

Microbial dynamics and flagellate grazing during transition to winter in Lakes Hoare and Bonney, Antarctica

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Abstract

The planktonic microbial communities of Lakes Hoare and Bonney were investigated during transition into winter. We hypothesized that the onset of darkness induces changes in the functional role of autotrophic and heterotrophic microplankton. Bacteria decreased in Lake Hoare during March–April, while in Lake Bonney bacterial abundances varied. Heterotrophic nanoflagellates (HNAN), phototrophic nanoflagellates (PNAN) and ciliates showed no marked decline with the onset of winter. PNAN outnumbered HNAN in both lakes. Grazing rates of HNAN in Lake Hoare ranged up to 30.8 bacteria per cell day⁻¹. The HNAN community grazed between 3.74 and 36.6 ng of bacterial carbon day⁻¹. Mixotrophic PNAN had grazing rates up to 15.2 bacteria per cell day⁻¹, and their daily community grazing exceeded bacterial production. In Lake Bonney East, PNAN grazing rates ranged up to 12.48 bacteria per cell day⁻¹ and in Lake Bonney West up to 8.16 bacteria per cell day⁻¹. As in Lake Hoare, the mixotrophic PNAN grazing rates (up to 950 ng C day⁻¹) usually exceeded bacterial production. HNAN grazing rates were generally similar to those in Lake Hoare. As winter encroaches, these lakes move progressively towards heterotrophy and probably function during the winter, enabling populations to enter the short austral summer with actively growing populations.

Introduction

Antarctic lakes are arguably among the most extreme lacustrine ecosystems on the planet. They are characterized by constant low temperatures, low annual levels of photosynthetically active radiation (PAR) and truncated simplified microbial food webs (Laybourn-Parry *et al.*, 1991; Priscu *et al.*, 1999). Such environments are physiologically challenging to the species that have succeeded in colonizing their water columns and benthic zones, and consequently, survival strategies and annual patterns of productivity have been a focus of investigation. The lakes of the McMurdo Dry Valleys are far south (77–78°S) presenting a difficult logistic environment for fieldwork; the sun disappears below the horizon in late April and reappears in mid-August, and temperatures range from 0 in summer to –60 °C in winter (average annual temperature *c.* –20 °C). Moreover, the lakes are some 100 km

distant from McMurdo Station. As a consequence, the lakes have been mainly investigated in summer and on a few occasions during the late winter–spring transition. One of these early studies began on 9 September 1991 and revealed that phytoplankton photosynthesis in Lake Bonney had already begun in late winter before the first sampling date (Lizotte *et al.*, 1996; Priscu *et al.*, 1999). In these ice-covered lakes where there is no turbulence, the initiation of spring growth is a function of increases in PAR. In Vestfold Hills lakes (68°S) where year-long investigations are possible, photosynthesis starts as soon as the light returns (Bayliss *et al.*, 1997). However, the Vestfold Hills lakes have highly transparent annual ice around 2 m thick, whereas the Dry Valley lakes have perennial ice covers up to 4–5 m thick containing dust and debris, so that only between 0.5% and 3.3% of surface PAR penetrates the ice in the Taylor Valley lakes (Fryxell, Hoare and Bonney; Howard-Williams *et al.*, 1998).

Among the phototrophic nanoflagellates (PNAN) that dominate the phytoplankton communities of Antarctic lakes, cryptophytes are particularly conspicuous (Bielewicz *et al.*, 2011). In a number of the Dry Valley lakes (Lakes Fryxell and Hoare) and in brackish marine-derived lakes in the Vestfold Hills (68°S), cryptophytes engage in mixotrophy that appears to be related to the levels of PAR (Marshall & Laybourn-Parry, 2002; Laybourn-Parry *et al.*, 2005). In Lakes Fryxell and Hoare, the proportion of the carbon budget derived from grazing upon bacteria, as opposed to photosynthesis, was higher in November and declined in December towards mid-summer (Marshall & Laybourn-Parry, 2002). *Pyramimonas gelidicola* is also mixotrophic in Antarctic lakes (Bell & Laybourn-Parry, 2003). The genus has not been reported as mixotrophic at lower latitudes, so it is likely that many species exploit this strategy in these extreme lakes.

The current study covered the period of sharply declining PAR in Lakes Hoare and Bonney (March–early April 2008); PAR was barely detectable beneath the lake ice in early April. Our specific aim was to assess how abundances of the microbial community change as winter encroaches and how the PNAN respond to decreasing PAR. Do they increase their dependence on mixotrophy or do they adopt a strategy of entering resting stages? While lake communities of the Vestfold Hills maintain active communities of bacteria, ciliates and flagellates during winter (Bell & Laybourn-Parry, 1999; Laybourn-Parry *et al.*, 2005), these lakes are much further north (68°S) than those of the Dry Valleys and are climatically less extreme.

