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Summer-winter transitions in Antarctic ponds II: Biological responses

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Abstract: We observed ice formation and water column attributes in four shallow Antarctic ponds between January and 7 April 2008. During that time ponds went from ice-free to > 80 cm thick ice, near-freshwater to hypersaline, well-lit to near darkness and temperatures fell to below zero. Here we examine shifts in biological activity that accompanied these changes. During February, freeze-concentration and ongoing photosynthesis increased dissolved oxygen concentration to up to 100 mg l^{-1}, with a near-equivalent decrease in dissolved inorganic carbon and a pH rise. Benthic photosynthesis was responsible for 99% of estimated biological oxygen production. Net oxygen accumulation ceased in late February, pH began to fall and inorganic carbon to increase, but the pool of dissolved oxygen was depleted only slowly. Anoxia had been attained in only one pond by April and there was little accumulation of indicators of anaerobic activity. The nitrogen and phosphorus balances of the ponds were dominated by organic forms, which, like DOC and CDOM, behaved conservatively. Conversely, inorganic nitrogen and phosphorus uptake was evident throughout the study period, at a molar ratio of 16N:1P in two of three ponds, consistent with uptake into biological material. We found no coupling between N and P uptake and photosynthesis.

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Introduction

Meltwater ponds are widespread in Antarctica and in many areas represent a significant component of the aquatic environment (Vincent & James 1996, Howard-Williams & Hawes 2007, Quesada *et al*. 2008). When melted, conditions in these ponds can be benign for microbial growth, with water temperature reaching up to 10°C and abundant solar radiation available for photosynthesis (Hawes *et al*. 1997, 1999). Thick, species diverse, perennial microbial mats often develop, which can deplete overlying water of nutrients and inorganic carbon and drive pH to values of over 10 (Howard-Williams & Hawes 2007, Hawes *et al*. 2008 and citations therein). The ponds are often hydrologically isolated, and influx of nutrients from outside is dependent on localized melting of snow and ice. However, within and below the microbial mats a suite of aerobic and anaerobic heterotrophic bacteria are important in recycling nutrients to maintain productivity (Mountfort *et al*. 2003, Howard-Williams & Hawes 2007).

The extreme stresses imposed by winter freezing must play a role in structuring biological communities in these ponds (Schmidt *et al*. 1991, Hawes *et al*. 1999), yet detailed knowledge of ecosystem processes in Antarctic ponds is largely restricted to the relatively benign summer period. To address this gap in understanding, in 2008 we followed the freezing process from January (maximum melt) through to April (~ 90% frozen) in a selection of ponds on the McMurdo Ice Shelf close to Bratina Island, Antarctica. This is the second of a series of papers describing the findings from this research. The first report has shown how liquid water persisted in these ponds for many weeks after ice formation has begun, but that the physical and chemical conditions under ice cover were markedly different to those during the ice-free period (Hawes *et al*. 2011). In this contribution we focus on changes in metabolic processes in pond biota during the ice formation process.

We examine pond metabolism using a mixture of "rate" and "state" observations. We assume that, after pond ice has formed, the ponds can be treated as closed systems and that depletion or accumulation of biologically active materials in the remaining water are evidence of metabolic activity - analogous to the whole pond approach of Ramlal *et al*. (1994). Thus, by recording the concentrations of biologically active substances through time, microbially-mediated transformations can be tracked. To supplement these data, three key metabolic processes: photosynthetic activity of the planktonic communities, photosynthetic activity of the benthic communities, and water column bacterial productivity, were estimated independently.

Study site

Full details of the site and physical characteristics of the four main study ponds are given in the first of this series of papers (Hawes *et al*. 2011). In brief, four small ponds on the McMurdo Ice Shelf close to Bratina Island (78°S, 165.5°E) were selected for the study: Legin, JA, P70 and Bambi ponds (all unofficial names). The selected ponds were of similar size and depth, all were ice-free during summer, and they had slightly different water chemistries. The ponds were sampled regularly from January–April 2008, during which time ice cover increased to ~ 80 cm thickness and irradiance fell to near darkness. Partitioning of salts into the remaining liquid phase during ice thickening resulted in a rise in conductivity (Hawes *et al*. 2011).

Of the ponds selected, Legin was the only one that proved to be strongly density stratified at the start of the experiment. A pocket of warm, saline water was trapped beneath a much larger, well-mixed volume of water. This meromictic state has been recognized in other Antarctic ponds as a consequence of the persistence of freeze-concentrated brine pools (e.g. Healy *et al*. 2006, Wait *et al*. 2006). The other three ponds, JA, Bambi and P70, were all well mixed, though in each case a few litres of saline water existed at the very base of the pond. These brine pools were such small targets that they could not be sampled reliably. Traces of stratification disappeared from the three "mixed" ponds over the course of ice formation, due to convective mixing that continued throughout the study period, but not from Legin pond.

Methods

Water sampling

Water samples were initially collected from 10, 40 and 80 cm in JA and P70 and at 10, 50 and 100 cm depth in Bambi and Legin. Once it became obvious that Bambi and Legin were deeper, and that the latter was stratified, an extra sample was taken from 120 cm depth in both ponds and at greater depths in Legin. As the ice-water interface descended through the ponds, the 10 cm samples were replaced with a sample from immediately beneath the ice cover.

Water samples were taken using an acid rinsed, 5 mm internal diameter plastic hose inserted to the required depth and connected to a hand-driven peristaltic pump. The pond end of the tube was plugged and water was drawn in through a 10 mm diameter side opening. From March onwards, the tubing passed through a thermos flask of hot water immediately after exiting the pond to prevent freezing. Samples were pumped into thrice-rinsed plastic bottles and kept in an insulated box containing warm water bottles. Despite these precautions, ice crystals occasionally formed and these were allowed to melt before subsamples were taken for analysis or experiment.

A Metler portable pH meter, calibrated using standard buffers was used for measuring the pH of freshly collected pond water and for estimation of alkalinity by Gran titration using standardized HCl (Mackereth *et al*. 1978).

Dissolved inorganic carbon (DIC) was estimated by infra-red gas analysis within 24 h of collection. 0.5 or 1 ml subsamples were injected into pre-washed, partially evacuated 4 ml Vacutainers containing 0.2 ml of 10% phosphoric acid. After vigorous shaking to displace DIC as CO_2, the headspace was equilibrated to ambient pressure and a subsample injected into a stream of air flowing through a LiCor infra-red gas analyser (IRGA). DIC concentration was estimated from peak height after calibration using standard solutions of sodium bicarbonate.

Samples for total dissolved organic carbon (DOC) were filtered though a pre-combusted, glass fibre filter (Whatman GF/F) and stored, unfrozen, in pre-combusted brown glass bottles. Samples were returned to New Zealand for analysis as CO_2 by IRGA after catalytic oxidation to carbon dioxide. Chromophoric dissolved organic material (CDOM) was determined at the field laboratory. Water was injected through a 0.22 μm filter into a 1 m path length optical cell linked to an Ocean Optics S2000 fibre-optic spectrometer. Absorbance at 440 nm minus absorption at 750 nm (g440) was recorded as an index of CDOM concentration.

