Significant changes in the bacterioplankton community structure of a maritime Antarctic freshwater lake following nutrient enrichment

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Nutrient enrichment is known to increase bacterioplankton population density in a variety of Antarctic freshwater lakes. However, relatively little is known about the associated changes in species composition. In this study, the bacterioplankton community composition of one such lake was studied following natural nutrient enrichment to investigate the resistance of the system to environmental change. Heywood Lake is an enriched freshwater maritime Antarctic lake, with nitrogen and phosphorus concentrations significantly higher than its more oligotrophic neighbours (by at least an order of magnitude). This major change in lake chemistry has occurred following large increases in the fur seal population over the last 30 years. Using analysis of 16S rRNA gene fragments, fatty acid methyl ester analysis, denaturing gradient gel electrophoresis and fluorescence *in situ* hybridization, significant changes are reported in lake microbiology which have resulted in a distinct bacterioplankton community. In comparison to its more oligotrophic neighbours, nutrient-enriched Heywood Lake has a high bacterioplankton population density, reduced species richness and an increasing evenness among key groups. Only 42.3% of the clones found with $\geq 97\%$ similarity to a named genus were also present in adjacent oligotrophic lakes, including three of the dominant groups. Critically, there was an apparent shift in dominance with trophic status (from the β -*Proteobacteria* to the *Actinobacteria*). Other key observations included the absence of a dominant group of *Cyanobacteria* and the presence of marine bacteria. The significant impact of natural nutrient enrichment on the microbiology of Heywood Lake, therefore, suggests that low-temperature oligotrophic freshwater lake systems might have low resistance to environmental change.

Received 20 April 2004

Revised 14 July 2005

Accepted 18 July 2005

INTRODUCTION

There have been a number of investigations of nutrient enrichment in high latitude limnetic systems, which have demonstrated profound effects on the productivity and species composition of the phytoplankton (Bell & Laybourn Parry, 1999; Unrein & Vinocur, 1999; Izaguirre *et al.*, 2001). In these studies, natural nutrient enrichment has resulted in significantly higher nitrate, phosphate and chlorophyll *a* concentrations, higher bacterioplankton population densities, higher heterotrophic nanoflagellate and phototrophic

nanoflagellate abundances, dramatic shifts in species composition within the phytoplankton and high productivities creating seasonal anoxia under ice. In addition to these changes, Laybourn-Parry *et al.* (1996), Butler (1999a, b) and Butler *et al.* (2000) were able to show that significant changes in the protozooplankton community structure could occur over relatively short periods of time.

It is known from temperate and tropical areas that increased nutrient loading is often accompanied by a change in species composition (Hawes, 1990). Also, in polar environments, the character of freshwaters can be radically changed (e.g. switched from an oligotrophic to a eutrophic state), by quite minor events such as interference with an inflow or through the introduction of small amounts of inorganic or organic substances, especially if the volume is small (Fogg, 1998). Despite these observations, however, the community

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Abbreviations: DGGE, denaturing gradient gel electrophoresis; DRP, dissolved reactive phosphate; FAME, fatty acid methyl ester; FISH, fluorescence *in situ* hybridization; TDP, total dissolved phosphate.

composition and taxonomy of the bacterioplankton has not been considered in detail, even though these communities are sensitive to disturbance and have been shown using fingerprinting techniques, to change with environmental conditions (Crump *et al.*, 2003; Pearce, 2005). In lower latitude limnetic systems, differences in the bacterioplankton community composition between lakes of different trophic status have already been observed (Lindström, 2000), and it has been shown that the availability of nutrients and seasonal changes in nutrient concentrations can affect the bacterioplankton community composition (Donner *et al.*, 1996; Yannarell *et al.*, 2003). In this study, we examine the bacterioplankton community composition in Heywood Lake, Antarctica, and compare it to observed patterns in neighbouring Signy Island lakes with little (Sombre Lake) or no (Moss Lake) natural nutrient enrichment.

The significance of this particular system is that Heywood Lake is situated in a region of the globe subject to extremely rapid environmental change. Quayle *et al.* (2002) report data for the Signy Island lakes showing that mean lake temperatures in winter have increased by 0.9 °C 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 approx. 45 % since 1951, while lake ice records indicate that the open-water period has increased significantly. In this way, Heywood Lake might indicate the type of changes that could take place in analogous systems subjected to environmental change.

METHODS

Study site. Heywood Lake is situated in Three Lakes Valley on Signy Island in the South Orkney Islands (60° 43' S, 45° 38' W), which lie at the northern extremity of the Maritime Antarctic. The island has the longest continuous record of biological research in Antarctica (Smith, 1997). It has been the subject of a number of investigations and is the main study site for long-term change. Glacial erosion has divided the island into six major catchments, in which there are 16 recognized lakes, representing all of the maritime Antarctic lacustrine environments. These small shallow lakes have formed following progressive deglaciation of Signy Island (Hawes, 1990). Most are oligotrophic (Heywood *et al.*, 1980), but several are enriched and of these Heywood Lake is the best known and most extensively studied (Fig. 1a).

Heywood Lake itself is situated in a pre-glacial valley, it is the largest of the Signy Island Lakes and has been isolated from the ice cap for hundreds of years – moss fragments in basal sediments have been AMS-radiocarbon dated to 5890 ± 60 ^{14}C years BP (Jones *et al.*, 2000). The lake is ice-covered to a maximum depth of 1 m for at least 8 months of the year, when the lake becomes anoxic and the water column clears. There are several small inflow streams, most notably from adjacent Knob Lake. The planktonic metazoan population of Heywood Lake consists of a few small crustaceans and rotifers (Butler, 1999a, b), but it contains no higher predators, such as fish or insect larvae, and lacks vascular and emergent plants.

Heywood Lake is 427 m long and 137 m wide with an area of 41 730 m². The lake comprises two basins separated by a shallow neck region. The larger north basin has a maximum depth of 6.4 m and a

Fig. 1. (a) Heywood Lake, in Three Lakes Valley, on the north eastern side of Signy Island, in the South Orkney Islands, Antarctica (60° 43' S, 45° 38' W). These islands lie at the northern extremity of the maritime Antarctic. (b) The dramatic impact of Antarctic fur seal populations on Signy Island moss communities, which has resulted in compaction and death of the indigenous moss flora and colonization by a nitrophilous alga characteristic of sites frequented by seals and birds. Note the seal exclusion fence installed to prevent further damage.

mean depth of 2 m. Its drainage basin is composed of approximately 1 % snow, 93 % rock, 5 % lichen and 1 % moss cover, so the ice-free terrain is composed of frost-shattered rock and rudimentary soils formed from glacial diamiction (Noon *et al.*, 2002). The lake lies only 4 m above sea level and is separated from the sea by 200 m of gently sloping ground. This permits ready access to the catchment by Antarctic fur seals (*Arctocephalus gazella*) and Southern elephant seals (*Mirounga leonina*). So Heywood Lake, although adjacent and physically similar to the oligotrophic Moss and Sombre Lakes, is different principally because its catchment is accessible to seals, which contribute considerable amounts of organic matter to the water. Unlike its more oligotrophic neighbours, 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, so that the total annual carbon gain in Moss Lake is less than an eighth of that in Heywood Lake (catchment data from Heywood *et al.*, 1980; Ellis-Evans, 1982, 1990; and Fogg, 1998). Butler (1999a) obtained values of 0.7–5.1 mg dissolved organic carbon l⁻¹ and 0.8–6.4 mg total organic carbon l⁻¹ for Heywood Lake. A striking example of the impact on Signy Island moss communities is demonstrated by the presence of a seal exclusion fence (Fig. 1b). The

once widespread moss carpet has been compacted and is now largely moribund; it has been recolonized by a community dominated by the nitrophilous alga *Prasiola crispa*, which is characteristic of sites frequented by seals and birds (Ellis-Evans, 1990).