Methods

Study sites and sampling

Lake Hoare and Lake Bonney are situated in the Taylor Valley (77°43'S, 162°23'E). Lake Hoare has a maximum depth of 34 m, a surface area of 1.94 km² and a maximum conductivity near 0.113 S m⁻¹ (Spigel & Priscu, 1998). Lake Bonney West has an area of 0.99 km² and a maximum depth of 40 m, and Lake Bonney East an area of 3.32 km² and a maximum depth of 37 m. Lake Bonney is meromictic with strong chemical and physical gradients. Maximum conductivity at the bottom of Lake Bonney East is 18 S m⁻¹ and Lake Bonney West 15 S m⁻¹ (Spigel & Priscu, 1996, 1998). Lake Hoare and the two lobes of Lake Bonney were sampled with a 5-L Niskin bottle at their deepest points between 1 March and 5 April 2008 for bacteria and nanoflagellates and between mid-November 2007 and 5 April 2008 for ciliates. A further set of samples was collected on 10 April from Lake Bonney East for grazing experiments. Samples

were collected from 5/6, 12/13 and 20 m in Lake Hoare from 5, 10 and 13 m in Lake Bonney West and from 5/6, 13 and 20 m in Lake Bonney East. Samples for bacteria and nanoflagellates were fixed in glutaraldehyde to a final concentration of 2%. One-litre samples for ciliates, dinoflagellates and rotifers were fixed with Lugol's iodine. *In situ* PAR was measured using a LICOR L1-193 spherical quantum sensor moored 10 m beneath the surface of the ice. Data were logged at 20-min intervals throughout the year with a Campbell CR 10× data logger located on the surface of the lake.

Analysis of samples

Bacterial abundance was determined by staining with SYBR gold (Molecular Probes, Invitrogen, Paisley, UK) after 10-mL aliquots were filtered onto 0.2-µm black polycarbonate filters. Enumeration was carried out under epifluorescence microscopy using a blue filter at ×1000. Bacterial cell volumes were determined from measurements on 50 cells. A carbon conversion figure of 0.22 pg carbon µm⁻³ was applied (Bratbak & Dundas, 1984). For nanoflagellates, 40-mL aliquots were stained with 4'6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich Co. Ltd, Gillingham, UK) and filtered onto 2.0-µm polycarbonate filters. Analyses for abundances were undertaken using epifluorescence microscopy (×1000) using both the UV and blue filters for the separation of heterotrophic (HNAN) and phototrophic (PNAN) nanoflagellates. Lugol's iodine-fixed samples were concentrated by settling and counted in a Sedgewick-Rafter counting chamber at ×200.

HNAN and PNAN grazing experiments

Grazing rates were determined using fluorescently labelled bacteria (FLB) derived from cultures of bacteria isolated from Dry Valley lakes following the protocol of Sherr & Sherr (1993). Experiments were conducted in replicate Twirlpaks each containing 100 mL of lake water over 60-min incubations with FLB concentrations 20% of natural bacterial abundance. At 5-, 10-, 20-, 30-, 40-, 50- and 60-min intervals, the contents of three replicate Twirlpaks were fixed in ice-cold glutaraldehyde to a final concentration of 2%. Experiments were conducted under light conditions that replicated those prevalent in the field at 2 °C. Forty millilitres of water from each replicate was stained with DAPI and filtered onto 2.0-µm polycarbonate filters with replicate washings using 0.2-µm filtered lake water to flush uningested FLBs through the filter. A minimum of 20 HNAN and 100 PNAN cells were examined from each replicate using epifluorescent microscopy, and the number of FLBs contained within the cells was

noted. Ingestion rates were calculated from the average number of FLBs per protozoan cell over time, using regression analysis over the linear part of the uptake curve.

To determine whether HNAN and PNAN were taking up dissolved organic carbon (DOC), we incubated water samples with fluorescein (FITC)-labelled dextrans (Sigma) with molecular weights of 4, 9, 38 and 580 kDa for 60 min at concentrations of 2 mg L^{-1} . Incubations were set up as described for FLB uptake. Slide preparations for analysis were made as described above for FLB uptake analysis. One hundred cells on each preparation were viewed under epifluorescence microscopy to determine whether FITC dextrans had been ingested.

Bacterial production

Bacterial productivity was estimated as ^3H -leucine incorporation into cold trichloroacetic acid (TCA)-insoluble products on 1.5-mL samples collected from the depths where grazing experiments were performed. Following the addition of $20 \text{ nM } ^3\text{H}$ -leucine, triplicate samples were incubated for 20 h in the dark at $4 \text{ }^\circ\text{C}$. The concentration of ^3H -leucine applied was derived from saturation experiments. Two TCA killed controls were also included in each experiment. The reaction was terminated by the addition of cold TCA (6.7% final concentration), and the cells were centrifuged, resuspended in cold 5% TCA, centrifuged again and resuspended in cold 80% ethanol. Following final centrifugation, the pelleted bacteria were dried and scintillation cocktail added; final radioactivity was determined using a calibrated Beckman 6500 scintillation counter. Leucine incorporation was converted to carbon-based productivity using conversion factors of 1.42×10^{17} cells (mol leucine) (Kirchman & Gerardo, 1988) and 11 fg carbon per cell (Takacs & Priscu, 1998; Takacs *et al.*, 2001). Rates from the $4 \text{ }^\circ\text{C}$ incubation were converted to *in situ* temperatures using an energy of activation of $12\,600 \text{ kcal mol}^{-1}$ (Takacs & Priscu, 1998).

Results

PAR levels dropped progressively to very low levels over the study period (Fig. 1). Mean daily PAR levels at 10 m below the ice surface in the water column ranged from $6.62 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in mid-January to $0.19 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in early April.