Chloride was determined on unfiltered samples returned unfrozen to New Zealand, by high pressure ion chromatography, with a Dionex AS11, 4 mm diameter column and 10 mM NaOH as eluent. Dissolved species of nitrogen and phosphorus were determined on samples filtered through acid-rinsed Whatman GF/F filters, then frozen for return to New Zealand. NO_3-N, NH_4-N and dissolved reactive phosphorus (DRP) were determined using an Astoria autoanalyser; dissolved organic nitrogen and phosphorus (DON and DOP) were analysed as NO_3-N and DRP after persulphate digestion, using a Lachat flow injection analyser.

Samples for estimating planktonic chlorophyll *a* (chl *a*) concentration were collected onto Whatman GF/F filters and stored frozen for return to New Zealand. Filters were ground in cold 95% acetone, extracted at 4°C for four hours, then the supernatant separated by centrifugation. Chlorophyll *a* was estimated using a Perkin Elmer spectrofluorometer, calibrated with standard solutions of chl *a*. The phaeopigment form of chl *a* was estimated by acidification and re-measuring of fluorescence of the extract. Two values were calculated, the concentration of undegraded chl *a* and the proportion of phaeopigment.

To separate changes in measured variables due to the effects of freeze-concentration from changes due to metabolic processes, we examined the ratios of each to chloride. Previous studies (Wait *et al*. 2006, 2009) have suggested that chloride behaves in a largely conservative manner during the freezing process in these ponds. We first log-transformed all variables, to allow for the near-exponential change in most, including the conservative tracer. To determine which variables were primarily driven by freeze-concentration, we then looked for significant

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positive correlations between all log transformed variables and ln(chloride) but because this was a time series and the number of correlation pairs was small, significant correlations at the $P < 0.05$ level were interpreted with caution.

Temperature, conductivity and dissolved oxygen concentration

Depth profiles of temperature and conductivity were obtained using a recently calibrated C-90 conductivity-temperature meter (TPS Ltd, Springwood, Australia) (Hawes *et al*. 2011). Initially, dissolved oxygen (DO) concentration was estimated using a Presense fibre-optic oxygen sensor (optode, http://www.Presense.com). When unexpectedly high concentrations were recorded shortly after ice formation, DO was checked using a Unisense, Clarke-type glass microelectrode connected to a portable picoammeter (http://www.Unisense.com). Both optode and electrode were subject to a 2-point calibration on each day of use (0 and 100% saturation at 0°C). The optode and microelectrode showed similar concentrations and, when the optode proved too brittle for use at low sub-zero field air temperature, the microelectrode became the primary instrument for determining DO concentration profiles. Profiling involved the measurement of DO concentration at 5–10 cm intervals down the water column. Summary statistics derived from these data were the average DO concentration in mixed layers and the total mass of oxygen in each pond (see below).

Estimation of mass balances

Measurement of pond bathymetry and ice thickness, and estimation of residual water volume have been described elsewhere (Hawes *et al*. 2011). In this contribution we have used those data to calculate total mass of determinands in the ponds, by summing volume-weighted concentrations across 10 cm thick "slices" of residual water. In Legin pond, the masses of oxygen in the mixolimnion and the density gradient were calculated separately.

Phytoplanktonic photosynthetic activity

Estimates of planktonic photosynthesis were obtained by incubating freshly collected pond water in 20 ml glass vials, to which 10 μCi of ^{14}C-labelled bicarbonate was added (Amersham International Ltd). Triplicate vials were suspended at the collection depth for 24 h, with dark controls for each wrapped in foil and incubated simultaneously at 0–2°C. At the end of each incubation, a 10 ml sample was preserved by the addition of 0.2 ml 10% HCl. On return to New Zealand, a 5 ml subsample was mixed with 10 ml Aquasol liquid scintillation fluor and counted in a RackBeta liquid scintillation counter. Daily net carbon uptake (mg m^{-3} d^{-1}) was estimated as:

$$(\text{dpm light} - \text{dpm dark})/(\text{dpm added})\cdot(\text{ml incubated})/(\text{ml counted})\cdot(\text{mg m}^{-3}\ \text{DIC})\cdot 1.05,$$

where 1.05 is a correction for isotopic discrimination.

Bacterial numbers and activity

Samples (1.5 ml) for enumeration of bacteria were preserved using filtered formaldehyde at a final concentration of 1%. On return to New Zealand, numbers were determined by flow cytometry using a FACSCalibur instrument (Becton Dickinson, Mountain View, CA) with CellQuest v3.1 software. All samples were counted within five months of collection. Samples were stained using SYBRII stain (Molecular Probes Inc) buffered in DMSO and then incubated in the dark at ~4°C for 10–15 min before being analysed (Lebaron *et al*. 1998). The sheath fluid was double distilled water and the analysed volume was calculated using TrucountTM (Becton Dickinson) beads as a tracer.

Bacterioplankton production was measured with the ^{3}H-thymidine incorporation method as described in Smith & Azam (1992). Triplicate 1.7 ml subsamples were incubated in the dark, either *in situ* or, from late February onwards, in the laboratory at ambient pond temperature. Incubation tubes had a final concentration of 16.6 nM of ^{3}H-thymidine (85.0 Ci mmol^{-1}) and were incubated for 8–16 hours to obtain enough radioactivity, but maintain incubation linearity (e.g. Leakey *et al*. 1996). Incubations were stopped by adding 89 μl of ice cold 100% TCA and kept cold for transport to New Zealand. Blanks were prepared by adding 100% TCA to the incubation tube prior to incubation. In New Zealand, samples were centrifuged at 16 000 g for 7 min at +4°C and washed once each with refrigerated 5% TCA and 80% ethanol. 0.5 ml of OptiPhase HiSafe (Wallac) scintillation cocktail was added and the microcentrifuge tubes vortexed before radioactivity was measured with a Wallac 1409 scintillation counter.

Benthic biomass

Samples of benthic microbial mat were collected through ice cover using a rod-mounted piston corer, with a 2.84 cm^2 cross sectional area. In each pond, in mid-February, triplicate cores were taken at 30 cm depth intervals along a profile from the pond edge to the deepest part. Cores were extracted into 95% acetone, separated by filtration (Whatman GF/F), then absorption was measured at 675 and 750 nm using an Ocean Optics S2000 spectrometer (http://www.OceanOptics.com). Chlorophyll *a* concentration, as mg m^{-2}, was calculated using equations of Marker *et al*. (1980). Benthic sampling disrupts the sediment-water interface, and some leakage of anoxic (nutrient-rich) sediment inevitably occurs during retrieval. To avoid the risk that repeated core sampling would compromise the time series of water column measurements, no time series of benthic biomass was obtained.