Antarctic fur seal numbers in the Heywood Lake catchment area have increased markedly since 1974, and particularly since 1984, including a tenfold increase between 1976 and 1988 (see Fig. 2 and data in Ellis-Evans, 1990). Peak numbers (in February) have been monitored on Signy Island and have been found to increase from only a few animals each year in the 1950s and 1960s, to a few dozen in the early 1970s, but then to 2000–3000 between 1976 and 1983, 8000–17 000 between 1984 and 1993, 20 500 in 1994 and 1995 (Smith, 1997) until, most recently, 10 004 in 2003 (British Antarctic Survey, unpublished data). This expansion of the fur seal population has also been reported elsewhere in the Southern Ocean (Bester *et al.*, 2003). The presence of the fur seals has resulted in considerable nutrient input, biological change and perturbation of the lake sediments, which are between 5000 and 7000 years old. Seal impact has been severe and both physical disturbance and nutrient toxicity have resulted in significant or complete destruction of Antarctic plant communities surrounding the lake. In addition to fur seal excrement and fur wash, plant and organic accumulations are being compressed and eroded into the lakes (Smith, 1997).

Sampling regime. Lake water samples were taken above the deepest point in Heywood Lake on 9 February 2000. Samples of 2 l at each depth were taken over the following depth profile: at the surface (approx. 0.3 m), from depths of 1, 2, 3, 4 and 5 m and just above the bottom (approx. 5.5 m). Water samples were obtained from an inflatable boat 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.

Physical and chemical analyses. Vertical profiles of temperature, oxygen concentration and conductivity were taken at 1 m intervals with a Solomat WP4007 water quality meter connected to a 803PS Sonde (Zellweger Analytics). Light penetration was measured using a Grant 1000 series Squirrel logger connected to three SKP

Fig. 2. Annual numbers of the Antarctic fur seal (*Archtocephalus gazella*) in February in the Heywood Lake catchment area, which have increased markedly since 1974, and particularly since 1984, including a tenfold increase between 1976 and 1988. The presence of the fur seals has resulted in considerable nutrient input, biological change and perturbation of the lake sediments.

215 flat quantum sensors (Skye Instruments). Subsamples of lake water (500 ml) were filtered through Whatman GF/C filters using a Mityvac hand pump. Part of the 500 ml subsamples were used for fluorometric analysis of chlorophyll *a* concentrations, according to Welschmeyer (1994). Measurements of ammonium-N, nitrate-N, dissolved reactive phosphate (DRP), total dissolved phosphate (TDP) and chloride were measured using GF/C-filtered water and an Alpkem FS 3000 auto-analyser (Alpkem) with a model 5027 autosampler attached. Total dissolved nitrogen 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).

Determination of total population density. The total population density in each sample was determined by direct microscopical counts of DAPI stained cells, using the method described by Porter & Feig (1980).

Analysis of 16S rRNA gene fragments. Enzymic amplification of 16S rDNA was performed on DNA extracted directly from Heywood Lake water using the method described by Pearce (2000). For these amplifications, each PCR mixture (50 µl) contained 10 ng extracted DNA as template, 10 pmol each primer, 20 nmol each dNTP, 1 U SuperTaq polymerase (HT Biotech) and the SuperTaq buffer supplied with the enzyme. Primers used were 8F, 5'-AGAGTTTGATCCTGGCTCAG-3' (Dunbar *et al.* 1999), and 1500R, 5'-AGAAAGGAGGTGATCCAGCC-3' (Andrade *et al.* 2002), generating a 1492 bp fragment. Amplification reactions were performed with a Genius thermocycler (Techne) using the following conditions: an initial denaturation step consisting of 94 °C for 5 min, 30 cycles consisting of 94 °C for 45 s, 55 °C for 45 s, 72 °C for 70 s, and a final elongation step consisting of 72 °C for 5 min. Controls containing no DNA were also used to ensure that contaminants were not being amplified. The PCR products were cleaned using GFX PCR clean up columns (Pharmacia). Cleaned products were ligated into the pGEMT-Easy vector (Promega) and ligation mixtures were transformed into competent JM109 cells as recommended by the manufacturer. Transformants were screened using black/white selection on Luria agar containing S-Gal/IPTG and 50 µg ampicillin ml⁻¹ (Sigma). Putative positive colonies were transferred to individual tubes containing 50 µl sterile water. The cell suspensions were subjected to two freeze/thaw cycles and 1 µl aliquots were used as templates in a PCR reaction containing the M13F/M13R universal primers (M13F, 5'-CGCCAGGGTTTCCCAGTCACGAC-3'; M13R, 5'-GAGCGGATAACAATTTCACACAGG-3'). PCR conditions were as described above, except that the annealing temperature was raised to 58.5 °C. A length (760 bp) of each clone obtained was sequenced with the M13F primer using the Big Dye terminator kit v.2 (Applied Biosystems). Sequence reactions were carried out at the British Antarctic Survey using a Megabase 500 capillary sequencer. Clone sequences were compared with the GenBank nucleotide database using GAPPED-BLAST searches (<http://www.ncbi.nlm.nih.gov/blast/blast.cgi>) (Altschul *et al.*, 1997) to determine their closest phylogenetic neighbours. Identification to the genus level was defined as a 16S rRNA gene sequence similarity of ≥97% with that of a sequence deposited in the EMBL database (Drancourt *et al.*, 2000).

Direct culture of bacteria. Cultures were obtained by making a dilution series of lake water and pipetting 200 µl onto solid casein-peptone-starch (CPS), tryptic soy broth agar (TSBA) and R2A agar plates in three replicates. Plates were incubated at 4 °C for 2 months.

Fatty acid methyl ester (FAME) analysis. The phenotypic diversity and identity of individual strains isolated by direct culture was determined by FAME analysis, as described by Thompson *et al.* (1993) and van der Gast *et al.* (2001). The samples were injected into a Hewlett Packard model 5890 series II gas chromatograph.

Fatty acid peaks were named by the Microbial Identification System (MIS) software (Microbial ID) and isolates were identified using the MIS 'Aerobe Library'.

Fluorescence *in situ* hybridization (FISH). A sample (10 ml) of each lake-water sample was filtered through a black polycarbonate 0.2 µm screen membrane filter (Poretics). Cells were fixed with 2 ml 4% paraformaldehyde in PBS for 30 min. A gentle vacuum was then applied and cells were rinsed, initially in 5 ml PBS, then in 5 ml distilled water. Filters were removed from the filtration apparatus, air-dried, placed on a glass microscope slide and stored at -20 °C. Five different *in situ* hybridization probes were used specific for the domain *Bacteria*, the α -, β - and γ -subclasses of the *Proteobacteria* and the *Cytophaga-Flavobacterium* group as described by Pearce *et al.* (2003).

Denaturing gradient gel electrophoresis (DGGE). Enzymic amplification of 16S rDNA was performed on extracted community DNA using the Muyzer primer pair (Muyzer *et al.*, 1993) which amplifies corresponding *Escherichia coli* positions 341–534 bp of the 16S rRNA gene. A volume of 25 µl was separated by DGGE as described by Pearce (2000). The gel was run for 80 min at 60 °C and 10 V cm⁻¹, before staining for 45 min in a solution containing 0.5 µg ethidium bromide ml⁻¹. This was visualized on a UV trans-illuminator (UVP). Photographs were taken with a Gelcam (Polaroid) using Polaroid 665 professional positive/negative instant pack film. Gel images were analysed using Gelcompar II software (Applied Maths) and bands were determined using band recognition software. Bands were then excised, reamplified and cleaned using GFX PCR clean up columns (Pharmacia), ligated into the pGEMT-Easy vector (Promega) and transformed into competent JM109 cells as described for the rDNA clone libraries.

RESULTS

Physical and chemical analyses

Physical and chemical analyses showing the effects of nutrient enrichment, particularly on nitrogen and phosphorus concentrations, are given in Table 1.

