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The structure and stability of the bacterioplankton community in Antarctic freshwater lakes, subject to extremely rapid environmental change

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Abstract

In this study, variation in the bacterioplankton community structure of three Antarctic lakes of different nutrient status, was determined in relation to physical and chemical gradients at depth and at time intervals, across the seasonal transition from winter ice-cover to the summer ice-free period. The three lakes studied were: Moss Lake (low nutrient, with typical nutrient concentrations of 80 $\mu g\, l^{-1}$ nitrate and 10 $\mu g\, l^{-1}$ dissolved reactive phosphate), Sombre Lake (low nutrient, but becoming progressively enriched, with typical nutrient concentrations of 185 $\mu g\, l^{-1}$ nitrate and 7 $\mu g\, l^{-1}$ dissolved reactive phosphate) and Heywood Lake (enriched, with typical nutrient concentrations of 1180 $\mu g\, l^{-1}$ nitrate and 124 $\mu g\, l^{-1}$ dissolved reactive phosphate). Bacterioplankton community structure was determined using a combination of PCR amplification of 16S rRNA gene fragments and denaturing gradient gel electrophoresis (DGGE). Results indicated marked changes in this bacterioplankton community structure, which were particularly associated with the transition period. However, significant changes also occurred during the period of holomixis. Comparison of the results from lakes of different nutrient status suggest that increased levels of nutrient input, and in the timing and duration of ice cover will lead to marked changes in the structure and stability of the bacterioplankton community at existing levels of environmental change.

Keywords: Bacterioplankton; Antarctic; Community structure; DGGE; Seasonal cycle; Environmental change; Climate change

1. Introduction

Antarctic limnological studies to date have generally focussed on the phytoplankton and zooplankton communities [1,2]. On Signy Island, for example, Laybourn-Parry et al. [3] and Butler [4–6] were able to show that significant changes in the protozooplankton community structure could occur over relatively short periods of time. With few exceptions, however, the bacterioplankton community structure in Antarctic systems has not been considered, and has often been regarded as a 'black box' [7]. Relatively few studies have attempted to describe either spatial or temporal patterns in this structure, even though recent investigations have demonstrated that environments differing in important physico-chemical parameters support communities differing in species composition [8]. These communities are sensitive to disturbance and in some cases will change with environmental conditions [9].

A study of the bacterioplankton community is timely, as Quayle et al. [10] report data for Signy Island lakes showing extremely rapid environmental change. Mean lake temperatures in winter have increased by 0.9 °C

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between 1980 and 1995 and nutrient levels at some sites exhibit order of magnitude increases per decade. In addition, photographic estimates suggest that permanent ice cover on these lakes has receded by approximately 45% since 1951, and lake ice records indicate that the open-water period has increased significantly.

The lakes of Signy Island, in the South Orkney Islands (60°43′ S, 45°38′ W), lie at the northern extremity of the maritime Antarctic. The lakes also form part of a latitudinal gradient of environmental conditions (temperature, light and nutrient levels), complexity in their microbiology [11], and have been extensively studied for over 30 years [12]. There are 16 recognized lakes on Signy Island in a range of different catchment types, all of which are freshwater and ice-covered to a depth of 1 m for 8–9 months each year. Lake temperatures range from 0–6 °C and the nutrient content of individual lakes varies considerably, largely as a result of animal activity in accessible lakes nearer to the beaches. By monitoring past records and current trends in these lake systems, it is possible to make predictions about how such systems might change in the future, and in response to current observed trends in climate change.

Although PCR amplification of 16S rRNA gene fragments and denaturing gradient gel electrophoresis (DGGE) [13] has already been used to study bacterioplankton community structure in polar aquatic ecosystems [14–16], seasonal changes in bacterioplankton community structure have only been studied in temperate systems, and the stability of bacterioplankton community structure appears to vary with lake system studied and geographical location. Examples do exist of very stable communities [17–20] but most studies reveal some form of microbial community dynamics [21–29]. Such changes have been attributed to a wide variety of possible mechanisms, including biotic factors: grazing [30], viral lysis [31], biomass of microzooplankton, cryptophytes, chrysophytes and diatoms [32,33], response to episodic bloom events [34], competitive exclusion [35], and also to the physico-chemical factors: water column stability, depth [36] or seasonal change [28]. The application of alternative molecular techniques have also suggested that seasonal patterns of substrate utilization [37], concentrations of available trace metals and dissolved organic matter, chemistry of infiltrating hydrothermal waters, irradiation by high levels of UV [38], changing environmental conditions [39], physico-chemical parameters [40], disturbance [41], presence of lake ice [42] and temperature [43] can all contribute to bacterioplankton community structure. However, the typical ranges of annual bacterioplankton community variability remain to be established for Antarctic freshwater lakes [39]. In this study, the bacterioplankton community structure of three freshwater lakes on Signy Island, selected to represent lakes of different nutrient status, were determined at depth and time intervals over the seasonal transition from winter ice cover to the well-mixed summer ice-free period.