Bacterial abundances in Lake Hoare during March showed an overall decline, while in both lobes of Lake Bonney, there was no clear trend over the study period. Overall numbers were higher in Lake Hoare (range $2.67\text{--}8.40 \times 10^8 \text{ cells L}^{-1}$) and in early to mid-March were highest at 12/13 m (Fig. 2). In Lake Bonney East,

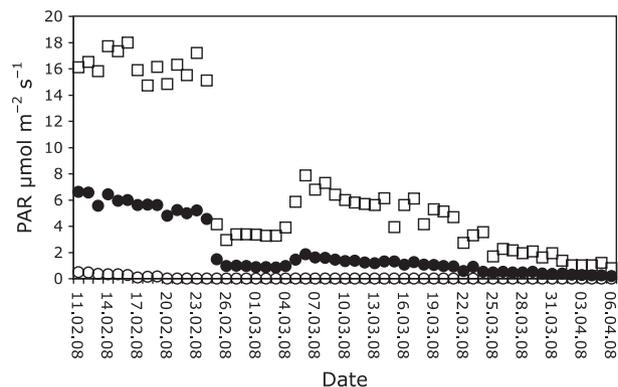


Fig. 1. PAR in Lake Bonney. Automatic readings were taken every 20 min. Filled circles mean daily PAR, open circles mean minimum daily value, squares mean maximum daily value.

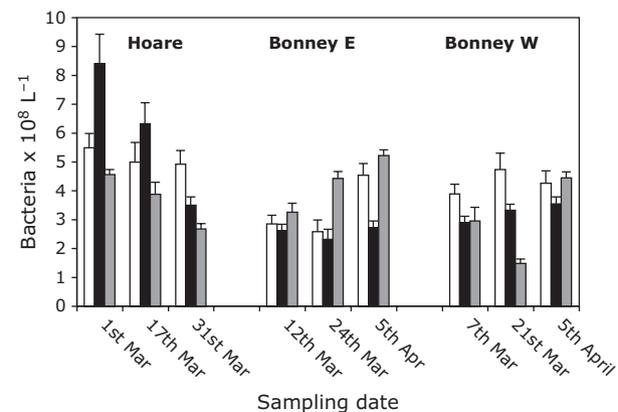


Fig. 2. Bacterial abundances in Lake Hoare and in the two lobes of Lake Bonney on three sampling dates during March and April. Each set of three histograms relates to depth as follows: Lake Hoare unfilled 5/6 m, grey 12/13 m and black 20 m; Bonney East unfilled 5/6 m, grey 13 m and black 20 m; Bonney West unfilled 5 m, grey 10 m and black 13 m. All dates from 2008.

bacterial abundance ranged between 1.87 and $5.22 \times 10^8 \text{ cells L}^{-1}$ with highest bacterial concentrations at 20 m. There was no consistent depth pattern in Lake Bonney West (Fig. 2) where bacterial numbers ranged between 1.48 and $4.73 \times 10^8 \text{ cells L}^{-1}$. HNAN concentrations increased in Lake Hoare at the end of March coinciding with a decrease in their bacterial prey numbers (Fig. 3); however, there was no significant correlation between HNAN and bacterial numbers ($r = 0.0354$). HNAN abundances showed no consistent pattern over the study period in Lake Bonney (Fig. 4), nor was there any correlation between bacterial and HNAN concentrations (Lake Bonney East $r = 0.107$, Lake Bonney West $r = 0.18$).

PNAN concentrations in Lake Hoare and Lake Bonney were considerably higher than HNAN numbers (Figs 3

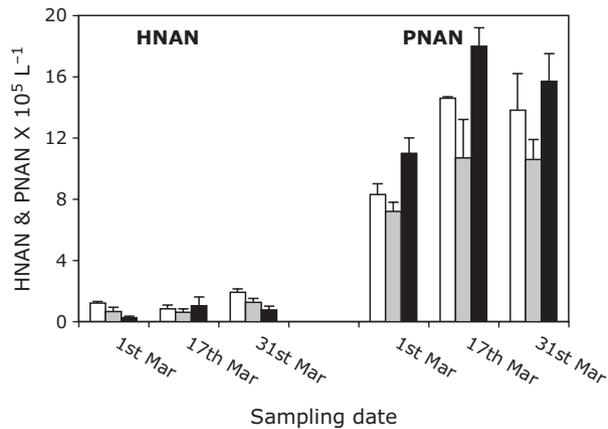


Fig. 3. HNAN and PNAN abundances in Lake Hoare during March 2008. In each set of three histograms: unfilled 5/6 m, grey 12/13 m and black 20 m.

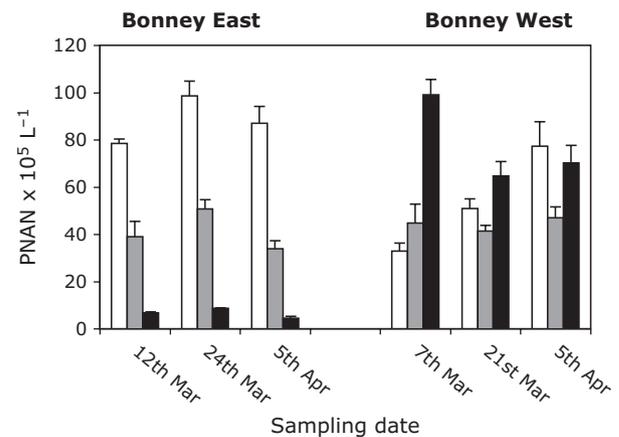


Fig. 5. PNAN abundances in Lake Bonney East and West lobes. Each set of three histograms relates to depth as follows: Bonney East unfilled 5/6 m, grey 13 m and black 20 m; Bonney West unfilled 5 m, grey 10 m and black 13 m. All dates from 2008.

