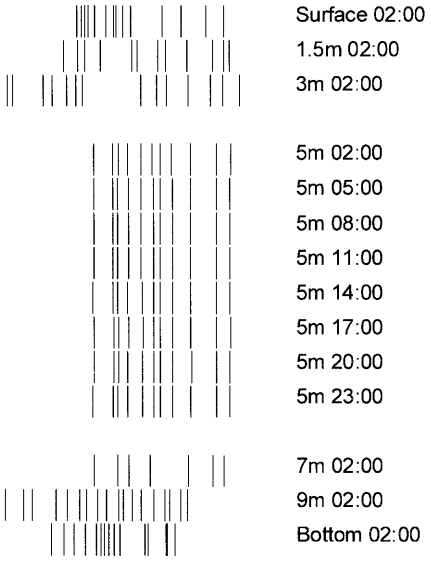

Fig. 7. DGGE depth profiles at 3-h intervals over the 24-h study period. The full time sequence is given for the 5-m sample

in chemical concentrations, a feature common to many Antarctic lakes. In most cases, concentrations of individual ions were not low enough to suggest that nutrient concentrations were a significant growth-limiting factor for the phytoplankton at this time. However, on the 27th, the complete lack of ammonium nitrogen at shallower depths suggested that nitrogen availability may have become growth-limiting.

Microbial community structure

In this study, a well-defined "deep chlorophyll maximum" (DCM) was observed between 5 and 7 m, a common observation in stratified lakes both in the Antarctic (Lizotte and Priscu 1994; Bell and Laybourn-Parry 1999; Priscu et al. 1999; Purdy et al. 2001) and elsewhere (Adrian et al. 2001; Klausmeier and Litchman 2001). The DCM represents a compromise between higher light intensity nearer to the surface and greater nutrient availability at depth, and has been observed regularly in Sombre Lake (Hawes 1985; Butler 1999b). The highest chlorophyll-*a* concentrations were found at the same depth as high population densities of photosynthetic nanoflagellates, dinoflagellates (*Gymnodinium* sp.) and chlorophytes (especially *Ankistrodesmus* sp. and *Chlorella* sp.). The change in the distribution of chlorophyll-*a* with depth between 1500 hours and 0300 hours suggested some degree of diel vertical migration of the phytoplankton (particularly *Chlorella* sp.) between these depths, in response to changing light levels. However, clear vertical migration of the phytoplankton in temperate systems has previously been observed with frequencies below 24 h, for cyanobacteria (Walace et al. 2000), Cryptophyceae (Pithart 1997), algae (Arvola et al. 1992; Hansson 1995) and including flagellate species (Jones 1991). In a similar study, Gervais (1997) observed a regular diel vertical migration of phytoflagellates with day-time ascent and night-time descent.

There was also some evidence of diel vertical movement of the protozooplankton. The maximum number of HNF was found at the surface at 0300 hours, but the counts fluctuated with depth at 1500 hours. Similarly, at 1500 hours, ciliate numbers were high between 3 and 5 m and low at 7 m, whilst at 0300 hours, ciliate numbers were low between 3 and 5 m and high at 7 m. This concentration at 7 m probably occurred because many of the ciliate species present prey on phytoplankton, which were particularly abundant at this depth. Again, clear vertical migration of zooplankton has been observed to occur elsewhere with frequencies below 24 h (DeMeester and Vyverman 1997; Morgan et al. 1997; Kuczynska-Kippen 2001). The maximum number of bacteria (from DAPI counts) occurred at 7 m between 2000 hours and 0200 hours, but there was little change in their distribution with depth and time. This is a likely explanation for the lack of pattern in the distribution of HNF, since HNF prey mainly on bacteria.

Bacterioplankton community structure was shown to be stable over the 24-h period studied, despite fluctuations in the total population density. Up to 18 bands were detected in DGGE profiles, with maximum numbers in the upper layers and at the oxic-anoxic boundary. If band number is representative of the maximum diversity of bacteria present (it actually represents 16S rRNA gene copy number, and only the most common sequences amplify), then maximum diversity of bacterial species occurred at the oxic-hypoxic boundary. This is in

agreement with Rodrigo et al. (1999) who observed that most microorganisms were concentrated at the oxic-anoxic boundary during the day and dispersed through the hypolimnion at night. A wide range of heterotrophic bacteria have been previously identified in other Antarctic lakes. There is no clear evidence from the work of Franzmann, Bowman and McMeechin that there appears to be endemicity among the Antarctic bacteria. However, in this study, only a relatively small number of dominant groups were found. This observation of relatively low numbers of dominant groups has also been made using DGGE for lake systems elsewhere (Lindström 2001). This could be because many of the isolates cultured from Antarctic lakes have been washed in from the surrounding catchment (Ellis-Evans and Wynn-Williams 1985) and are not necessarily active once in the lake, although they are readily recovered in culture. In addition, the change in bacterial abundance with constant community structure suggests that bacterial predation in Antarctic lakes is not species specific.

Elsewhere in the Antarctic, using lower sampling frequencies, Ellis-Evans and Saunders (1988), Laybourn-Parry et al. (1991, 1992), McMinn and Hodgson (1993) and Izaguirre et al. (1993, 2001) all observed between two and sevenfold differences in population density between successive sample dates. Indeed, Takacs and Priscu (1998) found that total bacterioplankton biomass could decrease by as much as 88% between successive sampling dates. Each of these studies demonstrates that any sampling strategy that is not random or simultaneous introduces dependencies among the observations, which must be taken into account during the analysis and interpretation of the data (Avois et al. 2000).

DGGE analysis of 16S rRNA gene fragments has already been used extensively to profile complex microbial communities and to infer the phylogenetic affiliation of community members (Muyzer et al. 1993; Muyzer and de Waal 1994; Ferris et al. 1996). It is particularly appropriate for detecting sequence differences, as variations of only about 2% in 16S rRNA sequence have been shown to correspond to ecologically significant physiological diversity (Moore et al. 1998). The technique's limitations have been summarised by Muyzer and Smalla (1998). The main benefit is that it is now recognised that many microorganisms in the aquatic environment are not culturable (Ward et al. 1992). PCR amplification of 16S rRNA genes followed by DGGE separation of the fragments generated removes the need for culturing. DGGE has already proved appropriate for assessing bacterial diversity in Antarctic lakes (Pearce 2000) and microbial mats (Voytek et al. 2000). In this study, it provided a particularly fast and convenient way to monitor changes in bacterioplankton community structure.

Short-term stability in Sombre Lake

This study showed some evidence of diel changes in chlorophyll-*a* concentrations although, with the exception of *Chlorella* sp., no obvious diel migration of any particular group of phyto- or protozooplankton was observed. In addition, the bacterioplankton community structure in Sombre Lake was constant throughout the 24-h study period. This observed stability is particularly significant, as the generation times of pico- and microplanktonic organisms are often much shorter than the sampling intervals of seasonal investigations. Even in relatively cold Antarctic lakes, in summer open-water conditions (when lake temperatures are highest), bacterial doubling times can range from 3.29 to 8.4 day^{-1} (Ellis-Evans 1981a, b). Therefore, short-term studies are vital to the understanding of functional (e.g. trophic) interactions between populations and communities (Pernthaler et al. 1996) and how individual species might respond to environmental change.

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