2. Materials and methods

2.1. Study lakes

The physical characteristics of the Signy Island Lakes are given in Table 1. Moss Lake (low nutrient) is situated in Paternoster Valley on the north west corner of Signy Island. The drainage basin of 9 ha is composed of approximately 10% snow and ice, 60% rock, 5% lichen and 1% moss cover. There is no evidence of nutrient input from seals, but up to 1% of the catchment is subject to nutrient input from birds. Much of the nutrient input into Moss Lake is derived from melt water running down from the central ice cap. Sombre Lake (low nutrient but subject to progressive enrichment) is also situated in Paternoster Valley. The drainage basin is composed of approximately 74% snow and ice, 14% rock, 9% lichen cover and 2% moss, with >2% subjected to seal derived and a further 2% bird derived nutrient input. Heywood Lake (high nutrient) is situated in Three Lakes Valley, parallel to, and just to the west of Paternoster Valley. There is evidence of high levels of nutrient input from seal activity over 33% of the catchment area, and up to 1% from bird activity (catchment data from Refs. [44,45]).

2.2. Sampling regime

Samples were taken from above the deepest part of the lake at two-week intervals over a 77 day Signy Island field season (sample date 0–14th November 1999, sample date 1–24th November 1999, sample date 2–14th December 1999, sample date 3–28th December 1999, sample date 4–13th January 2000, sample date 5–24th January 2000 and sample date 6–9th February 2000). Samples of 2 l per depth were taken at the surface and at depth intervals of between 1 and 2 m. During the period of ice cover, sampling equipment was deployed

Table 1
Physical characteristics of the three Signy Island study lakes

	Lake		
	Moss	Sombre	Heywood
Nutrient status	Low	Low (increasing)	High
Length (m)	235	250	427
Width (m)	125	150	137
Area (m^2)	16,680	24,300	41,730
Depth (m)	10.5	11.5	6
Altitude (m)	48	5	4
Distance from sea (m)	800	50	200
Ice cover (months)	9	9	8

through a hole previously drilled through the lake ice with a motorised ice auger, otherwise it was deployed from a boat in an equivalent position marked by polypropylene ropes permanently fixed to the shore. Water samples were obtained using a hand-operated diaphragm pump and a rigid Durapipe tube of appropriate length. Samples were transported to the laboratory within 2 h in dark, acid washed, pre-chilled Nalgene bottles, each rinsed three times with lake water from the appropriate sample depth before filling to overflowing. Alkalinity and chlorophyll analyses were prepared straight away. The remaining filtering proceeded over the next 24 h.

2.3. Physical analyses

Light penetration was measured using a Grant 1000 series Squirrel logger (Cambridgeshire, England) connected to three SKP 215 quantum sensors (Skye Instruments, Llandridnod Wells, Wales). A cantilever arm, described by Light [46], was used to position the sensor away from the sampling hole during the period of ice cover. Vertical profiles of temperature were taken at 1 m intervals with a Solomat WP4007 water quality meter connected to a 803PS Sonde (Zellweger Analytics, Dorset, England).

2.4. Chemical analyses

Sub-samples (500 ml) were filtered through Whatman GF/C filters using a Mityvac hand pump. Measurements of nitrate-N and dissolved reactive phosphate (DRP) were measured using GF/C-filtered water and an Alpkem FS 3000 auto-analyzer (Alpkem, Wilsonville, Oregon) with a model 5027 autosampler attached. Total dissolved nitrogen and dissolved reactive silicate were determined spectrophotometrically using the methods of D'Elia et al. [47] and Mackereth et al. [48] respectively. An Orion 250A pH meter was used to measure pH and alkalinity by the Gran titration technique [48]. Vertical profiles of oxygen concentration and conductivity were taken at 1 m intervals with the Solomat water quality meter as described in the previous section.

2.5. Community DNA extraction

Sub-samples of water (1 l) were filtered through 0.2 μm cellulose nitrate filters (Whatman, Maidstone, England) using a 250 ml filter unit (Sartorius, Goettingen, Germany). The filters were placed in a 30 ml sample tube (Sterilin, Feltham, Middlesex, UK) with 18 ml of the original lake water sample, which was then mixed for 3 min. The resulting suspension was divided into twelve 1.5 ml Eppendorf tubes and centrifuged for up to 60 min at 14,000 rpm (Force 14 Microcentrifuge, Denver Instruments, Denver, USA). The pellets from each of four Eppendorf tubes were combined in triplicate and re-suspended in 1 ml lake water, which was then centrifuged for a further 60 min. The resultant pellet was re-suspended in 10 μl of lake water and subjected to five, 5 min freeze–thaw cycles alternating between −80 and 40 °C. Five microlitres of the supernatant was removed from immediately above the cell debris for use in the PCR reaction [15].