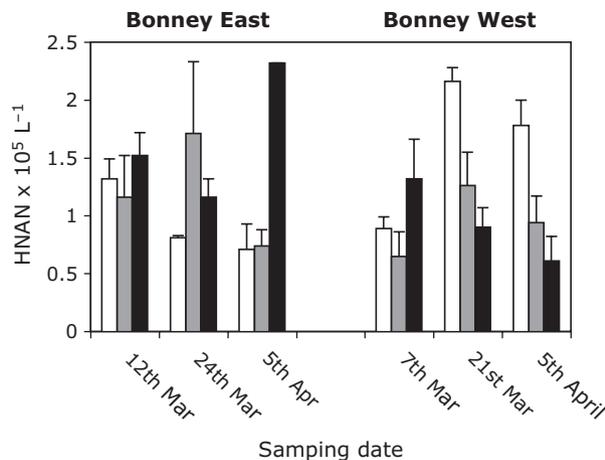


Fig. 4. HNAN abundances in Lake Bonney East and West lobes. Each set of three histograms relates to depth as follows: Bonney East unfilled 5/6 m, grey 13 m and black 20 m; Bonney West unfilled 5 m, grey 10 m and black 13 m. All dates from 2008.

and 5). Lake Bonney had higher PNAN concentrations compared with Lake Hoare. Among the PNAN community of Lake Bonney, there were 2–3 species of cryptophyte, *Chlamydomonas* and *Ochromonas*, while in Lake Hoare 3–4 species of cryptophytes dominated with lesser numbers of *Chlamydomonas*, *Pyramimonas* and *Ochromonas*. There was an increase in PNAN in Lake Hoare through March, at a time when daylight hours and PAR were decreasing and interestingly highest concentrations occurred in the deeper water at 20 m. Mean PNAN abundances in both lobes of Lake Bonney were consistent from mid-March to early April, but there was a distinct difference in the depth profile. In Lake Bonney East, highest concentrations occurred at 5/6 m (7.69 –

9.86×10^6 cells L^{-1}) with lowest numbers at 20 m (0.45 – 0.86×10^6 cells L^{-1}), while in Lake Bonney West the pattern was less clear with high concentrations both at depth and immediately below the ice cover (Fig. 5; range 3.28 – 9.90×10^6 cells L^{-1}).

Ciliated protozoan concentrations in Lake Hoare and Lake Bonney East showed an increase over the period November to March/April. Total numbers in Lake Hoare ranged between 44 and 265 cells L^{-1} and in Lake Bonney East from 56 to 248 cells L^{-1} (Fig. 6a and c). In Lake Bonney West, there was no clear pattern in abundance with concentrations ranging between 98 and 202 cells L^{-1} (Fig. 6b). Among the genera noted were *Monodinium*, *Askenasia*, *Vorticella*, *Euplotes*, *Plagiocampa*, *Urostyla*, *Frontonia* and the suctorian *Sphaerophyra* that preys on other ciliates. Scuticociliates that feed on bacteria were also common. In Lake Hoare, the dominant genera were *Monodinium*, *Askenasia* and a number of hypotrich species including *Euplotes* (Fig. 6a). In Lake Bonney, hypotrichs were less common, while *Monodinium* and *Askenasia* dominated the ciliate community (Fig. 6b and c). A number of small dinoflagellates 10–15 μm (*Gymnodinium* spp.) were relatively common in Lake Bonney and increased in February, March and April (Fig. 6b and c). Dinoflagellates were noted in epifluorescence counts for PNAN and HNAN with some possessing autofluorescence, while others lacked chlorophyll and were presumably heterotrophic. *Gymnodinium* spp. were only found in Lake Hoare on one occasion at the end of March.

Rotifers were present in Lake Hoare between November and the end of March reaching a maximum of 19 individuals L^{-1} in mid-March. The majority belonged to the genus *Philodina*. In comparison, rotifers were very

