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Microbial population responses in three stratified Antarctic meltwater ponds during the autumn freeze

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Abstract: The planktonic microbial communities of three meltwater ponds, located on the McMurdo Ice Shelf, were investigated from the end of January 2008 to early April, during which almost the entire pond volumes froze. The ponds were comprised of an upper mixed layer overlying a salt-stabilized density gradient in which planktonic communities were primarily embedded. Plankton comprised all components of the "microbial loop", though carnivorous protists were rare. As the ponds froze and light became increasingly limited, it was expected conditions would induce physiological changes altering the functional role of autotrophic and heterotrophic microplankton within the ponds. The results showed that microbial groups responded to the onset of winter by declining in abundance, though an exception was the appearance of filamentous cyanobacteria in the water column in March. As freezing progressed, autotrophs declined more rapidly than heterotrophs and grazing rates and abundances of mixotrophic and heterotrophic organisms increased. Grazing pressure on bacteria and picophytoplankton also increased, in part explaining their decline over time. The results indicate that stressors imposed during freezing select for increasing heterotrophy within the remaining microbial communities, although all components of the food web eventually decline as the final freeze approaches.

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Key words: bacteria, ciliates, flagellate grazing, food webs, planktonic communities

Introduction

In the cold desert of continental Antarctica, meltwater ponds, though liquid for only a few weeks or months in summer, can be havens of microbial activity (Vincent & James 1996, Howard-Williams & Hawes 2007). Organisms that inhabit these ponds must, however, tolerate many months of winter freezing as well as exploiting the much briefer period when liquid water is available and conditions suit growth and development (Vincent & Vincent 1982, Howard-Williams & Hawes 2007, Vincent *et al.* 2009).

The transition between open water in summer and total freezing in winter is gradual with a slowly diminishing pool of liquid water persisting beneath the ice cover for several months before complete, or near complete freezing occurs (Schmidt *et al.* 1991, Hawes *et al.* 2011a, 2011b). The transition is characterized by a rapid decline in irradiance and temperature, increasing concentration of dissolved salts, and rises and falls in dissolved oxygen (DO) and inorganic carbon concentrations. An understanding of how pond ecosystems are affected by these annual changes is needed to complete the picture of how communities in one of Antarctica's commonest inland habitats (Howard-Williams & Hawes 2007) are structured by the extreme climate.

While the biomass of Antarctic ponds is usually dominated by benthic communities (Vincent & James 1996, Hawes *et al.* 2011b), simple planktonic communities are also present and active during the summer. These communities have simplified trophic structures in which crustacean zooplankton and other higher food web components are absent. Instead they are comprised mostly of a diverse array of bacteria, picophytoplankton, phytoplankton (often mostly flagellates) and heterotrophic microzooplankton (James *et al.* 1995, Hawes *et al.* 1997). The microzooplankton are often dominated by flagellates and a diverse range of small ciliates. The ciliates, although primarily bacterivorous, are thought to be the top trophic level of the food web in these systems (James *et al.* 1995). In these ponds, as it is in many Antarctic and Arctic freshwater habitats, mixotrophy is a common strategy within the flagellate population, which adds complexity to the microbial food web (Laybourn-Parry *et al.* 1997, James *et al.* 1998, Laybourn-Parry & Marshall 2003).

All of these organisms need to tolerate a wide range of environmental conditions driven by the extreme seasonal changes found in Antarctica. Summer conditions are recognized as the most benign (Howard-Williams *et al.* 1989) with open water and temperatures consistently above freezing resulting in a period of maximum photosynthetic growth. In contrast, in winter it has recently emerged that many of the planktonic organisms are frozen into pond ice, and remain dormant, with flagellates primarily surviving as