2.6. PCR amplification of 16S rRNA gene fragments

Enzymatic amplification of the 16S rRNA genes was performed on DNA extracted from the total community DNA. 16S rRNA gene fragments were amplified using the polymerase chain reaction (PCR) with primers designed by Muyzer et al. [13] for amplification from bacteria. Cell lysate (5 μl) was added to 50 pmol of Muyzer primers M2: 5′-ATT ACC GCG GCT GCT GG-3′ and M3 (incorporating a GC clamp) 5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′ to target the variable V3 region of the 16S rRNA gene and amplify a fragment corresponding to positions 341–534 in *E. coli*. Two hundred micromoles per litre of each of the deoxyribonucleotide triphosphates (HT Biotechnologies, Cambridge, England) and 10 μl of 10x PCR buffer (HT Biotechnologies, Cambridge, England) 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 sterilized water (Sigma, St Louis, USA). The reaction mixture was covered with 70 μl mineral oil and placed in a PHC-3 temperature cycler (Techne, Cambridge, England). 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 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. A final annealing step of 5 min at 72 °C was added at the end of the reaction. All DNA extraction procedures and manipulations were carried out in a laminar flow hood to minimize aerial contamination and all plasticware and equipment were exposed to 254 nm UV radiation for 15 min in a UV crosslinker (UVTech, Cambridge, England). All PCR products (10 μl volumes) were analyzed by electrophoresis in 2% (wt/vol) agarose gels before DGGE analysis was performed. The optimal template concentration was determined by using serial dilutions and subsequent amplification of 1 μl as template DNA in a PCR. A control

oligonucleotide was used to determine the efficiency of amplification.

2.7. Denaturing gradient gel electrophoresis

Amplification products were separated by DGGE [13,49]. The linear denaturing gradient of urea and formamide which ranged from 0% to 50%, and which was 20 cm in length and 1 mm thick 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 visualized on a UV transilluminator (UVP, Cambridge, England). Photographs were taken with a Gelcam (Polaroid, Cambridge, USA).

2.8. Data analysis

DGGE banding patterns were used to determine bacterioplankton community structure. A photograph taken was scanned to produce a TIFF file, which was analysed with Gelcompar II (Applied Maths, Kortrijk, Belgium). Images were reversed, and the contrast of some individual lanes was balanced with respect to other lanes on the same gel. All clear DGGE bands, representing either individual DNA fragments or groups of DNA fragments, were scored independently, when recognised by the band recognition search in Gelcompar II. This included bands of identical electrophoretic mobility that differed in intensity, and bands at the same position in the gel, which had the same melting behaviour but not necessarily the same sequence [7]. The computer program CANOCO [50] was used to perform canonical correspondence analysis, and a weighted correlation matrix was used to link the variation in DGGE band pattern with environmental variation [51].

3. Results

3.1. Moss Lake (low nutrient)

In Moss Lake, both the physical and chemical analyses suggested stratification on sample dates 1 and 2, a transition phase on sample date 3 and almost complete vertical mixing on sample dates 4–6 (Table 2 and Fig. 1(a)). DGGE profiles on sample date 1 differed at each depth analysed (Fig. 2). On sample date 2, the surface and 1.5 m bands were similar and the 7 and 9 m bands were similar, otherwise the profiles differed with depth. Sample date 3 showed similarity between bands at 1.5, 3, 5 and 7 m and between bands at 9 m and the bottom, but both sets differed from each other and with the surface. Similar banding patterns occurred at each depth on sample dates 4, 5 and 6, suggesting consistent bacterioplankton community profiles with depth. A canonical correspondence analysis ordination diagram from Moss Lake results (Fig. 3(a)) suggested that most of the variation in DGGE band profiles from sample dates 2–4 could be explained by chemical parameters and sample dates 5 and 6 with physical parameters. A weighted correlation matrix identified temperature and dissolved reactive phosphate (Table 3) as the most significant environmental variables associated with changes in DGGE band pattern in Moss Lake.

Table 2
Temperature profiles (in °C) of the three Signy Island study lakes, suggesting the presence of stratification in the water column until 28/12 in Moss and Sombre Lakes and only 24/11 in Heywood Lake

Depth (m)	Sample date (day/month)					
	24/11	14/12	28/12	13/1	24/1	9/2
Moss Lake						
0	Nd	0.4	1.2	2.2	5.5	3.7
1.5	Nd	0.6	1.7	2.2	5.5	3.7
3	Nd	0.7	1.9	2.2	5.5	3.5
5	Nd	1.0	2.3	2.2	5.5	3.5
7	Nd	1.7	2.1	2.3	5.5	3.5
9	Nd	1.7	2.0	2.4	5.4	3.5
Sombre Lake						
0	0.5	0.3	1.7	2.5	4.6	4.5
1.5	0.6	0.7	1.6	2.5	4.6	4.5
5	1.0	1.0	1.7	2.5	4.6	4.8
7	1.0	1.0	1.7	2.5	4.6	4.8
9	1.0	1.0	1.4	2.5	4.6	4.9
10	1.0	1.1	1.2	2.9	4.6	4.8
10.5	1.1	1.2	1.2	2.9	4.6	4.9
Heywood Lake						
1	1.9	2.3	5.2	3.9	6.2	6.2
2	1.9	2.3	5.1	3.9	6.2	6.2
3	1.9	2.3	5.0	3.9	6.2	6.1
4	1.5	2.3	5.0	3.9	6.2	6.1
5	1.3	2.3	5.1	3.9	6.2	6.0

Abbreviation: Nd – Not determined (due to logistical constraints).