Benthic photosynthetic activity

Benthic photosynthetic activity was interpreted from high-resolution profiles of DO concentration through the water-microbial mat-sediment system. This required collection of a

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Table I. Characteristics of the surface waters of the four ponds close to the end of the summer open water period (25 January 2008).

Pond	Cl g m^{-3}	Chl *a* mg m^{-3}	pH	DIC g m^{-3}	DRP mg m^{-3}	NH_4 mg m^{-3}	NO_3 mg m^{-3}	DOC g m^{-3}	DON mg m^{-3}	DOP mg m^{-3}	CDOM (g440)
Bambi	875	1.32	10.1	54	119	9	11	30	2163	160	0.15
JA	687	0.25	8.8	19	2	6	14	26	1032	21	0.10
P70	1788	1.60	10.1	14	17	12	8	25	1829	51	0.17
Legin	804	1.07	9.5	14	6	5	4	10	910	34	0.08

Note: all pond names are unofficial.

number of cores and, in order to minimize disruption to the main study ponds, a fifth pond, Orange pond (unofficial name), was used for these measurements. This pond is located between JA and P70 ponds and has a similar ice cover and microbial mat to both. It was assumed that the pattern of response of the microbial mat to changing irradiance in this pond would be similar to that in the four primary ponds.

For laboratory determination of the effect of irradiance on mat-water oxygen profiles, three cores, each of 50 mm diameter and extending to at least 7 cm into the sediment but retaining overlying pond water, were obtained from 10 cm water depth in Orange pond. These were acclimated to 400, 200, 100 and 40 μmol photons m^{-2} s^{-1}, provided by a fibre-optic, quartz-halogen light source. Two profiles from above the mat into the anoxic underlying sediment were obtained from each core, at each irradiance, with vertical resolutions of 0.2–0.5 mm. All DO measurements were obtained using a Unisense, glass Clarke-type oxygen microelectrode, with a tip diameter of 50 μm, coupled to a picoammeter and mounted in a micromanipulator. Positions of the microelectrodes were then normalized to the surface of the microbial mats and mean DO concentrations for the six profiles at each irradiance were calculated. Three-point boxcar averaging was applied to DO concentrations to smooth profiles. Calculation of net oxygen flux from the mat (J) was from the concentration gradient within the diffusive boundary layer. $J = -D_O \cdot \delta C/\delta z$, where D_O is the free-solution diffusion coefficient of oxygen in water at ambient temperature, C is dissolved oxygen concentration and z is depth. A value for D_O of 1.13×10^{-5} cm^2 s^{-1} was used throughout (Vopel & Hawes 2006).

Field measurements of DO profiles through benthic mats in Orange pond were obtained on 12 and 23 February, through 15 mm diameter holes drilled in the ice cover, with the micromanipulator positioned on the ice above these holes. On each date, four DO profiles were obtained at 1 mm vertical resolution through water-mat-sediment interfaces that were 10–15 cm below the underside of the ice cover. Under field conditions, finer resolution could not be obtained.

Results

Water chemistry

At the end of the open water period, the mixed layers of all ponds had high pH and measurable concentrations of inorganic carbon, nitrogen and phosphorus, though in all cases the dominant component of dissolved N and P were the organic fractions and all ponds contained ≥ 10 mg l^{-1} of DOC (Table I). Bambi was disproportionately rich in DIC, DRP and DOP. All had low concentrations of planktonic chl *a*, ranging from 0.25 to 1.60 μg l^{-1}. As freezing progressed, the water columns of JA, P70 and Bambi became convectively mixed and we were able to treat them as single water bodies that were getting steadily smaller over time. In contrast, Legin retained a mixed layer overlying a density gradient and could not be so treated.

Of the water quality variables investigated in this study, three associations emerged from the correlation of ln-transforms against chloride. The first group, conductivity, DOC, CDOM, DON and DOP showed strong correlations to chloride in all three of the mixed ponds ($P < 0.01$). The increases in concentration over time could therefore be attributed to physical concentration during the freezing process and no metabolic processes needed to be invoked. A second group of variables, dissolved oxygen, chl *a*, DRP

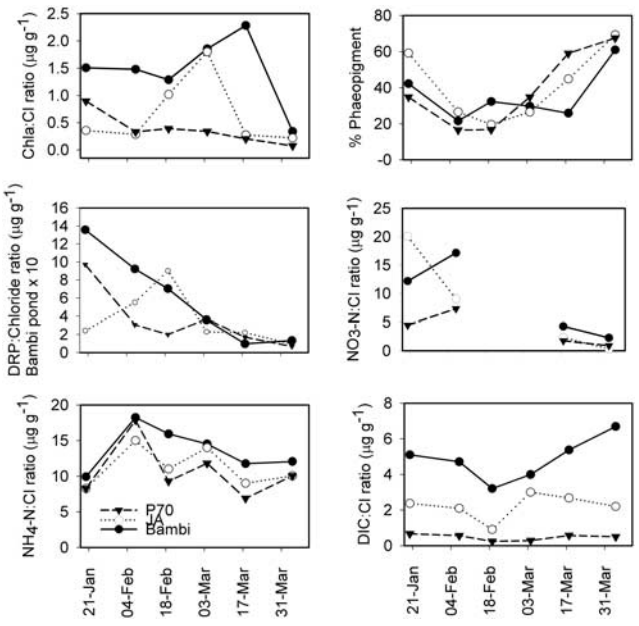

Fig. 1. Average ratios of mixed layer variables to chloride ($n = 3$) over the freezing process in the three McMurdo Ice Shelf ponds. For nitrate (centre right) values in late February and early March were below detection and could not be plotted.

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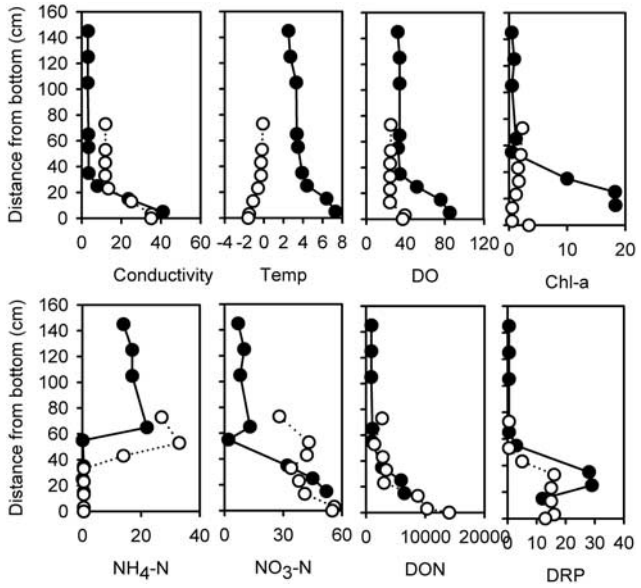

Fig. 2. Changes in indicator variables with depth in Legin pond (McMurdo Ice Shelf) between 12 February (solid symbols) and 31 March 2008 (open symbols). Conductivity mS cm^{-1}, temperature °C, DO mg l^{-1}; all others μg l^{-1}.

and NO_3-N did not behave conservatively. They were not correlated to chloride, or only weakly (in JA $0.05 > P > 0.01$ for DRP), suggesting that other processes were more important than physical concentration in determining the pattern of concentration change. A third group, DIC and NH_4-N, were weakly correlated to chloride in some ponds only, suggesting that a mix of freezing and non-freezing effects were important.