Determination of total population density

Total bacterioplankton population density in Heywood Lake ranged from a mean of 9.57×10^6 ($\pm 1.98 \times 10^6$) DAPI stained cells ml⁻¹ at 1 m to 1.5×10^7 ($\pm 1.0 \times 10^6$) DAPI stained cells ml⁻¹ at 3 m.

Direct 16S rDNA sequencing from environmental clones

DNA from 60 environmental clones could be identified to within $\geq 97\%$ of a named genus based on sequence comparison of 16S rRNA genes. Using this information, 73.3% of the bacteria were affiliated with the β -*Proteobacteria*, 15.0% with the α -*Proteobacteria* and 11.8% with the *Actinobacteria*. The specific taxa found were the β -*Proteobacteria* genera *Acidovorax*, *Alcaligenes*, *Bordetella*, *Janthinobacterium*, *Polaromonas*, *Polynucleobacter*, *Pseudomonas* (*mephitica*), *Rhodferax* and *Variovorax*, the α -*Proteobacteria* genera *Caulobacter*, *Rhodobacter* and *Sphingomonas*, and the *Actinobacteria* taxa *Aeromicrobium*, *Cryobacterium*, *Frigoribacterium* and *Actinomyces* (Table 2). Coverage was estimated to be 71.7% (Good, 1953).

Table 1. Signy lake characteristics, physical and chemical analyses

All values are the mean over a depth profile during holomixis (13 January 2000) $\pm$ SD. Abbreviation: PAR, photosynthetically active radiation.

Character	Heywood	Sombre	Moss
Reference	This study	Pearce <i>et al.</i> (2003)	Pearce (2003)
Area (ha)	4.5	2.7	1.5
Volume ($10^{-3} \times \text{m}^3$)	96	133	52
Depth (m)	6	11	10
Distance from the sea (m)	200	50	800
Height above sea level (m)	4	10	48
Fur seal activity in the lake	High	Some	None
Nitrate ($\mu\text{g l}^{-1}$)	1180 ± 50	185 ± 7	79 ± 5
Ammonium ($\mu\text{g l}^{-1}$)	161 ± 6	31 ± 4	38 ± 2
DRP ($\mu\text{g l}^{-1}$)	124 ± 5	7 ± 3	8 ± 2
TDP ($\mu\text{g l}^{-1}$)	157 ± 19	10 ± 3	15 ± 8
pH	6.5	6.7	6.1
Alkalinity (meq l^{-1})	0.1	0.2	0.1
Chlorophyll <i>a</i> ($\mu\text{g l}^{-1}$)	7 ± 1	11 ± 1	2
Chloride (mg l^{-1})	35 ± 4	34 ± 9	28 ± 3
Silicate ($\mu\text{g l}^{-1}$)	167 ± 2	296 ± 7	118 ± 2
Temperature (°C)	4	3	2
Dissolved oxygen (mg l^{-1})	3	13	13
Conductivity ($\mu\text{S cm}^{-1}$)	160	157	134
PAR (% transmission)	12	15	23

Table 2. Heywood Lake clone 16S rRNA gene sequences from extracted environmental DNA

Abbreviations: Lg, fragment length; Sm, similarity.

Heywood Lake clone accession no.	Heywood Lake clone closest phylogenetic affiliation following BLAST search against the EMBL database	Accession no. of BLAST match	Lg (bp)	Sm (%)	Class/phylum	Heywood Lake clone closest phylogenetic affiliation to a named taxon
(a) Matches $\geq 97\%$ similarity						
AJ878636	Unidentified eubacterium GKS16	AJ224987	688	99	β -Proteobacteria	<i>Variovorax</i>
AJ878625	β -Proteobacterium Wuba72	AF336361	646	99	β -Proteobacteria	<i>Acidovorax</i>
AM049199	β -Proteobacterium MWH-MoK4	AJ550654	685	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049186	β -Proteobacterium MWH-MoK4	AJ550654	685	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049187	β -Proteobacterium MWH-MoK4	AJ550654	685	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049188	β -Proteobacterium MWH-MoK4	AJ550654	673	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049189	β -Proteobacterium MWH-MoK4	AJ550654	677	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049190	β -Proteobacterium MWH-MoK4	AJ550654	658	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049195	β -Proteobacterium MWH-MoK4	AJ550654	667	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049192	β -Proteobacterium MWH-MoK4	AJ550654	675	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049197	Uncultured bacterium FukuS99	AJ290020	673	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049193	Uncultured bacterium FukuS99	AJ290020	678	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049200	Uncultured bacterium FukuN33	AJ289997	673	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049284	<i>Polynucleobacter necessarius</i>	X93019	665	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049178	<i>Polynucleobacter necessarius</i>	X93019	666	99	β -Proteobacteria	<i>Polynucleobacter</i>
AM049229	β -Proteobacterium HTCC528	AY584576	657	99	β -Proteobacteria	<i>Polaromonas</i>
AM049226	β -Proteobacterium HTCC528	AY584576	662	98	β -Proteobacteria	<i>Polaromonas</i>
AM049227	β -Proteobacterium HTCC528	AY584576	678	98	β -Proteobacteria	<i>Polaromonas</i>
AM049228	β -Proteobacterium HTCC528	AY584576	689	98	β -Proteobacteria	<i>Polaromonas</i>
AM049230	β -Proteobacterium HTCC528	AY584576	673	98	β -Proteobacteria	<i>Polaromonas</i>
AM049231	β -Proteobacterium HTCC528	AY584576	685	98	β -Proteobacteria	<i>Polaromonas</i>
AM049232	β -Proteobacterium HTCC528	AY584576	683	98	β -Proteobacteria	<i>Polaromonas</i>
AM049194	Uncultured bacterium FukuS99	AJ290020	676	98	β -Proteobacteria	<i>Polynucleobacter</i>
AJ878613	<i>Polynucleobacter necessarius</i>	X93019	681	98	β -Proteobacteria	<i>Polynucleobacter</i>
AM049179	<i>Polynucleobacter necessarius</i>	X93019	667	98	β -Proteobacteria	<i>Polynucleobacter</i>
AM049180	<i>Polynucleobacter necessarius</i>	X93019	684	98	β -Proteobacteria	<i>Polynucleobacter</i>
AM049181	<i>Polynucleobacter necessarius</i>	X93019	662	98	β -Proteobacteria	<i>Polynucleobacter</i>
AM049182	<i>Polynucleobacter necessarius</i>	X93019	684	98	β -Proteobacteria	<i>Polynucleobacter</i>
AM049185	<i>Polynucleobacter necessarius</i>	X93019	663	98	β -Proteobacteria	<i>Polynucleobacter</i>
AM049183	<i>Polynucleobacter necessarius</i>	X93019	686	98	β -Proteobacteria	<i>Polynucleobacter</i>
AJ878615	<i>Janthinobacterium lividum</i>	AF174648	747	98	β -Proteobacteria	<i>Janthinobacterium</i>
AM049184	<i>Bordetella trematum</i> DSM 11334 ^(T)	AJ277798	673	98	β -Proteobacteria	<i>Bordetella</i>
AM049210	<i>Bordetella trematum</i>	AJ27779	683	98	β -Proteobacteria	<i>Bordetella</i>
AM049211	Unidentified eubacterium GKS98	AJ224990	660	98	β -Proteobacteria	<i>Bordetella</i>
AJ878633	Uncultured β -proteobacterium Elb24	AJ421913	678	98	β -Proteobacteria	<i>Rhodoferrax</i>
AM049233	<i>Pseudomonas mephitica</i> ATCC 33665 ^T	AB021388	744	98	β -Proteobacteria	<i>Pseudomonas</i>
AM049201	β -Proteobacterium MWH-MoK4	AJ550654	643	97	β -Proteobacteria	<i>Polynucleobacter</i>
AM049236	<i>Janthinobacterium</i> sp. HHS6	AJ846271	467	97	β -Proteobacteria	<i>Janthinobacterium</i>
AM049225	β -Proteobacterium HTCC528	AY584576	681	97	β -Proteobacteria	<i>Polaromonas</i>
AM049224	Uncultured rape rhizosphere bacterium wr0041	AJ295499	785	97	β -Proteobacteria	<i>Polaromonas</i>
AM049234	<i>Pseudomonas mephitica</i> ATCC 33665 ^T	AB021388	795	97	β -Proteobacteria	<i>Pseudomonas</i>
AJ878624	<i>Pseudomonas mephitica</i> ATCC 33665 ^T	AB021388	804	97	β -Proteobacteria	<i>Pseudomonas</i>
AM049207	Unidentified eubacterium GKS98	AJ224990	670	97	β -Proteobacteria	<i>Alcaligenes</i>
AJ878620	Unidentified eubacterium GKS98	AJ224990	672	97	β -Proteobacteria	<i>Alcaligenes</i>
AM049217	Marine arctic deep-sea bacterium FD10	AJ557858	762	99	α -Proteobacteria	<i>Sphingomonas</i>
AJ878617	Marine arctic deep-sea bacterium FD10	AJ557858	751	98	α -Proteobacteria	<i>Sphingomonas</i>
AM049218	Marine arctic deep-sea bacterium FD10	AJ557858	764	98	α -Proteobacteria	<i>Sphingomonas</i>
AM049215	Antarctic bacterium R-8358	AJ440993	786	98	α -Proteobacteria	<i>Caulobacter</i>
AM049213	Antarctic bacterium R-8358	AJ440993	778	98	α -Proteobacteria	<i>Caulobacter</i>