3.2. Sombre Lake (low nutrient becoming progressively enriched)

In Sombre Lake, both the physical and chemical analyses also suggested stratification on sample dates 1 and 2, a transition phase on sample date 3 and almost complete vertical mixing on sample dates 4–6 (Table 2 and Fig. 1(b)). DGGE profiles on sample date 1 differed at each depth analysed (Fig. 2). However, there did appear to be some bands in common between the upper (1.5–7 m) and lower layers (9 m-bottom). On sample date 2, the surface and 5 m banding patterns were similar, as were the 7 and 9 m and the 10 m and bottom samples. This trend continued on sample date 3. Sample dates 4, 5 and 6, all showed similar banding patterns at each depth sampled. A canonical correspondence

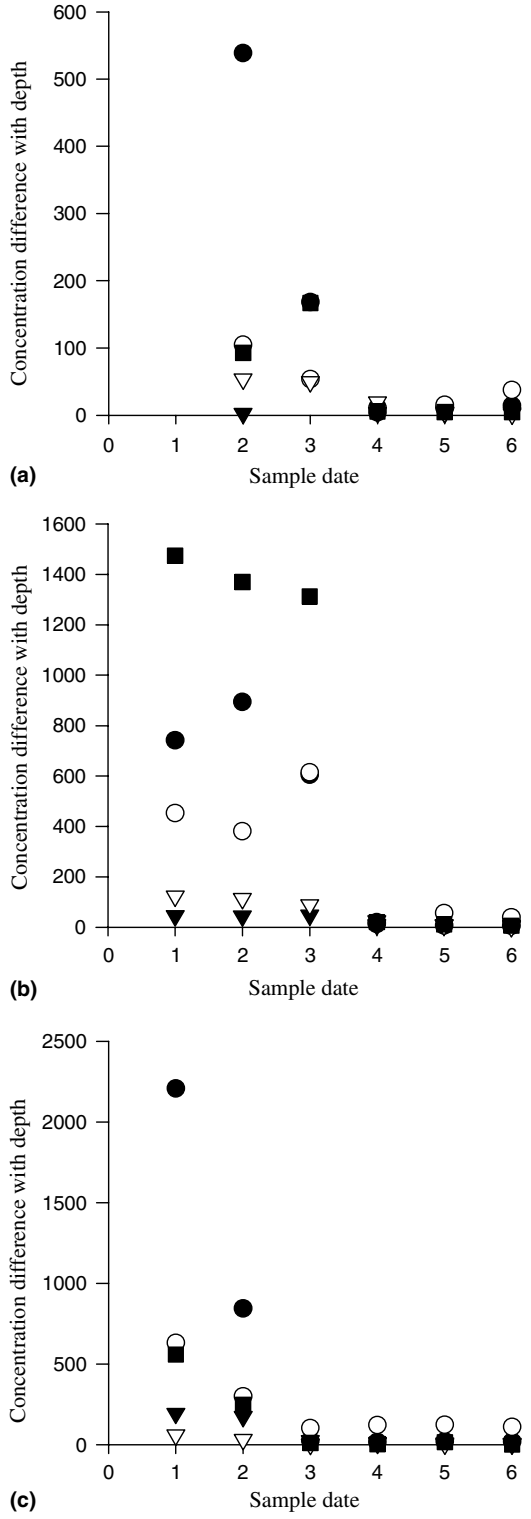

Fig. 1. The difference in concentration between the maximum and minimum concentrations of key ions in the water column at each sample date for (a) Moss lake, (b) Sombre Lake and (c) Heywood Lake, showing a clear structure in the water column early in the season, with little or no difference is during the period of holomixis. ●, Ammonium ion concentration (μg l^{-1}); ▽, Chloride ion concentration (mg l^{-1}); ▼, Dissolved reactive phosphate concentration (μg ml^{-1}); ○, Nitrate ion concentration (μg l^{-1}); ■, Silicate ion concentration (μg l^{-1}).

analysis ordination diagram from Sombre Lake results (Fig. 3(b)) also suggested that most of the variation in DGGE band profiles from sample dates 1–3 could be explained by chemical parameters and sample dates 4–6 with physical parameters. A weighted correlation matrix identified chlorophyll-*a*, dissolved oxygen and temperature (Table 3) as the most significant environmental variables associated with changes in DGGE band pattern in Sombre Lake.

3.3. Heywood Lake (high nutrient)

In marked contrast to the Moss and Sombre Lake profiles, Heywood Lake, showed stratification only on sample date 1, followed by a transition phase on sample date 2 (Table 2 and Fig. 1(c)). Almost complete vertical mixing occurred on days 3, 4, 5 and 6 at least two weeks before either Moss or Sombre Lakes (Table 4). DGGE profiles on sample date 1 differed at each depth analysed, although the 2 and 3 m sample, and the 4, 5 and bottom samples had bands in common (Fig. 2). On sample date 2, the banding patterns were similar with depth (with the exception of the surface sample). Sample date 3 onwards showed similarity between banding patterns at all depths. A canonical correspondence analysis ordination diagram from Heywood Lake results (Fig. 3(c)) suggested that most of the variation in DGGE band profiles from sample dates 1–3 could be explained by chemical parameters and days sample dates 4–6 with physical parameters. A weighted correlation matrix identified nitrate, dissolved oxygen, chlorophyll-*a* alkalinity and temperature (Table 3) as the most significant environmental variables associated with changes in DGGE band pattern in Heywood Lake.