When the chloride ratios of the non-conservative variables for the three mixed ponds were examined over time, different patterns emerged (Fig. 1). In P70, chl *a*:Cl, declined consistently over time while the other two ponds increased initially after ice formation before subsequently declining. Percentage phaeopigment showed a consistent pattern across ponds, declining immediately after ice formation, but gradually increasing again after mid-February. Two other non-conservative variables, DRP and NO_3-N, tended to show similar trends across ponds, with higher ratios to chloride in January–February than at the end of March (Fig. 1). The exception was DRP:Cl in JA, where DRP:Cl was unusually low during the open water period, but increased after ice formation before declining thereafter. NO_3-N:Cl trends were obscured in mid-February when one analytical batch returned values below detection limits, though a general declining trend can be inferred from Fig. 1. NH_4-N and DIC, were moderately correlated to chloride. NH_4-N:Cl showed an initial increase immediately after ice formation, followed by a gradual and slow decline, whereas DIC:Cl tended to decline up to mid-February in all ponds before increasing after late February (Fig. 1).

Table II. Changes in mass of inorganic N and P and DOC in "mixed" ponds between 7 February–18 March 2008. ΔDRP etc are calculated as the change in mass between samplings, and normalized to the average pond area for the two samplings.

		7 February 2008			18 March 2008		
		Bambi	JA	P70	Bambi	JA	P70
Residual volume m^3		99	39	46	34	9.43	13.38
Residual area m^2		230	120	125	103	51	42
NO_3-N	mg m^{-3}	16	12	15	17	10	12.5
NH_4-N	mg m^{-3}	17	18	19	32	29.5	20.5
DRP	mg m^{-3}	86	5	6.5	26	9.5	11
DOC	g m^{-3}	24	13	20	69	57	72
NO_3-N	g pond^{-1}	1.6	0.5	0.7	0.6	0.1	0.2
NH_4-N	g pond^{-1}	8.5	0.2	0.3	0.8	0.1	0.1
DRP	g pond^{-1}	1.7	0.7	0.9	1.1	0.3	0.3
DOC	kg pond^{-1}	2.4	0.5	0.9	2.3	0.5	1.0
ΔNO_3-N	mg m^{-2} d^{-1}	0.15	0.11	0.16			
ΔNH_4-N	mg m^{-2} d^{-1}	0.09	0.13	0.18			
ΔDRP	mg m^{-2} d^{-1}	1.17	0.03	0.05			
ΔDOC	g m^{-2} d^{-1}	0.01	-0.01	-0.01			

As noted above, a deep brine layer was found in Legin pond after sampling from the ice cover in February (Fig. 2). On 12 February 2008, ten days after ice formation, the density gradient began *c*. 35 cm from the pond base. It contained higher concentrations of oxygen, DIC, chlorophyll and all N and P forms except NH_4-N (which was below the limits of detection), than the mixed layer. Comparisons of profiles of Legin pond at the start and end of the time series (Fig. 2) show how temperature dropped to the freezing point, with the lowest temperature of almost -2°C at the base of the water column. Changes in conductivity show how the top of the density gradient was gradually entrained into the mixed layer as physical processes concentrated the latter, but how deeper parts of the density gradient remained unchanged by freeze-concentration processes. This entrainment means that normalisation of concentrations to chloride does not

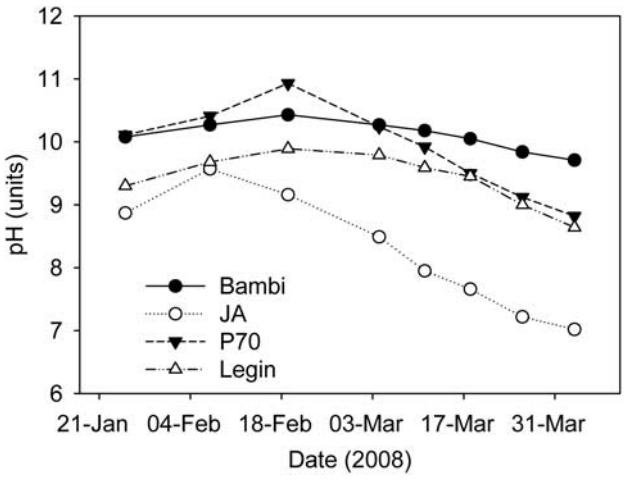

Fig. 3. Changes in average pH in the residual (unfrozen) mixed layer of three McMurdo Ice Shelf ponds over the course of pond freezing. (n = 2–3).

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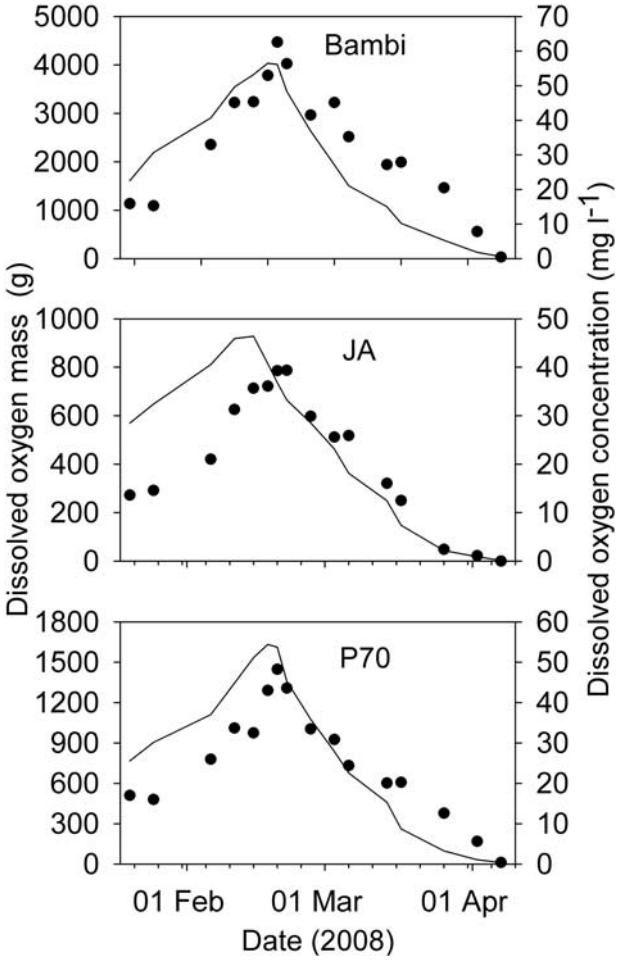

Fig. 4. Mass (lines) and average mixed-layer concentration (points) of dissolved oxygen in the three McMurdo Ice Shelf ponds during the freezing process. Mass is shown as a three-point running mean.