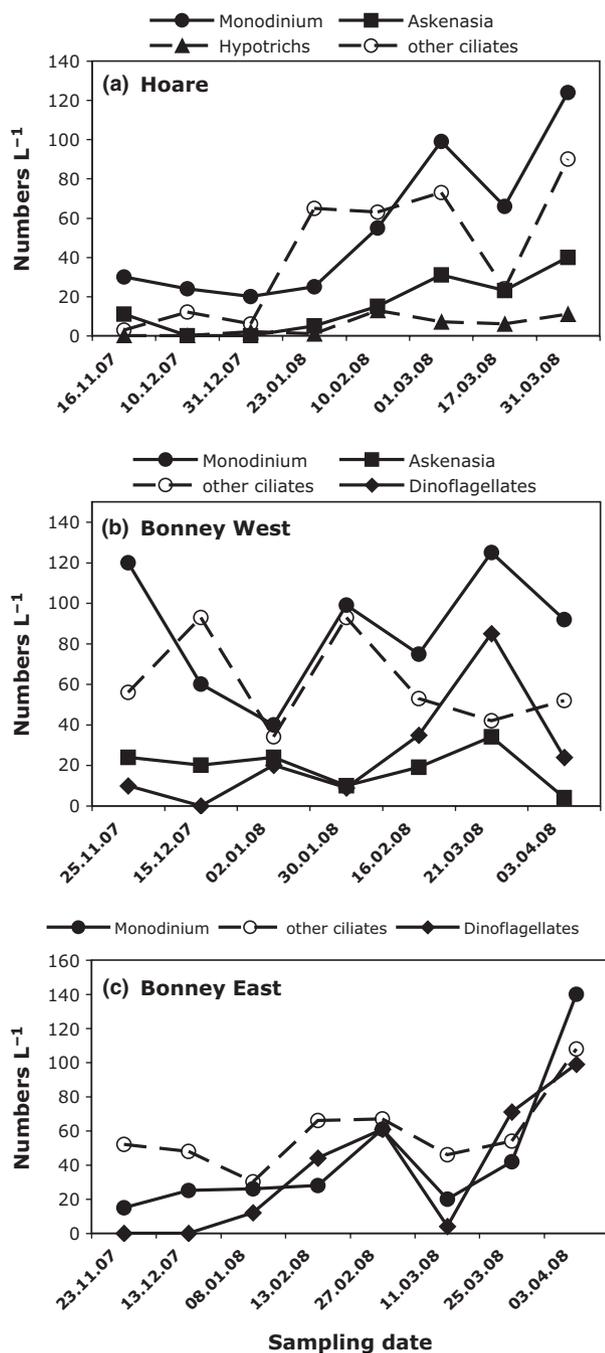


Fig. 6. Mean ciliate and dinoflagellate abundances in (a) Lake Hoare and (b) Lake Bonney West and (c) Lake Bonney East between November 2007 and April 2008.

sparse in Lake Bonney, and single specimens were noted in only three samples.

Mean HNAN grazing rates in Lake Hoare for the month of March ranged from 8.12 to 30.81 bacteria per cell day⁻¹, with the highest rates occurring at 12/13 m (Fig. 7). Mean PNAN grazing rates ranged from

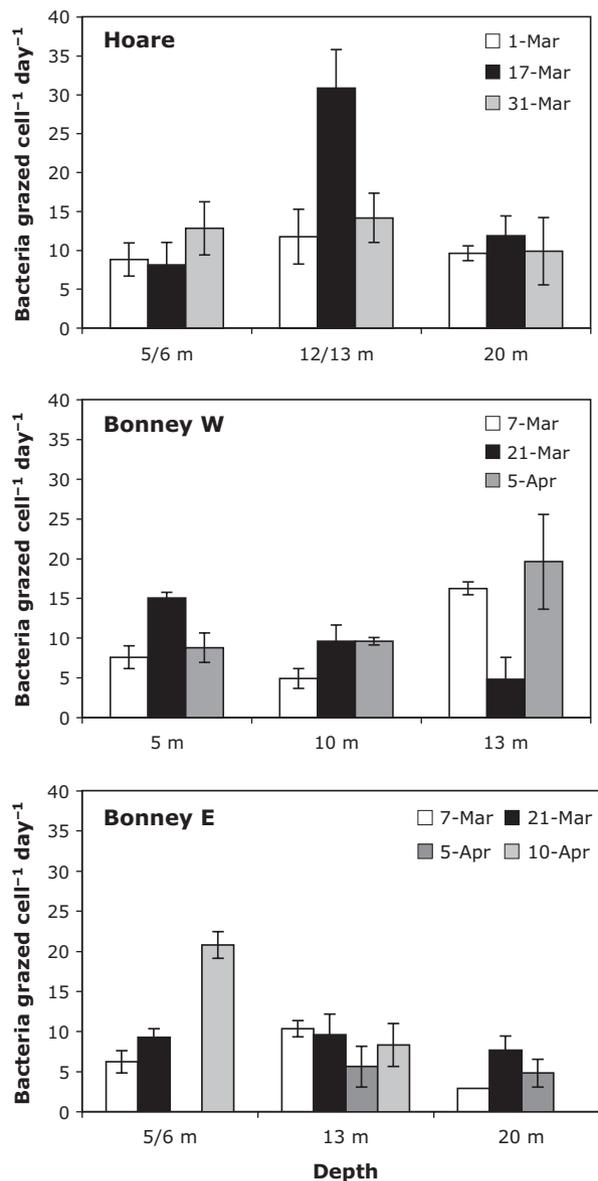


Fig. 7. Grazing rates of HNAN in relation to depth in Lake Hoare, Lake Bonney West and Lake Bonney East during March–April 2008.

3.94 to 15.20 bacteria per cell day⁻¹ (Fig. 8). The PNAN also showed higher rates at shallower depths (Fig. 8). Clearance rates for PNAN and HNAN varied considerably over the study period, showing a 2.5- to 3-fold variation (Table 1). Because HNAN concentrations were much lower than PNAN concentrations (Fig. 3), the PNAN had a much greater impact on the bacterial community removing up to 314 ng C L⁻¹ day⁻¹ (Table 2). In contrast, HNAN removed between 3.74 and 36.6 ng C L⁻¹ day⁻¹. On all occasions, PNAN grazing exceeded daily bacterial production at all the study depths in Lake Hoare.