4. Discussion

4.1. Patterns of community structure

In both low and high nutrient lake systems, bacterioplankton community structure was shown to be highly variable. When the lakes were stratified, the bacterioplankton community was found to differ significantly with lake depth and when the water column was mixed, the bacterioplankton community structure was found to be constant with depth (Fig. 4). However, significant variation during the period of holomixis, suggested that the bacterioplankton community structure was not stable, even when physical and chemical analyses suggested almost complete vertical mixing.

4.2. Early season

In a variety of lake systems elsewhere, bacterioplankton diversity has often been found to correlate with the

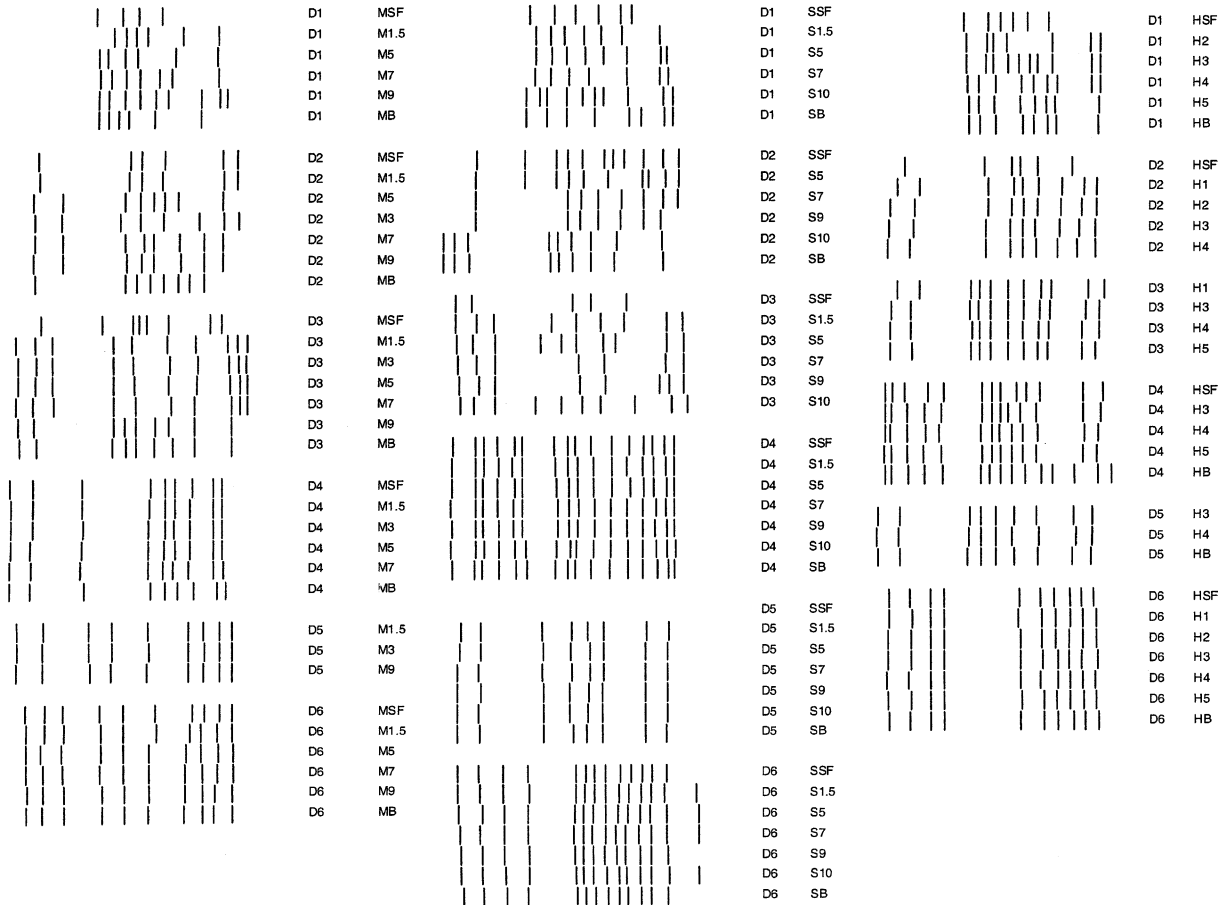

Fig. 2. DGGE profiles with depth in the lake on each of the six sample dates. D – sample date. M – Moss Lake. S – Sombre Lake. H – Heywood Lake. B – Bottom sample. SF – Surface sample.