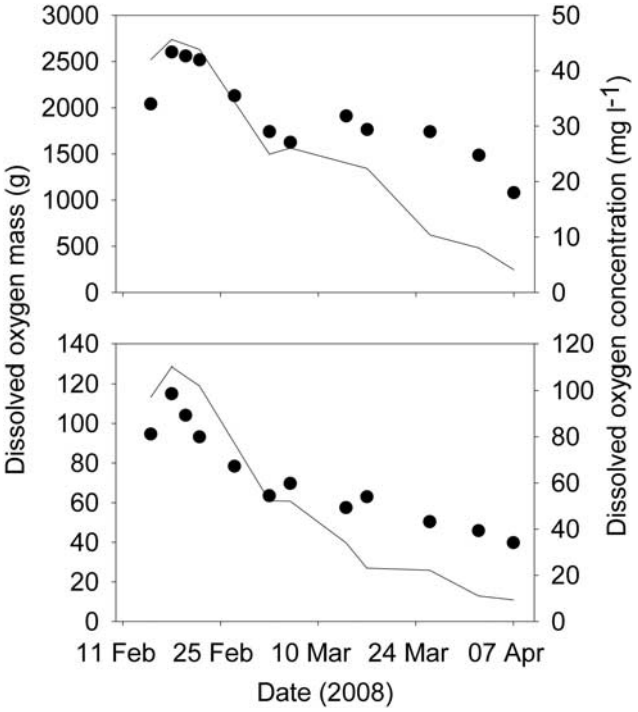

Fig. 5. Mass (line) and concentration (points) of dissolved oxygen in Legin pond (McMurdo Ice Shelf) during the freezing process. The top panel shows the mixolimnion (average concentration), the lower the density gradient (maximum concentration). Mass is shown as a three-point running mean.

completely remove the physical concentration effect. However, we can assume that variables that duplicated the pattern of change seen for chloride were affected primarily by physical processes, and those that did not were affected by biotic ones. Dissolved oxygen, chl *a* and DRP concentration decreased in both parts of the water column over the period, indicating non-conservative behaviour (Fig. 2). In contrast, changes to DON, NO_3-N and NH_4-N concentration with time tended to parallel changes in the conductivity profile.

Nutrient mass balance

In the three mixed ponds, ratios of DRP, NH_4-N and NO_3-N to chloride all tended to show generally declining trends after ice formation, indicating some degree of in-pond uptake. Estimates of mass in these mixed ponds shortly after freezing and later during the study allow uptake rates to be estimated. For these calculations we have used the dates of 7 February and 18 March. After 18 March, ponds had less than 50% remaining water, changes in the % remaining volume of the ponds began to diverge, the ratio of sediment area to volume was increasing rapidly and declining dissolved oxygen may have begun to affect biogeochemistry. Table II shows that areal rates of removal of NH_4-N and NO_3-N mass were rather

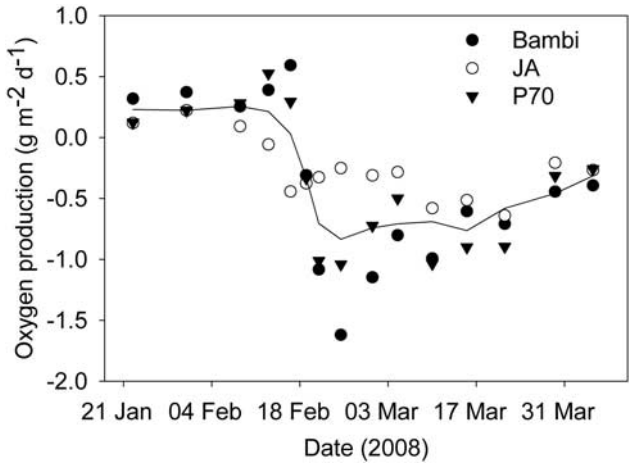

Fig. 6. The rates of change of oxygen mass, normalized to remaining unfrozen pond sediment area, for the three McMurdo Ice Shelf ponds. The points are for each pond, the line represents a three-time point running mean for data from the three ponds combined.

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Table III. Daily rates of phytoplankton carbon fixation during the early phase of the freezing process, expressed per unit volume and per unit chl *a*. After 20 February, carbon uptake rates were below detection limits. Each number is the mean of three replicates.

Date	Depth (cm)	Carbon fixation rate mg C m^{-3} d^{-1}			Chlorophyll-specific fixation rate mg C mg^{-1} chl *a* d^{-1}		
		Bambi	JA	P70	Bambi	JA	P70
20/01/08	10	4.9	1.7	12.5	0.15	0.36	0.32
	40	8.2	1.8	8.3	0.25	0.38	0.21
	80	6.5	1.5	11.6	0.25	0.31	0.32
26/01/08	10	6.6	4.6	14.2	0.27	0.97	0.39
	40	11.8	5.1	15.2	0.49	0.85	0.41
	80	9.3	4.6	11	0.34	1.17	0.32
07/02/08	10	12.9	1.3	5.6	0.41	0.18	0.39
	40	10.3	1.3	5.5	0.33	0.27	0.37
	80	7.5	13.1	5.4	0.22	0.32	0.32
20/02/08	10	Ice	Ice	Ice	Ice	Ice	Ice
	40	305	8.1	11.7	7.49	0.31	0.38
	80	175	5.4	9	3.47	0.1	0.29
	100	131			2.6		

similar in the three ponds, at 0.09–0.18 mg m^{-2} d^{-1} over 39 days, DRP removal was at 0.03 and 0.05 mg m^{-2} d^{-1} in JA and P70, while DRP removal was substantially higher in Bambi at 1.17 mg m^{-2} d^{-1}, where initial concentration was also highest.

pH

Mixed layer pH was high in all ponds at the start of the observations (Fig. 3). In all cases, immediately after initial ice formation pH increased still further, reaching a maximum in mid–late February. The pH change was smallest in Bambi pond which, having the highest DIC content, was probably the best buffered by carbonate equilibria. The biggest pH drop was seen in JA, which was close to neutral at the end of its freezing process.

Dissolved oxygen

Dissolved oxygen emerged as a variable for which physical processes appeared to play only a small role in determination of concentration. Mass and concentration of oxygen increased rapidly in all mixed layers after ice formation, reaching high, supersaturated concentrations, nearly two (mass) and four (concentration) times higher than open water by mid–late February (Figs 4 & 5). During this period of gas supersaturation, gas bubbles were observed to accumulate in the ice of the ponds, resulting in ice "whitening" and decreased light transmission. In late February, change of mass and concentration both rapidly switched from accumulation to loss, and from there onwards a near exponential decline was observed with time. Near anoxia was reached in JA and P70 by April.