Table 2. cont.

Heywood Lake clone accession no.	Heywood Lake clone closest phylogenetic affiliation following BLAST search against the EMBL database	Accession no. of BLAST match	Lg (bp)	Sm (%)	Class/phylum	Heywood Lake clone closest phylogenetic affiliation to a named taxon
AM049216	Marine arctic deep-sea bacterium FD10	AJ557858	814	97	α -Proteobacteria	<i>Sphingomonas</i>
AJ878623	<i>Caulobacter</i> sp. CB66	AJ227788	813	97	α -Proteobacteria	<i>Caulobacter</i>
AM049212	Uncultured rape rhizosphere bacterium wr0066	AJ295562	725	97	α -Proteobacteria	<i>Caulobacter</i>
AJ878632	Aquatic bacterium R1-A7	AB195725	678	97	α -Proteobacteria	<i>Rhodobacter</i>
AJ878614	<i>Aeromicrobium marinum</i>	AY166703	774	97	Actinobacteria	<i>Aeromicrobium</i>
AM049204	<i>Aeromicrobium marinum</i>	AY166703	792	98	Actinobacteria	<i>Aeromicrobium</i>
AM049205	<i>Aeromicrobium marinum</i>	AY166703	791	98	Actinobacteria	<i>Aeromicrobium</i>
AM049202	<i>Aeromicrobium marinum</i>	AY166703	813	97	Actinobacteria	<i>Aeromicrobium</i>
AJ878618	<i>Actinomycetales</i>	AY145533	688	97	Actinobacteria	<i>Actinomycetales</i>
AJ878627	<i>Cryobacterium psychrophilum</i>	AJ297438	770	97	Actinobacteria	<i>Cryobacterium</i>
AJ878631	Antarctic bacterium R-9112	AJ441003	787	97	Actinobacteria	<i>Frigoribacterium</i>
(b) Matches <97% similarity						
AM049238	Unidentified β -Proteobacterium HTC018	AB010865	491	96	β -Proteobacteria	<i>Janthinobacterium</i>
AM049221	Uncultured rape rhizosphere bacterium wr0041	AJ295499	762	96	β -Proteobacteria	<i>Polaromonas</i>
AM049223	Uncultured rape rhizosphere bacterium wr0041	AJ295499	811	96	β -Proteobacteria	<i>Polaromonas</i>
AM049209	<i>Bordetella trematum</i> DSM 11334 ^(T)	AJ277798	681	96	β -Proteobacteria	<i>Bordetella</i>
AM049222	Unidentified eubacterium GKS16	AJ224987	809	96	β -Proteobacteria	<i>Polaromonas</i>
AM049206	Uncultured bacterium FukuS93	AJ290018	675	96	β -Proteobacteria	<i>Alcaligenes</i>
AM049191	β -Proteobacterium MWH-MoK4	AJ550654	693	96	β -Proteobacteria	<i>Polynucleobacter</i>
AM049208	Uncultured bacterium FukuN65	AJ290001	389	96	β -Proteobacteria	<i>Alcaligenes</i>
AM049239	<i>Pseudomonas mephitica</i> ATCC 33665 ^T	AB021388	795	96	β -Proteobacteria	<i>Pseudomonas</i>
AJ878622	Uncultured bacterium FukuS93	AJ290018	674	95	β -Proteobacteria	<i>Achromobacter</i>
AM049237	<i>Janthinobacterium</i> sp. HHS6	AJ846271	622	95	β -Proteobacteria	<i>Janthinobacterium</i>
AM049235	<i>Janthinobacterium</i> sp. J43	AJ864852	495	94	β -Proteobacteria	<i>Janthinobacterium</i>
AM049198	Uncultured bacterium FukuN33	AJ289997	678	93	β -Proteobacteria	<i>Polynucleobacter</i>
AM049196	Uncultured bacterium FukuN33	AJ289997	676	93	β -Proteobacteria	<i>Polynucleobacter</i>
AM049220	<i>Sphingobium yanoikuyae</i>	AB120764	464	96	α -Proteobacteria	<i>Sphingobium</i>
AJ878626	<i>Afipia massiliensis</i>	AY029562	676	95	α -Proteobacteria	<i>Afipia</i>
AM049214	<i>Caulobacter</i> sp. CB66	AJ227788	456	95	α -Proteobacteria	<i>Caulobacter</i>
AM049219	<i>Sphingomonas</i> sp. EVA17	AY212804	541	94	α -Proteobacteria	<i>Sphingomonas</i>
AJ878634	<i>Sphingopyxis macrogoltabida</i> HXN-200	AJ784806	151	88	α -Proteobacteria	<i>Sphingopyxis</i>
AM049203	<i>Aeromicrobium marinum</i>	AY166703	730	96	Actinobacteria	<i>Aeromicrobium</i>
AM049241	<i>Actinomycetales</i> bacterium GP-5	AY145533	681	96	Actinobacteria	<i>Actinomycetales</i>
AJ878619	Unidentified bacterium rJ14	AB021332	676	94	Actinobacteria	<i>Actinoplanes</i>
AM049240	Unidentified bacterium rJ7	AB021325	659	92	Actinobacteria	<i>Actinomycetales</i>
AJ878635	Unidentified bacterium rJ7	AB021325	519	89	Actinobacteria	<i>Sporichthya</i>
AJ878630	<i>Frankia</i> sp. HR27-14	L40617	525	87	Actinobacteria	<i>Frankia</i>
AJ878629	<i>Flavobacterium xinjiangense</i> As1.2748	AF433172	182	95	Bacteroidetes	<i>Flavobacterium</i>
AJ878628	<i>Flavobacterium</i> sp. R-21935	AJ786797	470	77	Bacteroidetes	<i>Flavobacterium</i>
AJ878637	Uncultured bacterium FukuN18	AJ289992	658	91	Verrucomicrobia	<i>Verrucomicrobium</i>

FAME analysis

Using FAME analysis, 82.7% of bacteria were identified as *Actinobacteria*, 6.9% as *Firmicutes*, 6.9% as γ -*Proteobacteria* and 3.4% as α -*Proteobacteria*. The specific genera found were the *Actinobacteria* genera *Arcanobacterium*, *Cellulomonas*, *Micrococcus*, *Rhodococcus* and *Tsukamurella*, the *Firmicutes* genus *Staphylococcus*, the γ -*Proteobacteria* genera *Acinetobacter* and *Stenotrophomonas* and the α -*Proteobacteria*

genus *Sphingomonas*. Perhaps surprisingly, given their overall dominance in freshwaters, the β -*Proteobacteria* were not identified in Heywood Lake samples using FAME analysis. Isolation frequencies are given in Fig. 3.