chemocline, thermocline or oxycline [9,42,52–55]. In this study, lake chemistry also appeared to be the most significant factor in determining the structure of the bacterioplankton community under ice. Changes in DGGE profiles in Moss Lake were particularly associated with dissolved reactive phosphate concentration. Moss Lake receives much of its inflow as melt water directly from the ice cap, and there are few opportunities for nutrient addition. Changes in DGGE profiles in Sombre Lake were not particularly associated with lake nitrogen or phosphorus concentrations. Sombre Lake is in the process of nutrient enrichment and although nutrient concentrations are closer to Moss than Heywood Lakes, unlike Moss Lake, significant fur seal activity does occur in the Sombre Lake catchment. Changes in DGGE profiles in Heywood Lake were particularly associated with nitrate concentration. Large numbers of seals and seabirds in the catchment of Heywood Lake have increased its phosphate loading, the N:P ratio is low and nitrogen rather than phosphate appears to be the main limiting nutrient. Heywood Lake is nutrient rich, with nitrogen and phosphorus concentrations significantly higher than its more oligotrophic neighbours (by at least an order of magnitude). The water is very turbid in summer, when horizontal visibility can be as low as 20 cm. These findings are interesting in view of the observation [56] that the major factor limiting primary production in low nutrient Moss Lake was phosphorus, in Sombre Lake it was phosphorus and ammonium-N (nitrate-N was not used to the same extent and so did not decrease to such low concentrations) and in Heywood Lake, the major factor limiting primary production was nitrogen.

4.3. Transition period

In each of the three lakes studied, physical factors started to dominate through the period of transition. Significantly, in view of the magnitude of the change in environmental conditions observed, temperature appeared to be an important factor in explaining the variation in DGGE community profiles for all three Lake types. This is particularly interesting as mean winter temperatures for the Signy Island Lakes increased by 0.9 °C between 1980 and 1995 [10]. The differences in temperature that coincide with significant changes in community structure can be as low as 0.1 °C in Moss and Sombre Lakes and 0.2 °C in Heywood Lake. Critically, the difference seen between the results in Moss and