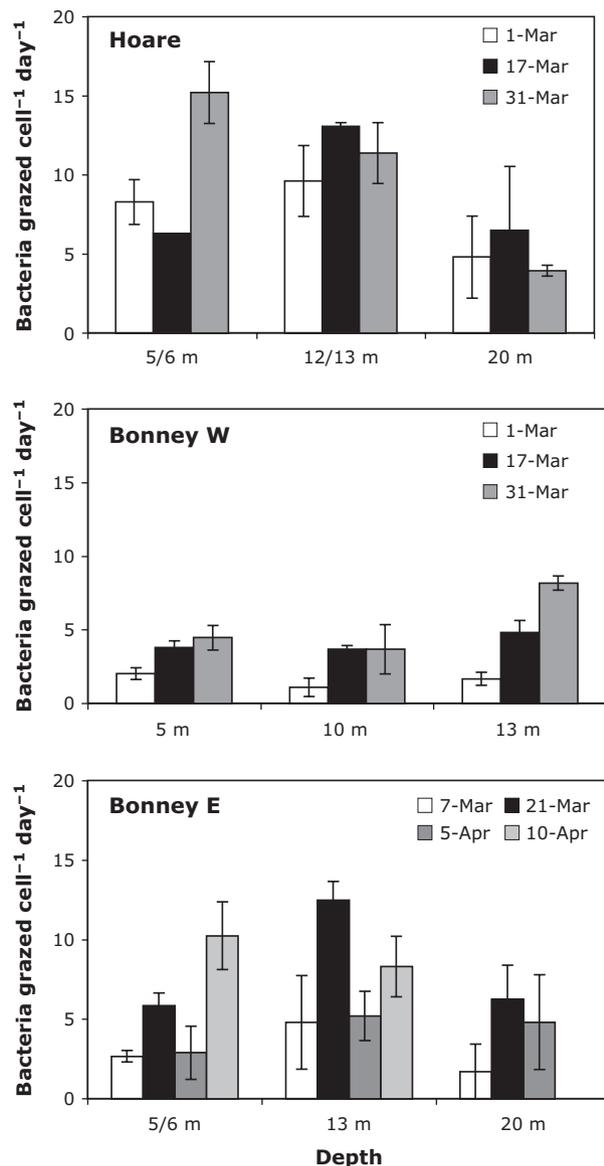


Fig. 8. Grazing rates of mixotrophic PNAN in relation to depth in Lake Hoare, Lake Bonney West and Lake Bonney East during March–April 2008.

The mean grazing rates of the HNAN in both lobes of Lake Bonney during March to April were similar to those in Lake Hoare with the exception of Lake Bonney East at 20 m (Fig. 7), where the rates were consistently lower. PNAN grazing rates were higher in the east lobe of Lake Bonney compared with west lobe (Fig. 8). Clearance rates in Lake Hoare and both lobes of Lake Bonney were similar with a wide range between the maximum and minimum rates (Table 1). As in Lake Hoare, the low concentrations of HNAN relative to PNAN resulted in the HNAN community having a much lower grazing impact on the bacterioplankton in comparison with the

PNAN. The HNAN grazed between 6.48 and 48.6 ng C L⁻¹ day⁻¹ in Lake Bonney West and between 6.5 and 24.6 ng C L⁻¹ day⁻¹ in Lake Bonney East. In contrast, the PNAN exerted a much greater effect on bacterial production, on most occasions grazing well in excess of daily bacterial production (Table 2). There was no evidence of either the PNAN or HNAN taking up fluorescently labelled dextrans in Lake Hoare or Lake Bonney, indicating that they do not appear to exploit the DOC pool as an energy and carbon source. Both HNAN and mixotrophic PNAN appear to be dependent on ingesting bacteria as an energy source.

Discussion

The current study is the first to investigate microbial processes during the transition period to winter in the McMurdo Dry Valley lakes. Year-long studies undertaken in lakes in the coastal Vestfold Hills at 68°S have demonstrated that contrary to what one might expect, many species continue to function over the period of winter darkness (Bell & Laybourn-Parry, 1999; Laybourn-Parry *et al.*, 2005). However, the Dry Valley lakes are much further south at 77°S and experience lower annual temperatures and lower annual PAR. Lakes Bonney and Hoare also possess perennial debris containing ice covers that severely attenuate PAR to the underlying water column during the period when sunlight is present. Bacteria, ciliate and flagellate numbers showed no radical reductions during March into April, suggesting that functional ecosystems are present in these lakes into winter. Low rates of phytoplankton photosynthesis were measurable between 12 and 16 March 2008 in Lake Bonney, with rates reaching 2.1 µg C L⁻¹ day⁻¹ in Lake Bonney East and 0.51 µg L⁻¹ day⁻¹ in Lake Bonney West (Kong *et al.*, 2012). As winter encroached in 2008, chlorophyll *a* continued to increase after photosynthesis had ceased, suggesting that part of the phytoplankton community was still active and switching to heterotrophy. Phytoplankton samples collected by an automatic sampler over winter in Lake Fryxell (Taylor Valley) provide evidence to support this assertion (McKnight *et al.*, 2000). These authors showed that while abundances were lower in winter than in spring and summer, vegetative cells were the most abundant form of all species observed in Lake Fryxell. One species, *Stichococcus*, was observed in winter and two of the cryptophyte species were more abundant in winter than in summer. McKnight *et al.* (2000) attributed winter survival to mixotrophy, a phenomenon that has been demonstrated among the PNAN of Taylor Valley lakes (Roberts & Laybourn-Parry, 1999; Marshall & Laybourn-Parry, 2002) and communities in the lakes of the Vestfold Hills (Laybourn-Parry *et al.*, 2005). Nutritional versatility