FISH

With a set of four probes for the major divisions within the domain *Bacteria*, it was possible to affiliate between 47.7 and

Fig. 3. Isolation frequency of different genera by FAME analyses of cultured strains isolated from Heywood Lake.

64.5 % of the EUB338-hybridized cells with known bacterial groups. With the exception of the 3 m sample, the β -*Proteobacteria* was clearly the most abundant group at all depths sampled and the α -*Proteobacteria* was the least abundant group at most depths studied (Fig. 4).

DGGE

DGGE analysis of Heywood Lake water at vertical depth intervals produced 13 distinct DGGE bands, with the exception of the bottom level water which may have included some sediment, suggesting the presence of up to 13 potentially dominant bacterioplankton groups (Fig. 5). The clones derived from DGGE products were dominated by operational taxonomic units belonging to uncultured bacteria, including representatives of the *Actinobacteria* (53.8 %), the *Bacteroidetes* (23.1 %), the Gram-positives (15.4 %) and the β -*Proteobacteria* (7.7 %). The specific clones identified were the actinobacterium MWH-VicMua1 and six uncultured

Fig. 5. DGGE profile of Heywood Lake during a period of holomixis. The DGGE image was captured as a TIFF file, which was then analysed with Gelcompar II (Applied Maths). All clear DGGE bands, representing either individual DNA fragments or groups of DNA fragments, were scored independently when recognized by the band recognition search in Gelcompar II. Abbreviations: D4, 9 February 2000; H, Heywood Lake; SF, surface sample; B, bottom sample. All numbers refer to the depth at which the respective sample was taken. H3 samples were excised for sequencing.

actinobacterium clones, including CL500-95, FBP402, TLM06, CLO-29, c270 and an unidentified clone. Within the *Bacteroidetes*-specific clones were *Flavobacterium xinjiangense* and an unidentified *Cytophagales* sp.; within the β -*Proteobacteria* was *Pelistega europaea*, and within the Gram-positives were uncultured GOBB3-CL171 and Z39. Significantly, a clone from Moss Lake DGGE band 1 (UBA520090) was also identified (Table 3).

DISCUSSION

Nutrient enrichment and bacterioplankton population density

Nutrient concentrations in Heywood Lake were significantly higher than in neighbouring oligotrophic Moss and Sombre

Fig. 4. Distribution of key bacterioplankton groups in Heywood Lake with depth, as determined by FISH. Error bars represent SD ($n=10$). Black bars, α -*Proteobacteria*; light grey bars, β -*Proteobacteria*; dark grey bars, γ -*Proteobacteria*; white bars, *Cytophaga*-*Flavobacterium* group.

Table 3. Identification of cloned and sequenced DGGE bands from a depth of 3 m in Heywood Lake

Abbreviations: Lg, fragment length; Sm, similarity; Uncl., unclassified.

DGGE band sequence accession no.	Moss Lake DGGE band closest phylogenetic affiliation	Accession no.	Lg (bp)	Sm (%)	Class/phylum	Closest phylogenetic affiliation with a named taxon	Source of matches
AJ865590	Actinobacterium MWH-VicMua1	AJ565417	156	98	Actinobacteria	<i>Microbacterium</i> sp.	Freshwater
AJ865591	<i>Flavobacterium xinjiangense</i>	AF433172	190	99	Bacteroidetes	<i>Flavobacterium</i> sp.	Glacier
AJ865592	Uncultured bacterium CL500-95	AF316665	158	99	Actinobacteria	Actinomycete	Crater lake
AJ865593	<i>Pelistega europaea</i>	UE16SRR	113	91	β -Proteobacteria	<i>Neisseria</i> sp.	Avian pathogen
AJ865594	Uncultured actinobacterium clone FBP402	AY250880	170	94	Actinobacteria	<i>Micrococcineae</i> sp.	Antarctic
AJ865595	Uncultured actinobacterium clone TLM06	AF534430	175	99	Actinobacteria	<i>Kineococcus</i> -like sp.	Arctic lake
AJ865596	Uncultured actinobacterium	AY562361	123	100	Actinobacteria	<i>Tetrasphaera</i> sp.	Delaware river
AJ865597	Uncultured bacterium CL0-29	AF316680	102	98	Actinobacteria	<i>Rhodococcus</i> sp.	Crater lake
AJ865598	Uncultured bacterium clone c270	AY154621	157	98	Actinobacteria	<i>Kineococcus</i> -like sp.	Earthworm cast
AJ865599	Uncultured firmicute GOBB3-CL171	AF388895	164	96	Uncl. Gram +ve	Actinomycete	Estuary
AJ865600	Moss Lake DGGE band 1	UBA520090	169	100	Bacteroidetes	<i>Flavobacterium</i> sp.	Adjacent lake
AJ865601	Uncultured bacterium clone Z39	AF488674	107	96	Uncl. Gram +ve	<i>Tetrasphaera</i> sp.	Freshwater
AJ865602	Unidentified cytophagales bacterium	AJ007870	121	91	Bacteroidetes	<i>Cytophaga</i> sp.	Swedish lake

Lakes. In particular, there were large differences in the concentrations of nitrogen and phosphorus (Table 1). Heywood Lake supported a higher bacterioplankton population density, with between 50- and 15-times the population densities in Moss and Sombre lakes, respectively.

Similarities with adjacent oligotrophic lakes

Overall, the bacterioplankton community in Heywood Lake was dominated by genera with characteristics that included the ability to utilize a wide variety of organic compounds and involvement in the mineralization of organic matter, they were all aerobic (or facultatively anaerobic), they contained species which produced pigment and were widely distributed globally in fresh waters. All of these properties are normally associated with oligotrophic aerobic pelagic environments (Pearce, 2003; Pearce *et al.*, 2003). The genera identified in Heywood Lake through 16S rDNA sequencing or FAME analysis that were also present in oligotrophic Sombre Lake (Pearce *et al.*, 2003) were *Acidovorax*, *Arcanobacterium*, *Bordetella*, *Cellulomonas*, *Flavobacterium*, *Janthinobacterium*, *Micrococcus*, *Pseudomonas*, *Rhodobacter*, *Sphingomonas* and *Staphylococcus*. This represents a Sørensen coefficient of 0.20, or 42.3% of the genera identified in this study.

Differences from adjacent oligotrophic lakes

Bacterioplankton taxa identified in nutrient-enriched Heywood Lake from direct 16S rDNA sequencing or FAME analysis included: *Acinetobacter*, *Actinobacteria*, *Actinomycetales*, *Aeromicrobium*, *Caulobacter*, *Cryobacterium*, *Frigoribacterium*, *Pelistega*, *Polaromonas*, *Polynucleobacter*, *Rhodococcus*, *Rhodoferrax*, *Stenotrophomonas*, *Tsukamurella*

and *Variovorax*. These groups were not found in the neighbouring oligotrophic lakes.

Heywood Lake bacterioplankton was dominated by bacteria most of which have yet to be cultivated (Table 3). Of the 13 dominant groups identified using DGGE, eight were closely related to uncultivated bacteria in the database, three were identified to genus level and only two could be identified to species level. This pattern is consistent with oligotrophic Moss and Sombre Lakes, and at least three of the dominant groups in Heywood Lake were identical to dominant groups in the oligotrophic systems. However, in nutrient-enriched Heywood Lake, there was a larger number of dominant groups: Moss Lake 9, Sombre Lake 8 and Heywood Lake 13.