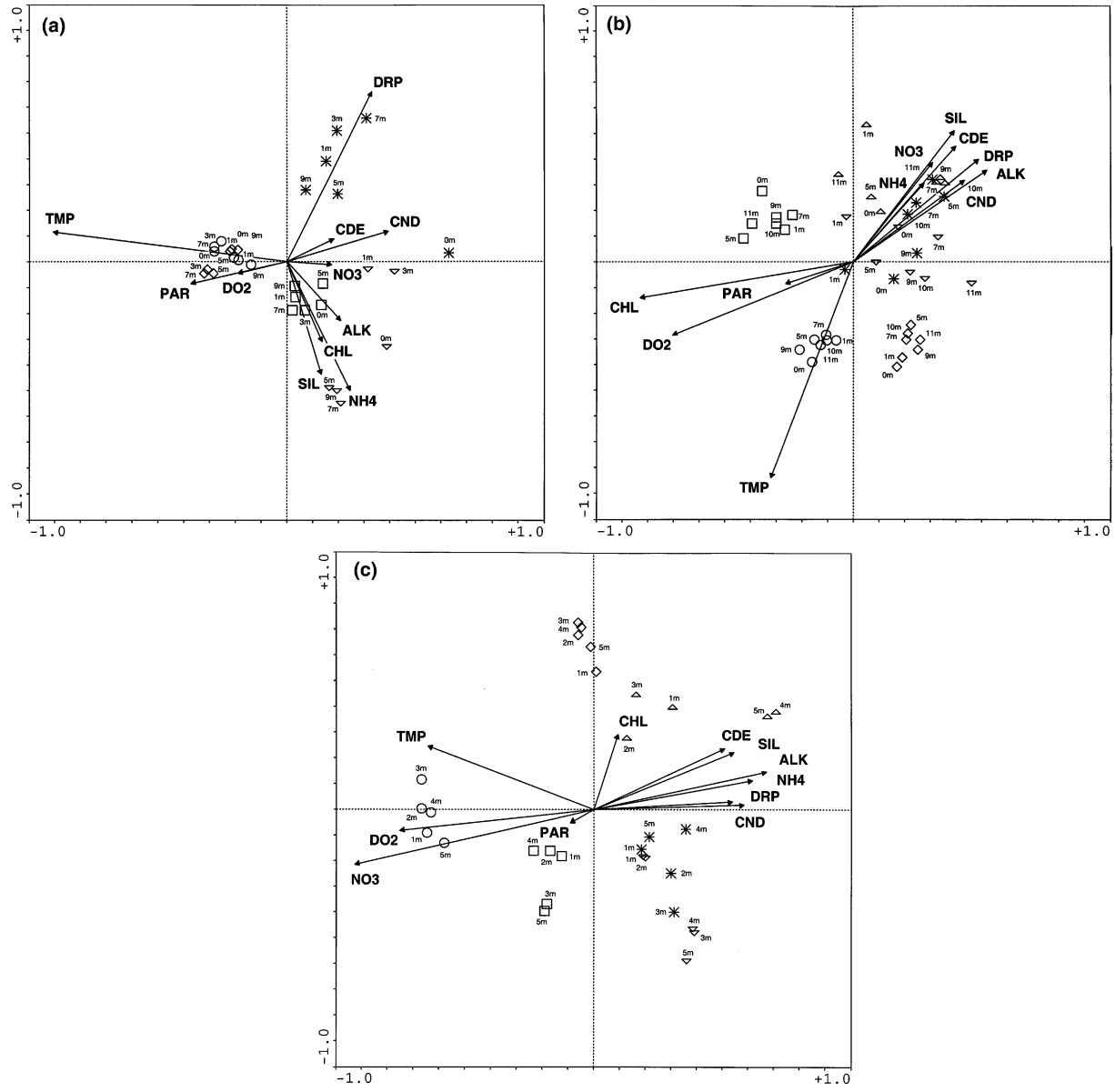

Fig. 3. Canonical correspondence analysis on the DGGE band and environmental data for (a) Moss Lake, (b) Sombre Lake and (c) Heywood Lake. Key to symbols: Sample date 1, △; Sample date 2, ▽; Sample date 3, ∗; Sample date 4, □; Sample date 5, ◇; Sample date 6, ○. SIL – silicate ion concentration, CDE – chloride ion concentration, DRP – dissolved reactive phosphate concentration, ALK – alkalinity, CND – conductivity, NH_4 – ammonium ion concentration, NO_3 – nitrate ion concentration, PAR – photosynthetically available radiation, CHL – chlorophyll-*a* concentration, DO_2 – dissolved oxygen concentration and TMP – temperature. The numbers represent the depth at which each sample was taken in metres.

Table 3
Correlation of > ± 0.6 between selected environmental factors and the bacterioplankton community structure for each of the Signy study lakes, determined by a weighted correlation matrix [65]

Moss Lake		Sombre Lake		Heywood Lake	
Temperature	−0.88	Temperature	−0.78	Nitrate	−0.91
DRP	0.62	Chlorophyll-*a*	−0.75	Dissolved O_2	−0.74
		Dissolved O_2	−0.64	Temperature	−0.63
				Chlorophyll-*a*	0.64
				Alkalinity	0.66

Abbreviation: DRP – dissolved reactive phosphate.

Sombre Lakes, and those in Heywood Lake, demonstrate the importance of the date of ice loss (Table 4). Ice cover appears to be very important in structuring the bacterioplankton community. Ice cover can affect lake systems both directly and indirectly in a variety of ways: it insulates the lake from temperature fluctuations, prevents freshwater dilution and the further entry of nutrient and organisms from the catchment, it influences photosynthetically available radiation and hence indirectly alters the compensation point and the chlorophyll

Table 4
The depth of ice, opaque ice and snow cover (in cm) on each sample date indicating the date of ice loss for each of the three study lakes

	Sample date					
	24/11	14/12	28/12	13/1	24/1	9/2
Moss Lake						
Ice depth	72.5	67	52	0	0	0
Opaque ice depth	35.5	35	7	0	0	0
Snow depth	12.5	0	0	0	0	0
Sombre Lake						
Ice depth	68	66	52	0	0	0
Opaque ice depth	28	31	10	0	0	0
Snow depth	2	5	0	0	0	0
Heywood Lake						
Ice depth	45	30	0	0	0	0
Opaque ice depth	3	0	0	0	0	0
Snow depth	1	0	0	0	0	0

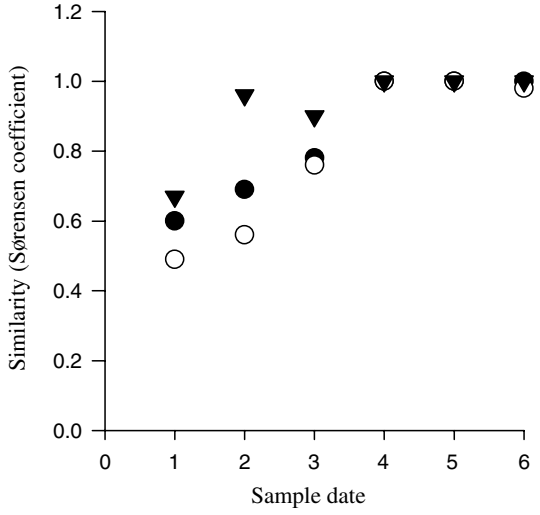

Fig. 4. A plot to show the similarity of DGGE profiles with sample depth through the season using a Sørensen coefficient. Again showing a difference in the bacterioplankton community structure early in the season, with little or no difference during the period of holomixis. Key to symbols: ● – Moss Lake. ○ – Sombre Lake. ▼ – Heywood Lake.

maximum, but perhaps most significantly, it reduces wind induced mixing allowing vertical stratification. In the McMurdo Dry Valley lakes, Takacs and Priscu [57] found that bacterial production was always greatest just below the ice cover at the beginning of the season. However, to date, research on the microbial ecology of the McMurdo Dry Valley lakes has concentrated primarily on phototrophs and relatively little is known about the heterotrophic bacterioplankton. In Crooked Lake, in the Vestfold Hills, Laybourn-Parry et al. [1] found that the bacterioplankton exhibited a seasonal pattern of abundance and that changes in bacterial abundance related to temperature and grazing. They observed that seasonal changes in abundance occurred – in the winter, the lake was inversely stratified below ice cover, however, in summer, the stratification broke down, so that in January and February the water column had a uniform temperature. Bell and Laybourn-Parry [58], also found that high productivity created seasonal anoxia during spring and winter below the ice cover. In each of these studies of Antarctic lake systems, the presence and absence of ice appeared to have a significant effect on the microbial community.