In the density gradient of Legin pond, generally similar trends of dissolved oxygen were observed to those seen in the mixed layer (Fig. 5). Depletion of oxygen was, however, slower in this pond than the other three, with concentrations in early April still above atmospheric saturation throughout the profile. The density gradient maximum concentration always exceeded that of the mixed layer. The highest concentration that we observed, at just over 100 mg l^{-1}, equivalent to > 700% saturation in this brackish brine, was in the density gradient of Legin pond in late February. As freeze-concentration did not affect conditions in the density gradient, high dissolved oxygen concentrations there must have been due to local photosynthesis.

Oxygen mass balance

The change of oxygen mass was assumed to be driven primarily by the balance of gross photosynthesis (P), respiration (R) and ebullition (E), such that change of oxygen mass (ΔDO) = P - (R+E). Our best estimate of R is obtained towards the end of the study period when the water was no longer supersaturated with oxygen, hence E was zero, near-darkness prevailed and ΔDO = R. This yields a rate of whole pond respiration (R) of ~ 0.45 g O_2 m^{-2} d^{-1} (Fig. 6). At the end of February, the rapid reversal in trajectory of oxygen mass change implicates this as a time when P was declining rapidly. Photosynthetic oxygen flux will not have immediately fallen to zero, but by assuming that it did we can obtain a conservative estimate of E at that time, since ΔDO = (R+E). Measured (R+E) was ~ 1.0 g O_2 m^{-2} d^{-1}, yielding a value of E for late February of ~ 0.55 g O_2 m^{-2} d^{-1}. Oxygen concentrations earlier in February, when gross photosynthesis was still positive, were similar to those prevailing when this estimate of E was made. If we assume that (E+R) estimated above as ~ 1.0 mg O_2 m^{-2} d^{-1} prevailed in early February, when ΔDO = 0.25 g m^{-2} d^{-1}, P must then have been at least 1.25 g m^{-2} d^{-1}.

Measured photosynthetic rates

Immediately after ice formation, ponds tended to show similar rates of planktonic carbon fixation to those estimated during open water (Table III). There was also

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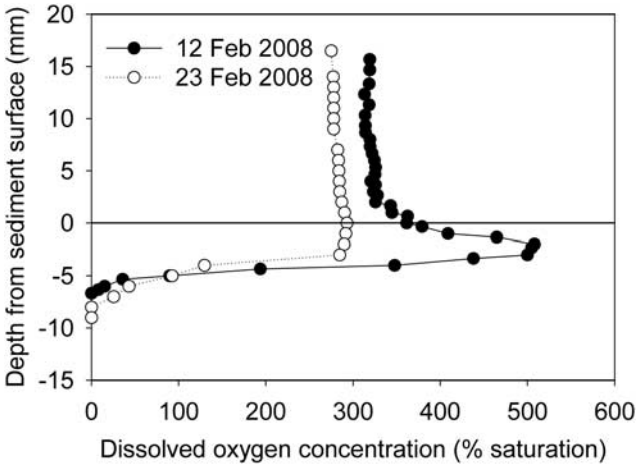

Fig. 7. Profiles of dissolved oxygen across sediment-water interface of Orange pond, (McMurdo Ice Shelf) at 30 cm depth. The point of origin on the y-axis is the estimated position of the sediment surface, which in this pond comprises a microbial mat.

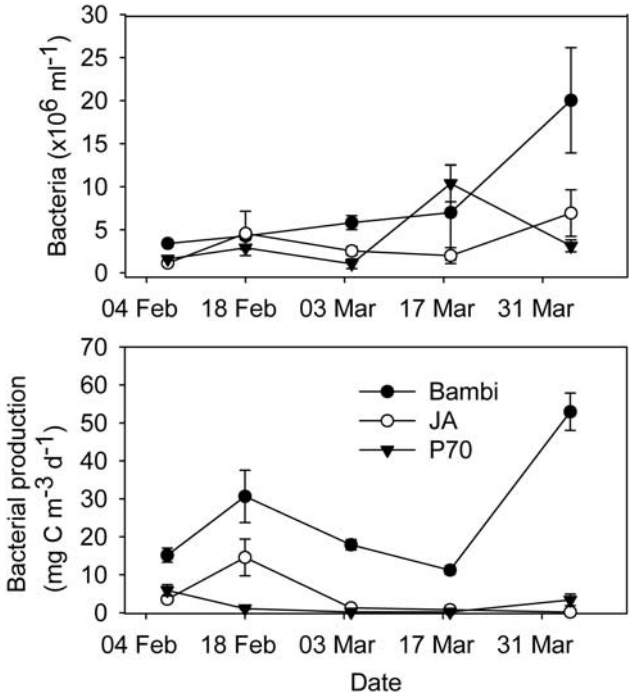

Fig. 8. Numbers and productivity of planktonic bacteria in three ponds on the McMurdo Ice Shelf.

little change in activity per unit chl *a*, with average rates mostly < 0.5 mg C mg^{-1} chl *a* d^{-1}, though Bambi showed a spike in photosynthetic activity on 20 February, the last date on which phytoplankton photosynthesis was detectable. This date also marked the rapid increase in the proportion of planktonic chl *a* in the "phaeopigment" form. Conversion of phytoplankton carbon uptake to oxygen evolution, on a mole-for-mole basis and normalized to residual pond area, shows that, apart from an anomalously high rate seen in Bambi pond on 20 February of 340 mg O_2 m^{-2} d^{-1}, the phytoplankton contributed 1–16 mg O_2 m^{-2} d^{-1}. On average, this was $< 1\%$ of the whole pond photosynthesis of ~ 1.25 g m^{-2} d^{-1}.

Benthic photosynthesis, estimated from rates of oxygen flux in Orange pond, can be combined with measured irradiance under ice to further indicate whether potential benthic photosynthesis and observed oxygen accumulation rates are compatible. Hawes *et al*. (2011) showed that early in February, mean daily irradiance immediately under ice was 225 W m^{-2}, approximately equivalent to ~ 450 μmol photons m^{-2} s^{-1} as photosynthetically active radiation (Kirk 1994), falling to 10 W m^{-2} (~ 20 μmol photons m^{-2} s^{-1}) by the beginning of March. Laboratory estimates of 827 ± 293 mg m^{-2} d^{-1} (average ± SD, $n = 6$) at 450 μmol m^{-2} s^{-1} are more than enough to fit the observed areal rates of increase (Fig. 6). Negative net photosynthesis was measured in laboratory incubations at 40 μmol photons m^{-2} s^{-1}, confirming that at the irradiance reported from the ponds in March, no net oxygen flux would have been possible. Plotting measured oxygen flux versus irradiance from our laboratory experiments gave a linear decrease between 40 and 200 μmol m^{-2} s^{-1}, from which we were able to extrapolate a near-dark respiration rate of ~ 250 mg m^{-2} d^{-1}.