Cyanobacteria

DGGE suggested the importance of the *Actinobacteria* and, unlike both of the oligotrophic lakes used for comparison, none of the dominant bacterioplankton were *Cyanobacteria*. This is unusual, as many of the microbial communities in non-marine ecosystems within polar regions are dominated by *Cyanobacteria*, with populations that are amongst the highest recorded in any natural environment (Vincent *et al.*, 2000). This observation is supported by the dominance (as determined by DGGE) of *Nostoc* sp. in Moss Lake (Pearce, 2003) and an uncultured cyanobacterium clone in Sombre Lake (Pearce *et al.*, 2003). The lack of cyanobacterial dominance might be linked to the high nitrogen concentration associated with fur seal enrichment, as relatively high N:P ratios in nutrient-rich lakes tend to favour non-cyanobacterial taxa (Smith, 1983, 1990). This might also be due to the observation that pico-cyanobacteria have a high nutrient-scavenging ability and can be an important part of the pelagic community in terms of biomass in more

oligotrophic conditions (Stockner, 1988; Stockner *et al.*, 2000).

Marine species

A particularly interesting feature of the Heywood Lake bacterioplankton was the presence of psychrophilic marine bacteria, including *Polaromonas vacuolata* (Irgens *et al.*, 1996) and *Rhodobacter sphaeroides* (Pinhassi *et al.*, 1997). In addition, the α -*Proteobacteria* were significant and, though not exclusively, are more traditionally associated with marine environments. This is probably due to the close proximity of the lake to the sea and frequent visits by marine mammals and birds. Although we cannot be certain that the marine bacteria were active within the lake, the relatively high levels of α -*Proteobacteria*, as determined by FISH, suggested that they might be (as the probes target rRNA). A similar phenomenon was observed by Bell & Laybourn-Parry (1999) in nutrient-enriched Rookery Lake, where the ciliate community resembled the marine community and was dissimilar to that found in older saline lakes within the Vesthold Hills.

Ubiquity of bacterioplankton genera

Although the Heywood Lake bacterioplankton community was not consistent with neighbouring oligotrophic lakes, many of the genera isolated from Heywood Lake represented ubiquitous aquatic bacteria and the bacteria most common in aquatic habitats, such as Gram-negative rods, particularly *Pseudomonas* and *Flavobacterium*. In addition, surface films formed during calm weather on lakes and ponds often comprise *Pseudomonas*, *Caulobacter*, *Flavobacterium* and *Micrococcus*. Significantly, many of the genera found have been identified in freshwater systems elsewhere (Table 4), including nutrient-enriched systems in temperate areas such as soil, marine, alkaline, sedimentary and estuarine systems, and as such it might be more appropriate, as suggested by Reynolds *et al.* (2002) for the phytoplankton and by Yannarell *et al.* (2003) for the bacterioplankton, to develop and use a functional classification of these aquatic micro-organisms.

Specific examples of the common occurrence of key genera identified in Heywood Lake are *Flavobacterium xinjiangense* and members of the *Actinobacteria*. *Flavobacterium xinjiangense*, widely distributed in fresh and marine waters (Zhu *et al.*, 2003) was also found in both Moss Lake (Pearce, 2003) and Sombre Lake (Pearce *et al.*, 2003). The *Flavobacteria* show a high abundance in aquatic ecosystems and these heterotrophic bacteria may have a specialized role in uptake and degradation of the high-molecular-mass fraction of dissolved organic matter in these environments (Kirchman, 2002). In addition, the predominant group, the uncultured *Actinobacteria*, have been described in freshwater habitats in Europe and Asia (Hahn, 2003). The ecological implications of this observation are that freshwater ecosystems contain similar bacterioplankton at the genus level, but that at the

Table 4. A comparison of taxa identified in Heywood Lake, by FAME, DGGE and 16S rRNA gene sequence analysis (where clones have $\geq 97\%$ similarity with the named taxon) to lake systems elsewhere

Abbreviations: s, Sombre Lake, Signy Island (Pearce *et al.*, 2003); a, Adirondack Mountain lakes (Hiorns *et al.*, 1997); b, Lake Baikal (Korsunov *et al.*, 2003); g, oligotrophic high mountain Lake Gossenköllesee, mesotrophic Lake Fuchskuhle and Lake Baikal (Glöckner *et al.*, 2000); l, Bugach and Lesnoi turbid Siberian ponds (Trusova & Gladyshev, 2002); o, oligotrophic crater lake, Oregon, USA (Urbach *et al.*, 2001); p, eutrophic priest pot (Finlay & Maberly, 2000); z, typical freshwater bacteria (Zwart *et al.*, 2002).

Taxon	s	a	b	g	l	o	p	z
<i>Acidovorax</i>	■				■			
<i>Acinetobacter</i>			■		■	■	■	
<i>Actinomycetales</i>								
<i>Aeromicrobium</i>								
<i>Arcanobacterium</i>	■							
<i>Actinobacteria</i>								
<i>Bordetella</i>	■							
<i>Caulobacter</i>		■	■		■			■
<i>Cellulomonas</i>	■					■		
<i>Cryobacterium</i>								
<i>Flavobacterium</i>	■	■	■	■	■		■	■
<i>Frigoribacterium</i>								
<i>Janthinobacterium</i>	■						■	
<i>Micrococcus</i>	■	■	■				■	■
<i>Polaromonas</i>						■		
<i>Polynucleobacter</i>				■		■		■
<i>Pseudomonas</i>	■	■	■		■		■	■
<i>Rhodobacter</i>	■			■				
<i>Rhodococcus</i>							■	
<i>Rhodoferrax</i>		■		■		■		■
<i>Sphingomonas</i>	■			■	■		■	■
<i>Staphylococcus</i>	■							
<i>Stenotrophomonas</i>								
<i>Tsukamurella</i>								
<i>Variovorax</i>		■		■	■		■	■

species level, differences related to the specific environment do occur.

Reduction in species richness

Using the Simpson diversity index (Simpson, 1949), from 16S rRNA gene sequences Heywood Lake has a lower bacterioplankton biodiversity at 0.92 than Sombre Lake at 0.94. The probability of selecting a clone with a new sequence from a random sample was 10.3% (1:10) for Heywood Lake and 16.0% (1:6) for Sombre Lake.

Increasing evenness

Lindström & Leskinen (2002) cite several investigations of bacterioplankton diversity in aquatic environments with

results indicating that although the species richness of these communities is high, only a few taxa (<20) dominate. In this study a larger number of dominant groups were detected than in the adjacent oligotrophic lakes, suggesting nutrient enrichment has led to greater evenness among dominant bacterioplankton. The use of FISH showed that the β -*Proteobacteria* was clearly the most abundant group, followed by the *Cytophaga*–*Flavobacterium* group, the γ -*Proteobacteria* and the α -*Proteobacteria* at most depths studied (Fig. 4). The β -*Proteobacteria*, however, were not as large a component of the Heywood Lake bacterioplankton as found in the Moss and Sombre Lake bacterioplankton and at a number of depths the *Cytophaga*–*Flavobacterium* were either equally or more abundant. In proportion to the overall population size, the γ -*Proteobacteria* and the α -*Proteobacteria* were also relatively more numerous, suggesting that the species evenness of Heywood Lake is higher than that of Moss or Sombre Lakes (Pearce, 2003; Pearce *et al.*, 2003).