4.4. Late season

The late season variability shown in the bacterioplankton community structure, cannot be explained by physico-chemical parameters alone, as wind induced vertical mixing during the austral summer open water period, disrupts physical and chemical stratification. It is, therefore, likely that biotic factors may become more important during this period. Lindström [32,33] found that the variables most strongly correlating with DGGE banding pattern in temperate lakes were: the biomass of microzooplankton, cryptophytes, chrysophytes, diatoms and temperature, suggesting that these biota had an impact on community structure. In the Vestfold Hills, Laybourn-Parry et al. [1] found that the bacterioplankton exhibited a seasonal pattern of abundance and that changes in bacterial abundance probably related to temperature and grazing by heterotrophic and mixotrophic flagellates. Other biotic factors which might affect the bacterioplankton community structure include: grazing and viral lysis [26,30,31], inflow of allothonous microorganisms following the spring melt [59], response to episodic bloom events [34] and particularly competitive exclusion of numerically inferior populations by numerically superior populations [35].

4.5. Biodiversity

The number of DGGE bands, and hence, the minimum number of dominant phylotypes is given in Table 5. The results obtained for the Signy lakes, therefore, reflect those obtained for lake systems at lower latitudes. Up to 15 bands were obtained from a temperate eutrophic freshwater lake by Overmann et al. [55] and Konopka et al. [54] found 8–23 bands in samples from 58 lakes (which included 10 thermally stratified lakes). This is perhaps not surprising, as Lindström and Leskinen [60] cite several investigations of bacterioplankton diversity in aquatic systems with results indicating that although the species richness of these communities is high only a few taxa (<20) dominate, furthermore, comparative 16S rRNA analysis of lake bacterioplankton has revealed up to 16 globally distributed phylogenetic clusters [61]. One observation which does not fit the lower latitude pattern, however, is that in Lake Saelenvannet, the distribution and diversity of bacterioplankton was shown to decrease with depth [52], whereas in

Table 5
DGGE band number with depth and sample date in the three study lakes

Sample date	Moss Lake		Sombre Lake		Heywood Lake	
	Band No.	Depth	Band No.	Depth	Band No.	Depth
24/11	4	0	6	0	6	0
	6	1.5	7	1.5	7	2
	6	5	8	5	10	3
	7	7	7	7	9	4
	9	9	10	10	8	5
	6	10	8	10.5	8	5.5
14/12	6	0	11	0	6	0
	6	1.5	10	5	9	1
	8	3	8	7	9	2
	8	5	7	9	9	3
	8	7	9	10	9	4
	8	9	9	10.5		
	8	10				
28/12	8	0	5	0	11	1
	10	1.5	9	1.5	11	3
	10	3	10	5	11	4
	10	5	7	7	11	5
	10	7	8	9		
	9	9	10	10		
	9	10				
13/1	9	0	16	0	13	0
	9	1.5	16	1.5	13	3
	9	3	16	3	13	4
	9	5	16	7	13	5
	9	7	16	9	15	5.5
	9	10	16	10		
			16	10.5		
24/1	9	1.5	8	0	9	3
	9	3	8	1.5	9	4
	9	9	8	5	9	5.5
			8	7		
			8	9		
			8	10		
			8	10.5		
9/2	10	0	13	0	10	0
	10	1.5	14	1.5	10	1
	10	5	14	5	10	2
	10	7	14	7	10	3
	10	9	13	9	10	4
	10	10	14	10	10	5
			13	10.5	10	5.5

the Signy study lakes, it appears to increase with depth in the early part of the season.

Although caution must be applied in the interpretation of DGGE band profiles, and the limitations of the method have been well documented [62,63], it has been shown that DGGE selectively amplifies the predominant members of the bacterioplankton community [64] and although each band may represent amplicons from different species with the same sequence or even a mix of sequences, the band number is a good indication of the minimum number of dominant phylotypes present.

4.6. Overall pattern

The physical and chemical analyses suggested that water column stratification is primarily responsible for the determination of the bacterioplankton community structure and overall seasonal stability, with lake water chemistry of primary importance earlier in the season, giving way to physical parameters through the transition period. Variation in community structure during holomixis also strongly suggests that factors other than the physical and chemical parameters tested, may determine which species dominate the bacterioplankton

community. However, the importance of the presence of an ice cover in these Antarctic lake studies, through the prevention of wind induced mixing, would suggest that the physico-chemical characteristics of Antarctic lake systems might have a greater impact on the actual community structure and its seasonal stability in a changing environment. The difference observed between the results in Moss and Sombre Lakes, and those observed in Heywood Lake, demonstrate that a change in climate or in the timing of ice cover may drastically change lake bacterioplankton community structure, particularly when substantial inter-year meteorological variability has already been demonstrated [10]. Such differences in bacterioplankton community structure could change the ecological relationships within the aquatic food web and hence influence ecosystem function.

5. Conclusions

This study has demonstrated that seasonal and spatial patterns of community composition in maritime Antarctic lakes are variable and can change significantly over a 14 day period at key times during the annual cycle, it has also demonstrated unexpectedly high levels of variation in community structure during the austral summer open water period. Critically, the bacterioplankton community structure was shown to change in response to physico-chemical parameters at magnitudes well below those currently observed as a result of climate change.

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