In situ dissolved oxygen profiles through the benthic mats of Orange pond showed significant net oxygen flux through the diffusive boundary layer to the water column on 12 February, but much less on 23 February (Fig. 7). Calculation of flux for quadruplicate profiles on each of these dates showed yields of 176 ± 13 and 8 ± 1 mg O_2 m^{-2} d^{-1} (average ± SD, $n = 4$). The first of these values is broadly consistent with the estimated flux to the water columns of JA, P70 and Bambi during the first half of February (Fig. 6). The low flux on 23 February indicates that a date for the cessation of net oxygen production by benthic communities of mid–late February is reasonable.

Attribution of oxygen accumulation under thickening ice to benthic photosynthesis assumes that activity measured in shallow samples would be similar to that at greater depth. While we could not test this assumption, we were able to confirm that similar, high concentrations of benthic chl *a* were recorded across all depths in all ponds. Bambi (300–600 mg m^{-2}) and P70 (300–400 mg m^{-2}) having the highest chl *a* biomass and JA (200–400 mg m^{-2}) and Legin (200–300 mg m^{-2} in the mixed layer, but only 50 mg m^{-2} near the base of the density gradient) the lowest.

Bacterial abundance and thymidine uptake

The number of bacteria in the three mixed ponds remained rather constant through February and much of March, increasing slightly in all cases towards the end of the study period (Fig. 8). Bambi pond, the mixed pond with the

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largest residual volume at the end of the study period, showed a particularly rapid increase in numbers towards the end. Bacterial productivity also tended to decline until a final rise on the last sampling, though both Bambi and JA did show a small rise in activity in late February. Highest numbers and activity developed consistently in Bambi, though when expressed as activity per bacterium, the ponds yielded similar trends, with values generally between 0.1 and 4 ng C $(10^6$ bacteria$)^{-1}$ d^{-1} and tending to decline over time. Bacterial abundance and activity in stratified Legin pond formed part of a comprehensive micro-plankton study which will be reported elsewhere.

Discussion

This study has investigated changes that accompany the freezing process in four ponds on the McMurdo Ice Shelf close to Bratina Island. A high degree of conformity was seen in the behaviour of the ponds, with the powerful effect of freeze-exclusion of solutes playing a controlling role in the dynamics of many variables. Other variables were affected by both physical and metabolic processes, and these provide insights into the dominant microbial processes that were ongoing after the initiation of ice cover on the ponds in February, as well as the kinds of stress that the organisms in the ponds were subject to during the freezing process. Several of the non-conservative variables showed a distinct break in late February, as the ponds shifted from net accumulators of oxygen to net consumers. This allows us to divide the freezing period into an early, oxygen accumulating and a later, oxygen consuming phase.

During the early phase of freezing, the accumulation of dissolved oxygen under ice cover, at rates greater than can be explained by freeze-concentration, is evidence of ongoing net autotrophic carbon fixation. In this phase the ice transmitted 20% or more of incident irradiance (Hawes *et al*. 2011) and irradiance within the ponds was still high with ongoing 24 hour daylight. The end of the phase coincided with planktonic and benthic net photosynthesis falling below zero and the rapid increase in the proportion of phaeopigments in the planktonic pigment analyses.

Photosynthesis, oxygen and DIC

Phytoplankton photosynthesis made only a minor contribution to overall oxygen balance, due to low biomass and low biomass-specific rates of carbon fixation. Similarly low rates have been observed previously in McMurdo Ice Self ponds phytoplankton (Rae *et al*. 2000), where they were associated with chronic photoinhibition at low temperature. Other authors have suggested that nutrients, particularly nitrogen, may limit planktonic productivity (Hawes *et al*. 1993).

The observed oxygen fluxes in the study ponds were, however, consistent with the photosynthetic capacity of benthic mats in nearby Orange pond. While measuring oxygen flux in Orange pond rather than in the ponds where oxygen concentration changes were measured is not ideal, the broad similarity of light regimes (Hawes *et al*. 2011), oxygen accumulation rate and benthic biomass across all ponds, gives a degree of confidence that patterns of benthic photosynthesis measured in one can be extrapolated to all. (Orange pond had an average chl *a* concentration at 10 cm depth of 399 ± 156 mg m^{-2}, $n = 3$.) Measured rates were also similar to those reported previously from these ponds under open water (Hawes *et al*. 1999). Quesada *et al*. (2008) reviewed rates of photosynthesis of polar microbial mats, reporting values that, converted from carbon to oxygen at a 1:1 molar ratio, lie between 25 and 250 mg O_2 m^{-2} h^{-1}. Conversion of these to daily rates, gives ample scope to suggest that the rates observed and calculated here are in agreement with those obtained for similar ecosystems by other methods. Benthic communities appear to dominate biomass, photosynthesis and respiration in these ponds. A similar benthic dominance is seen in shallow Arctic pond systems (Ramlal *et al*. 1994).

Dissolved inorganic carbon and pH showed similar phases through the freezing process to oxygen. Up until around 18 February, as oxygen mass increased, pH was also increasing and DIC in decline; after this date both trajectories reversed. When calculated in a similar way to oxygen flux, DIC in Bambi, JA and P70 ponds declined at rates of 0.4, 0.3 and 0.2 g C m^{-2} d^{-1} between 7–19 February. Expressed as oxygen equivalents, at a 1:1 molar ratio, these declines are equivalent to 1.2, 1.0 and 0.7 g O_2 m^{-2} d^{-1}, similar to the rate of net photosynthesis calculated from changes in dissolved oxygen concentration (P-R = 0.8 g O_2 m^{-2} d^{-1}). This supports the view that the first phase of the winter period is one when carbon is accrued by benthic photosynthesis, with a shift from net photosynthesis to net respiration in late February, whereafter there was an accumulation of DIC and a corresponding decline in pH.