Dominant bacterioplankton groups and methodology

The complementary results from each of the different methods stressed the importance of a polyphasic approach. 16S sequence analysis of two-thirds of the predicted diversity present suggested over 70 % of the sequences detected were from β -*Proteobacteria*, 15 % from α -*Proteobacteria* and 11 % from *Actinobacteria*. FAME analysis indicated that 80 % of cultured isolates were *Actinobacteria*, but as this technique is culture-dependent, this may indicate the *Actinobacteria* are taken into culture more readily, suggesting that they might be more resistant to environmental changes or have wider environmental tolerance limits. FISH indicated a predominance of β -*Proteobacteria*, followed by *Cytophaga*–*Flavobacterium*, γ -*Proteobacteria* and α -*Proteobacteria* (unfortunately we did not have access to an *Actinobacteria*-specific probe in the field), whilst over 50 % of DGGE bands were *Actinobacteria*, 23 % *Bacteroidetes* and 8 % β -*Proteobacteria*. Taken together, these results suggest that there is much greater biodiversity among the β -*Proteobacteria*, but that a small number of key *Actinobacteria* dominate the Heywood Lake bacterioplankton community.

Shift in dominance with trophic status

A key observation from this study was an apparent shift in dominance with trophic status. Moss Lake was dominated by the *Proteobacteria* (5/9 DGGE bands), Sombre Lake showed no clear dominance, with equal numbers of *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*, whilst the *Actinobacteria* dominated in Heywood Lake (7/13 DGGE bands), indicating a potential shift in dominant groups from β -*Proteobacteria* to *Actinobacteria* with increasing nutrient enrichment. The *Actinobacteria* are widespread and abundant in several lake systems.

Resistant groups

In Heywood Lake, three of the dominant groups were found to be dominant in neighbouring oligotrophic systems, suggesting that there are indeed important freshwater groups, or alternatively that there is a species-specific resistance to environmental perturbation in the form of nutrient enrichment. This is supported by the observation that Heywood Lake has a diversity of bacterioplankton which differs markedly from its neighbours, but this diversity does include some of the genera found in the oligotrophic systems, thus suggesting that specific groups may have a wider tolerance to environmental change.

Conclusion

Changes in the utilization of catchments by marine mammals or other perturbations of nutrient supply are likely to result in significant changes in the nutrient status and bacterioplankton communities of Antarctic lake systems. Nutrient-enriched Heywood Lake has a high bacterioplankton population density, reduced species richness and increasing evenness. Less than half of the genera found were also present in neighbouring oligotrophic lakes, including three of the dominant groups. A comparison of the three lakes with different nutrient levels suggested an apparent shift in dominance with trophic status. Other key observations included the absence of dominant *Cyanobacteria* and the presence of marine bacteria. Overall, the bacterioplankton community of Heywood Lake was distinct and differed markedly from its more oligotrophic neighbours. This significant impact of nutrient enrichment on Heywood Lake bacterioplankton communities, therefore, suggests that low-temperature oligotrophic freshwater lake systems may have low resistance to environmental change.

ACKNOWLEDGEMENTS

This research was supported by the Natural Environment Research Council through the British Antarctic Survey as part of the Terrestrial and Freshwater Biodiversity Project within the Antarctic Biodiversity, Past, Present and Future ASGC Programme. We would particularly like to thank Matt Edworthy for assistance in the field and for chemical analyses, Mairi Nicolson for assistance with sequencing and the many base members involved in the fur seal surveys. FAME analysis was supported by the Natural Environment Research Council through the Centre for Ecology and Hydrology, Oxford. We would also like to thank Cynan Ellis-Evans, Alex Rogers and the reviewers for helpful comments on the manuscript.

REFERENCES

- Altschul, S. F., Madden, T., Schäffer, A. A., Zhang, J., Zhang, Z. W., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Andrade, D. S., Murphy, P. J. & Giller, K. E. (2002). The diversity of *Phaseolus*-nodulating rhizobial populations is altered by liming of acid soils planted with *Phaseolus vulgaris* L. in Brazil. *Appl Environ Microbiol* **68**, 4025–4034.

- Bell, E. M. & Laybourn-Parry, J. (1999). The plankton community of a young, eutrophic, Antarctic saline lake. *Polar Biol* **22**, 248–253.
- Bester, M. N., Ryan, P. G. & Dyer, B. M. (2003). Population numbers of fur seals at Prince Edward Island, Southern Ocean. *Afr J Mar Sci* **25**, 549–554.
- Butler, H. G. (1999a). Seasonal dynamics of the planktonic microbial community in a maritime Antarctic lake undergoing eutrophication. *J Plankton Res* **21**, 2393–2419.
- Butler, H. G. (1999b). Temporal plankton dynamics in a maritime Antarctic lake. *Arch Hydrobiol* **146**, 311–339.
- Butler, H. G., Edworthy, M. G. & Ellis-Evans, J. C. (2000). Temporal plankton dynamics in an oligotrophic maritime Antarctic lake. *Freshw Biol* **43**, 215–230.
- Crump, B. C., Kling, G. W., Bahr, M. & Hobbie, J. E. (2003). Bacterioplankton community shifts in an Arctic Lake correlate with seasonal changes in organic matter source. *Appl Environ Microbiol* **69**, 2253–2268.
- D'Elia, C. F., Steudler, P. A. & Corwin, N. (1977). Determination of total nitrogen in aqueous samples using persulphate digestion. *Limnol Oceanogr* **22**, 760–764.
- Donner, G., Schwartz, K., Hoppe, H.-G. & Muyzer, G. (1996). Profiling the succession of bacterial populations in pelagic chemoclines. *Arch Hydrobiol Spec Issue Adv Limnol* **48**, 7–14.
- Drancourt, M., Bollet, C., Carliz, A., Martelin, R., Gayral, J. P. & Raoult, D. (2000). 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J Clin Microbiol* **38**, 3623–3630.
- Dunbar, J., Takala, S., Barns, S. M., Davis, J. A. & Kuske, C. R. (1999). Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. *Appl Environ Microbiol* **65**, 1662–1669.
- Ellis-Evans, J. C. (1982). Seasonal microbial activity in Antarctic freshwater lake sediments. *Polar Biol* **1**, 129–140.
- Ellis-Evans, J. C. (1990). Evidence for change in the chemistry of maritime Antarctic Heywood Lake. In *Antarctic Ecosystems, Ecological Change & Conservation*, pp. 77–82. Edited by K. R. Kerry & G. Hempel. Berlin: Springer.
- Finlay, B. J. & Maberly, S. C. (2000). *Microbial Diversity in Priest Pot: A Productive Pond in the English Lake District*. Edited by D. W. Sutcliffe. Ambleside: Freshwater Biological Association.
- Fogg, G. E. (1998). *The Biology of Polar Habitats*. Oxford: Oxford University Press.
- Glöckner, F. O., Zaichikov, E., Belkova, N., Denissova, L., Pernthaler, J., Pernthaler, A. & Amann, R. (2000). Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of Actinobacteria. *Appl Environ Microbiol* **66**, 5053–5065.
- Good, I. J. (1953). On the population frequencies of species and the estimation of population parameters. *Biometrika* **40**, 237–264.
- Hahn, M. W. (2003). Isolation of novel ultramicrobacteria classified as Actinobacteria from five freshwater habitats in Europe and Asia. *Appl Environ Microbiol* **69**, 1442–1451.
- Hawes, I. (1990). Eutrophication and vegetation development in maritime Antarctic lakes. In *Antarctic Ecosystems, Ecological Change & Conservation*, pp. 83–90. Edited by K. R. Kerry & G. Hempel. Berlin: Springer.
- Heywood, R. B., Dartnall, H. J. G. & Priddle, J. (1980). Characteristics and classification of the lakes of Signy Island, South Orkney Islands, Antarctica. *Freshw Biol* **10**, 47–59.
- Hiorns, W. D., Methé, B. A., Nierzwicki-Bauer, S. A. & Zehr, J. P. (1997). Bacterial diversity in Adirondack mountain lakes as revealed by 16S rRNA gene sequences. *Appl Environ Microbiol* **63**, 2957–2960.
- Irgens, R. L., Gosink, J. J. & Staley, J. T. (1996). *Polaromonas vacuolata* gen. nov., sp. nov. a psychrophilic, marine, gas vacuolated bacterium from Antarctica. *Int J Syst Bacteriol* **46**, 822–826.
- Izaguirre, I., Mataloni, G., Allende, L. & Vinocur, A. (2001). Summer fluctuations of microbial planktonic communities in a eutrophic lake – Cierva Point, Antarctica. *J Plankton Res* **23**, 1095–1109.
- Jones, V. J., Hodgson, D. A. & Chepstow-Lusty, A. (2000). Palaeolimnological evidence for marked Holocene environmental changes on Signy Island, Antarctica. *The Holocene* **10**, 43–60.
- Kirchman, D. L. (2002). The ecology of Cytophaga–Flavobacterium in aquatic environments. *FEMS Microbiol Ecol* **39**, 91–100.
- Korsunov, V. M., Namsaraev, B. B., Gonchikov, G. G., Kozireva, L. P., Zakiyan, S. M. & Zemskaya, T. I. (2003). *Phylogenetic diversity of extremophiles in Baikal region: ecological and biotechnological aspects*. http://www.bionet.nsc.ru/misc/ecopro/eng/1_12.html (accessed 8 May 2003).
- Laybourn-Parry, J., Ellis-Evans, J. C. & Butler, H. (1996). Microbial dynamics during the summer ice-loss phase in maritime Antarctic lakes. *J Plankton Res* **18**, 495–511.
- Lindström, E. S. (2000). Bacterioplankton community composition in five lakes differing in trophic status and humic content. *Microb Ecol* **40**, 104–113.
- Lindström, E. S. & Leskinen, E. (2002). Do neighbouring lakes share common taxa of bacterioplankton? Comparison of 16S rDNA fingerprints and sequences from three geographic regions. *Microbial Ecol* **44**, 1–9.
- Mackereth, F. J. H., Heron, J. & Talling, J. F. (1989). *Water Analysis: Some Revised Methods for Limnologists*. Ambleside: Freshwater Biological Association.
- Muyzer, G., de Waal, E. C. & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59**, 695–700.
- Noon, P. E., Leng, M. J., Arrowsmith, C., Edworthy, M. G. & Strachan, R. J. (2002). Seasonal observations of stable isotope variations in a valley catchment, Signy Island, South Orkney Islands. *Antarctic Sci* **14**, 333–342.
- Pearce, D. A. (2000). A rapid, sensitive method for monitoring bacterioplankton community dynamics, applied to Antarctic freshwater lakes. *Polar Biol* **23**, 352–356.
- Pearce, D. A. (2003). Bacterioplankton community structure in a maritime Antarctic oligotrophic lake during a period of holomixis, as determined by denaturing gradient gel electrophoresis (DGGE) and fluorescence *in situ* hybridisation (FISH). *Microb Ecol* **46**, 92–105.
- Pearce, D. A. (2005). The structure and stability of the bacterioplankton community in Antarctic freshwater lakes, subject to extremely rapid environmental change. *FEMS Microb Ecol* **53**, 61–72.
- Pearce, D. A., van der Gast, C., Lawley, B. & Ellis-Evans, J. C. (2003). Bacterioplankton community diversity in a maritime Antarctic lake, as determined by culture dependent and culture independent techniques. *FEMS Microbiol Ecol* **45**, 59–70.
- Pinhassi, J., Zweifel, U. L. & Hagström, A. (1997). Dominant marine bacterioplankton species found among colony-forming bacteria. *Appl Environ Microbiol* **63**, 3359–3366.
- Porter, K. G. & Feig, Y. S. (1980). The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* **25**, 943–948.
- Quayle, W. C., Peck, L. S., Peat, H., Ellis-Evans, J. C. & Harrigan, P. R. (2002). Extreme responses to climate change in Antarctic lakes. *Science* **295**, 645.