Table 1. Maximum and minimum clearance rates for HNAN and PNAN in Lakes Bonney and Hoare (SEM shown in brackets)

Lake	HNAN			PNAN		
	Clearance rate nL per cell h ⁻¹	Date 2008	Depth (m)	Clearance rate nL per cell h ⁻¹	Date 2008	Depth (m)
Lake Hoare	0.61 (0.15)	1 March	5/6	0.37 (0.15)	1 March	20
	1.53 (0.05)	17 March	12/13	1.16 (0.19)	31 March	5/6
Lake Bonney West	0.82 (0.19)	7 March	10	0.16 (0.08)	7 March	10
	2.34 (0.33)	7 March	13	1.16 (0.48)	21 March	13
Lake Bonney East	0.32 (0.18)	11 March	20	0.18 (0.07)	11 March	20
	1.94 (0.60)	10 April	5/6	2.28 (0.18)	24 March	13

Table 2. PNAN community grazing impact on bacterial production in Lake Bonney West at 5, 10 and 13 m, in Lake Bonney East at 5, 13 and 20 m and in Lake Hoare at 5, 13 and 20 m. All rates in ng carbon L⁻¹ day⁻¹

Lake Bonney West	5 m	10 m	13 m
7 March			
Bacterial production	151	124	71
PNAN grazing	99	72	246
21 March			
Bacterial production	161	111	83
PNAN grazing	287	225	465
5 April			
Bacteria production	37	59	57
PNAN grazing	516	214	859
Lake Bonney East	5 m	13 m	20 m
12 March			
Bacterial production	91	131	139
PNAN grazing	311	280	16
24 March			
Bacterial production	254	112	140
PNAN grazing	862	950	80
10 April			
Bacteria production	166	170	261
PNAN grazing	1181	516	ND
Lake Hoare	5 m	13 m	20 m
1 March			
Bacterial production	25	52	21
PNAN grazing	130	103	72
17 March			
Bacterial production	136	52	27
PNAN grazing	137	209	175
31 March			
Bacterial production	113	60	43
PNAN grazing	314	180	93

is an important survival strategy in a number of successful species in Antarctic lakes.

Winter studies on Arctic and subarctic lakes are also sparse, but limited investigations indicate that in the more complex food webs of these lakes, some species remain active in winter (Rigler & MacCallum, 1974; Rautio *et al.*, 2011; Karlsson & S awstr om, 2009). In Lake Saanaaj arvi (subarctic Finland), heterotrophic bacteria and nanoflagellates peaked in winter, and their growth sus-

tained by allochthonous carbon. In turn, these microorganisms sustained a growing population of *Daphnia umbra* (Rautio *et al.*, 2011). In contrast, in a northern Swedish lake, it was carbon generated by benthic algae channelled through heterotrophic bacteria that supported an active population of *Cyclops scutifer* (Karlsson & S awstr om, 2009). The carbon dynamics of northern lakes are much more complex than Antarctic lakes. In Antarctic lakes, allochthonous sources of carbon are minimal and systems are sustained by autochthonous carbon, whereas many Arctic and subarctic lakes receive significant carbon inputs from their catchments. Nevertheless, in both the northern and southern polar regions, populations of some organisms are able to function in winter by exploiting alternative sources of carbon to those used in summer.

One of the features of Antarctic lake communities is a marked interannual variation in the abundances of the bacteria and protozoan populations, making it difficult to place the data derived from this study into a seasonal context. Nevertheless, the bacterial abundances observed in the current study are within the range reported from summer investigations (Table 3), although they are in the lower sections of the reported ranges. PNAN and HNAN abundances are also variable (Table 3) but those found in the current study are within the ranges previously found. Indeed, the HNAN values are higher than those reported in 1997/1998 by Roberts *et al.* (2004) for Lake Hoare. Ciliates also showed considerable variability, not only in concentration, but also in species diversity, a trend shown by previous studies on the Dry Valley lakes (Kepner *et al.*, 1999). Between November 2007 and March 2008, abundances in Lake Hoare ranged between 36 and 265 cells L⁻¹, with higher numbers in March and in Lake Bonney East highest abundances occurred in April, while in Lake Bonney West, numbers remained much the same across the summer into the winter transition. There were evidently sufficient food resources (bacteria, flagellates) to support growing ciliate populations towards winter.