Stoichiometry of nutrient uptake

With the exception of Bambi pond, the ratio of accumulated mass of inorganic nitrogen:DRP in Table II was *c*. 7.5:1. This equates to a molar ratio of 16 and is close to the "Redfield Ratio" commonly ascribed to the average accumulation ratio of aquatic primary producers. It is therefore consistent with uptake of nutrients from the water column for incorporation into biomass. The molar ratio of N:P in Bambi pond was much lower, and there must have been additional processes that remove DRP from the water column. Webster-Brown *et al.* (in preparation) discuss a number of precipitation processes occurring during ice formation, which may be associated with this removal of DRP. While the N:P accumulation ratio matched the Redfield Ratio, matching N and P to C accrual was more problematic. The Redfield Ratio suggests a C:N accumulation at ~7:1 and a C:N ratio of ~9:1 has been reported for microbial mats from these ponds

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(Howard-Williams *et al*. 1989), supporting the prevalence of the ratio in mat biota. Uptake of inorganic nitrogen over the 39 day period in Table II was of the order of 0.2–0.3 mg m^{-2} d^{-1} and, if all of this nitrogen were incorporated into biota this would support a total accumulation of only 2–3 mg C m^{-2} d^{-1}, or 5–8 mg O_2 m^{-2} d^{-1} at a 1:1 molar equivalency. There is a clear discrepancy during the early phase of freezing between the net fixation of carbon, estimated from either DIC decline or DO increase (~ 300 mg C m^{-2} d^{-1}) and the uptake of nutrients. Should net carbon uptake have been being translated to growth, it must be dependent on recycled nutrient from the sediment underlying the mat, where high interstitial NH_4-N and DRP have been recorded (Hawes *et al*. 1993, Vincent *et al*. 1993).

It is not, however, uncommon for microbial mats to show high biomass and carbon fixation, but low growth rate. Elsewhere this has been related to limited external supply of "new" nutrient and intense internal nutrient cycling within mat communities (Ludwig *et al*. 2006). Such a situation may apply in the study ponds, with the "excess" fixed carbon providing an energy source for diverse microbial processes rather than simply supporting phototrophic growth (Nold & Ward 1996). Howard-Williams & Hawes (2007) argued that one reason for the success of microbial mats in Antarctic ponds is that, in the absence of bioturbation, they are able to gradually accumulate, retain and recycle nutrients from oligotrophic overlying water. It is possible that freeze-concentration was making these nutrients more available to the benthos. It is notable that in many cases DRP, NH_4-N and NO_3-N absolute concentrations varied little during February and March, suggesting that uptake was maintaining a "baseline" nutrient concentration. The small uptake of N and P that occurs for many weeks after ice formation may provide a significant opportunity for the sequestration of new nutrient into benthic mats, for immediate or subsequent growth.

As expected from the strongly conservative behaviour of the organic components of pond waters, we saw little change in the mass of DOC over time (Table II). CDOM was also found in high concentrations and showed conservative behaviour and Belzille *et al*. (2002) have previously shown that large organics are effectively excluded during lake ice formation. This supports previous suggestions that the majority of the dissolved organic material in these ponds, including DON and DOP, comprises accumulated refractory materials (Howard-Williams & Hawes 2007, Hawes *et al*. 2008). While a small dynamic pool of dissolved organics may have been present, any change was swamped by the high background concentrations of the apparently refractory organic material.

Bacterial activity

The absence of any clear tendency towards an increase in bacterial numbers or activity after ice formation (Fig. 8) is compounded as this figure contains no allowance for reduction in pond volume. The number of bacteria per pond is therefore showing a general decline over time, and suggests that bacteria are either included in the ice mass during freezing or were being removed from the water column by some other process. Only towards the end of the freezing period did bacterial numbers consistently increase. Whether this represents growth and/or concentration of the existing population or a pulse of bacteria with preferences for the conditions or darkness, increasing organic and inorganic solutes and declining oxygen developing at the time cannot be determined from our data. However, despite the high and increasing absolute DOC, DON and DOP concentrations, the pond environment was not conducive to planktonic bacterial proliferation and it seems unlikely that water column bacteria played a major role in evolution of water chemistry.

Markers of anaerobic microbial activity appeared slowly in the ponds, primarily at depth. High concentrations of H_2S only appeared late in the observational period and primarily in JA when dissolved oxygen had declined near to detection limits (Webster-Brown *et al.* in preparation) and N_2O and methane increased only slightly in the density gradient of Legin pond at the end of the study (authors' unpublished). Anaerobic processes including sulphate reduction, denitrification and methanogenesis are known to occur in sediment underlying microbial mats in these ponds (Downes *et al*. 2000, Mountfort *et al.* 2003) but may have remained restricted to underlying sediments because of high water column DO. Only relatively small parts of the deeper ponds will therefore have been subject, in a liquid state, to the highly reducing and potentially toxic conditions that would have ultimately accompanied anoxia.

Attempts to obtain cores in late March yielded microbial mat, but corers could not be pushed into the sediments, which were frozen. Loss of heat to the sediments was observed, implying temperatures below freezing in the sediments, and the possibility that they may freeze before mats and water cannot be discounted. Should this be the case it provides another possible explanation of the lack of products of anaerobic metabolism.

Implications for Antarctic pond ecosystems

An improved understanding of the stresses imposed on Antarctic pond biota during the process of freezing has emerged from this study. At the beginning of our study we proposed that physiological stresses imposed by darkness, osmotic stress and anoxia during the freezing process would result in the organisms that remain hydrated and metabolically active, being more challenged than those that are frozen and metabolically inactive. Contrary to our expectations, our results show that the majority of the volume and sediment surface area froze before the onset of strongly reducing conditions. This allowed most pond biota to avoid exposure to hypoxia and high concentrations of sulphide in the liquid, and presumably metabolically active,

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state. Instead, the conditions that most pond biota were exposed to before they froze allowed for ongoing photosynthesis, aerobic respiration and nutrient uptake. Rather than strongly reducing conditions, the stresses they endured were hyperoxia, high pH, low temperature and a gradual increase in osmolarity. Tolerance of the high concentrations of dissolved oxygen and pH that biota was exposed to during the early period of freezing is probably inherent in Antarctic mat communities, given that photosynthesis within the diffusion-dominated mat environment often produces very high concentrations of oxygen even in polar conditions (e.g. Hawes *et al*. 1999). Super-saturation can also be found in the deeper parts of stratified polar ponds under ice-free conditions (Hawes *et al*. 1993). Such extreme conditions are experienced in other ice-entombed environments in Antarctica, for example cryoconite holes (Tranter *et al*. 2004).

The severity of conditions during the onset of freezing in Antarctic ponds varies according to water depth. While freezing imparts its own physiological stresses, it may also protect the majority of the benthic community from exposure to the most extreme physical and chemical stresses in the liquid state. The areas that are exposed to extreme high conductivity, low oxygen and low temperature represent small fractions of the ponds with a depth of < 1 m. In ponds with a depth of > 1.5 m, however, a proportion of the sediment area will be exposed to more extreme conditions. Such parts of the deep ponds are rarely sampled, but may concentrate sufficiently to produce a hypersaline anoxic, sulphidic, super-cold brine over winter (Wait *et al*. 2006), while in summer they have reduced salinity, high temperature, are supersaturated oxygen, low in DIC. They may be amongst the most variably, stressful habitats in Antarctica, and perhaps on the planet. We noted that benthic biomass on the deepest pond that we sampled declined at extreme depth, but we were unable to undertake detailed examination of microbial communities in those deepest parts of the ponds. We are only beginning to understand how extreme that environment is and as yet have no understanding of what kinds of metabolic properties are present to allow organisms to persist there.

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