- Reynolds, C. S., Huszar, V., Kruk, C., Naselli-Flores, L. & Melo, S. (2002).** Towards a functional classification of the freshwater phytoplankton. *J Plankton Res* **24**, 417–428.
- Simpson, E. H. (1949).** Measurement of diversity. *Nature* **163**, 688.
- Smith, R. I. L. (1997).** Impact of an increasing fur seal population on Antarctic plant communities, resilience and recovery. In *Antarctic communities: Species, Structure & Survival*. Edited by B. Battaglia, J. Valencia & D. W. H. Walton. Cambridge: Cambridge University Press.
- Smith, V. H. (1983).** Low nitrogen to phosphorus ratios favour dominance by blue-green algae in lake phytoplankton. *Science* **221**, 669–671.
- Smith, V. H. (1990).** Nitrogen, phosphorus and nitrogen fixation in lacustrine and estuarine ecosystems. *Limnol Oceanogr* **35**, 1852–1859.
- Stockner, J. G. (1988).** Phototrophic picoplankton: an overview from marine and freshwater ecosystems. *Limnol Oceanogr* **33**, 765–775.
- Stockner, J. G., Callieri, C. & Cronberg, G. (2000).** Picoplankton and other non-bloom-forming Cyanobacteria in lakes. In *the Ecology of Cyanobacteria*, pp. 195–231. B. A. Whitton & M. Potts. The Netherlands: Kluwer.
- Thompson, I. P., Bailey, M. J., Ellis, R. J. & Purdy, K. J. (1993).** Subgrouping of bacterial populations by cellular fatty acid composition. *FEMS Microbiol Ecol* **102**, 75–84.
- Trusova, M. Y. & Gladyshev, M. I. (2002).** Phylogenetic diversity of winter bacterioplankton of eutrophic Siberian reservoirs as revealed by 16S rRNA gene sequences. *Microb Ecol* **44**, 252–259.
- Unrein, F. & Vinocur, A. (1999).** Phytoplankton structure and dynamics in a turbid Antarctic lake (Potter Peninsula, King George Island). *Polar Biol* **22**, 93–101.
- Urbach, E., Vergin, K. L., Young, L., Morse, A., Larson, G. L. & Giovannoni, S. J. (2001).** Unusual bacterioplankton community structure in ultra-oligotrophic Crater Lake. *Limnol Oceanogr* **46**, 557–572.
- van der Gast, C. J., Knowles, C. J., Wright, M. A. & Thompson, I. P. (2001).** Identification and characterisation of bacterial populations of an in-use metal-working fluid by phenotypic and genotypic methodology. *Int Biodeter Biodegr* **47**, 113–123.
- Vincent, W. F., Bowman, J. P., Rankin, L. M. & McMeekin, T. A. (2000).** Phylogenetic diversity of picocyanobacteria in Arctic and Antarctic ecosystems. In *Microbial Biosystems: New Frontiers. Proceedings of the 8th International Symposium on Microbial Ecology*, pp. 317–322. Edited by C. R. Bell, M. Brylinsky & P. Johnson-Green. Halifax, Canada: Atlantic Canada Society for Microbial Ecology.
- Welschmeyer, N. A. (1994).** Fluorimetric analysis of chlorophyll-*a* in the presence of chlorophyll-*b* and pheopigments. *Limnol Oceanogr* **39**, 1985–1992.
- Yannarell, A. C., Kent, A. D., Lauster, G. H., Kratz, T. K. & Triplett, E. W. (2003).** Temporal patterns in bacterioplankton communities in three temperate lakes of different trophic status. *Microb Ecol* **46**, 391–405.
- Zhu, F., Wang, S. & Zhou, P. J. (2003).** *Flavobacterium xinjiangense*, sp. nov. and *Flavobacterium omnivorum* sp. nov., novel psychrophiles from the China No.1 glacier. *Int J Syst Evol Microbiol* **53**, 853–857.
- Zwart, G., Crump, B. C., Kamst-Agterveld, M. P., Hagen, F. & Han, S.-K. (2002).** Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. *Aquat Microb Ecol* **28**, 141–155.