Of particular interest was the appearance of heterotrophic and autotrophic dinoflagellates in Lake Bonney and

Table 3. Abundances of bacteria, HNAN and PNAN in Lake Hoare to illustrate interannual variations

Year	Bacteria $\times 10^5 \text{ mL}^{-1}$	HNAN mL^{-1}	PNAN mL^{-1}
November–January 1993–1997*	3–70	–	–
Summer 1997/1998†	2.9–59.7	2.3–39.4	565
Summer 1997/1998‡	9.2–11.9	122	9966
November 2004§	7.15	–	650–3024
November/December 2000¶	5.0–22.0	620	600–4500
March 2008**	2.67–5.49	10–191	100–1346

*Takacs & Priscu (1998).

†Roberts *et al.* (2004).

‡Roberts & Laybourn-Parry (1999).

§J. Laybourn-Parry (unpublished data).

¶Marshall & Laybourn-Parry (2002).

**This study.

in Lake Hoare during the transition to total darkness. Dinoflagellates have not previously been noted as a conspicuous element in the protozooplankton of the Dry Valley lakes. Parker *et al.* (1982) noted a colourless dinoflagellate in Bonney East, but gave no concentrations for individual components of the plankton. Dinoflagellates are a common element in the brackish marine-derived lakes of the Vestfold Hills, so the limited previous reports from the Dry Valley lakes are surprising (Rengefors *et al.*, 2008; Logares *et al.*, 2009).

Grazing rates observed in Lake Hoare PNAN in the current study were similar to those reported for the summer of 1997/1998 when ingestion rates varied between 4.8 and 24.0 bacteria per cell day^{-1} (Roberts & Laybourn-Parry, 1999). However, in November and December 2000, grazing rates were lower than we observed in our present study, ranging from 5.28 to 10.08 bacteria per cell day^{-1} (Marshall & Laybourn-Parry, 2002). These differences lack simple explanations, but probably relate to the concentration of the bacteria prey and competition between both heterotrophic and PNAN. There is now evidence showing that protozoa can exercise selectivity in feeding and are able to discriminate on the basis of bacteria species or strain, not just prey size (Thurman *et al.*, 2010). Thus, the species make-up of the bacteria community at any given time is also likely to influence grazing rates.

The phytoflagellate grazing rates observed in Antarctic lakes are lower than rates reported from lower-latitude lakes, as one would expect given the extreme conditions that prevail in high-latitude waters. For example, reported rates for *Ochromonas* ranged from 1.2 to 120 bacteria per cell h^{-1} (Zubkov *et al.*, 2001; Wu *et al.*, 2004; Schmidtke *et al.*, 2006). Rates in the current study ranged from 0.21 to 0.47 bacteria per cell h^{-1} . In Swedish Lake

Örträsket, where the mixotrophic community was dominated by chrysoomonads, mixotrophs were always the major grazers with higher clearance rates (1–4 nL per flagellate h^{-1}) than the HNAN (Isaksson *et al.*, 1999). In the current study, PNAN outnumbered HNAN, a pattern that appears consistent in most of the studies previously undertaken in Dry Valley lakes (see Table 3). Consequently, when PNAN are practising mixotrophy, they can exert a much greater grazing impact on the bacterial community than the HNAN.

During March, PNAN grazing in Lake Hoare removed between 72 and 314 $\text{ng C L}^{-1} \text{ day}^{-1}$ (Table 2). These grazing rates exceeded bacterial production that ranged between 21 and 136 $\text{ng C L}^{-1} \text{ day}^{-1}$. This impact is greater than reported in November 1997 (3% of bacterial biomass day^{-1}) and January 1998 (> 1% of bacterial biomass day^{-1}) in Lake Hoare (Roberts & Laybourn-Parry, 1999). In November 1997, the PNAN removed more of the bacterial biomass than the HNAN. At the same time, the grazing impact of PNAN in Lake Fryxell was higher, reaching 13% of bacterial biomass day^{-1} . Here too, the PNAN exerted a greater impact than HNAN for most of the summer study period. As winter encroached in the current study, PNAN in Lake Bonney grazed in excess of daily bacterial production. At lower latitudes, it is usually the HNAN that are the major grazers of bacteria, but there are reports of the PNAN out-grazing them, for example in Lake Oglethorpe (Georgia) in February within top 3 m of the water column (Bennett *et al.*, 1990) and in Lake Örträsket (Isaksson *et al.*, 1999). Our data indicate that as the communities of PNAN in Lakes Hoare and Bonney move towards winter, there is an increasing dependence on mixotrophy as the capacity to undertake photosynthesis is curtailed, supporting the hypothesis put forward by McKnight *et al.* (2000). In the current study, there was no evidence of DOC uptake by PNAN or HNAN, whereas in a number of brackish lakes in the Vestfold Hills, cryptophytes and *P. gelidicola* survived in an active form during winter by grazing on bacteria, and in Highway Lake (Vestfold Hills, Antarctica) by taking up DOC over a range of molecular weights in addition to bacteria (Laybourn-Parry *et al.*, 2005). However, in the Vestfold Hills lakes, the grazing impact of mixotrophs on bacteria was always < 1% of bacterial biomass removed per day. Clearly, there are energetic benefits from adopting a mixotrophic strategy because as Raven (1997) has pointed out, mixotrophs have to invest energy in a phagotrophic and digestive apparatus in addition to sustaining a photosynthetic system. Our results show that, in what are some of the most extreme lacustrine systems on the planet, elements of the microbial community probably continue to function year around with the balance switching from autotrophy to heterotrophy during the polar night.

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