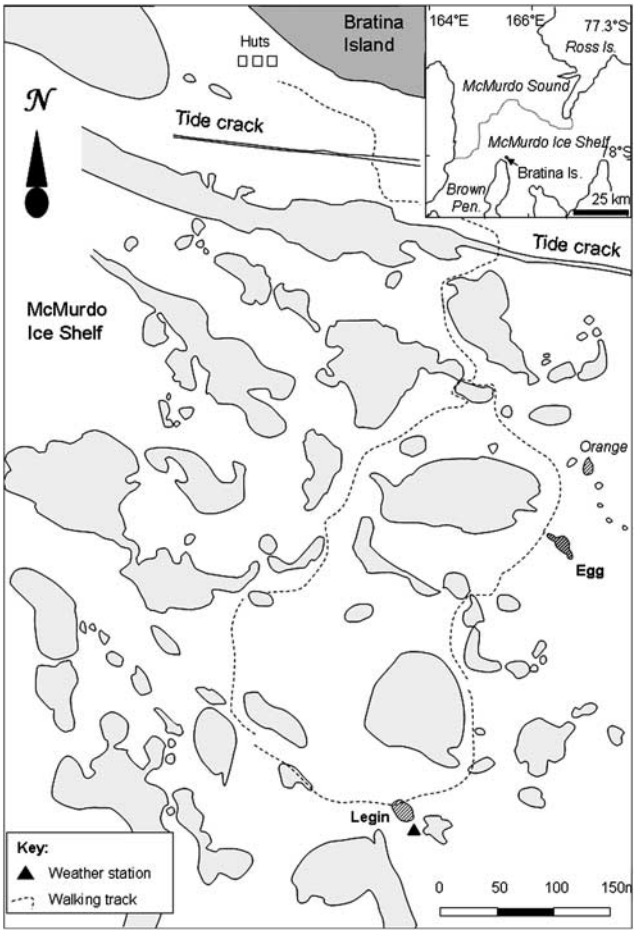

Fig. 1. Location map of study area, to the south of Bratina Island on the McMurdo Ice Shelf. Ponds are shown in grey, main study ponds are labelled.

encysted cells (Foreman *et al.* 2011). The transition periods of spring and autumn represent the most physiologically challenging times. During the spring melt rapid changes occur in such factors as temperature, salinity, and light as the ponds defrost. In the autumn transition extreme changes also occur as freezing progresses over several months. Slow freezing, however, results in a significant period when communities remain metabolically active while irradiance is reduced to virtual darkness in the remaining liquid water (Schmidt *et al.* 1991, Hawes *et al.* 2011a, 2011b). What happens to microbial populations during the transition from summer to winter is, however, unknown. How these organisms behave during the months of near-darkness may be significant to their annual energy balance, how they persist from year to year and also influence how communities are structured over the long-term. Because of access difficulties, there have been few attempts to follow changes over autumn.

This study addressed this information gap by determining how planktonic microbial populations respond to the changing environmental conditions during the two to three month period when liquid water remains under forming ice, following the open water summer period but before total winter freezing. Our working hypothesis was that as conditions change after ice formation there would be a corresponding decline in autotrophic numbers, shifts in species composition, and a change in nutritional strategies. As irradiance declines and photosynthesis is no longer effective, species of flagellates that can increase bacterivory will remain active, while those that cannot will decline and enter resting stages. This study forms part of a series of linked investigations made possible by the late-season extension of New Zealand and US Antarctic logistic support as part of the 2007–08 International Polar Year (Hawes *et al.* 2011a, 2011b, Webster-Brown *et al.* 2011).

Study site

The McMurdo Ice Shelf (MIS) is a small part of the Ross Ice Shelf, located in the south-west corner of the Ross Sea (Fig. 1). This study was conducted on a slow moving part of the MIS, trapped between Bratina Island and Brown Peninsula. This area has a rolling surface relief, *c.* 10 m high, with most of the surface covered by > 100 mm of marine-derived sediment. The dark sediment promotes locally elevated temperature and melting, giving rise to a large number of sediment-lined, meltwater ponds lying between rounded mounds of ice-coated sediment.

In this study we focus on three small ponds, unofficially named "Orange pond", "Egg pond" and "Legin pond", with surface areas of 30, 48 and 127 m^2 and summer depths of 115, 138 and 160 cm respectively. All three of the ponds were density stratified, due to a freeze-concentration of salts into bottom waters (Wait *et al.* 2006), which results in a mixed upper water column overlying a very steep, salt-induced density gradient in which stratified planktonic populations can develop (Hawes *et al.* 1997). All ponds were located within 40 minutes walk of our field laboratory on Bratina Island.

Methods

Pond sampling

Physical parameters of the three study ponds were sampled between late January, shortly before ice formed, to late March or early April 2008. Pond volumes were calculated using bathymetry data (Hawes *et al.* 2011a). Intensified chemical and biological sampling of each pond occurred on three occasions, early to mid-February (6–14 February), early March (5–11 March) and late March–early April (28 March–3 April) 2008. Because the ice surface varied during the course of the study, all sample depths were normalized to distance from the pond floor.

Dissolved oxygen, temperature and conductivity

Profiles of temperature and conductivity were obtained using a calibrated C-90 conductivity-temperature meter (TPS Ltd, Springwood, Australia) attached to a graduated

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pole and were conducted up to ten times per pond (Legin pond) over the study period. Dissolved oxygen concentration was measured, at 5 cm intervals down the water column, using either a Presense fibre optic oxygen sensor or a Unisense Clarke-type glass microelectrode connected to a portable picoammeter. Full methods are described in Hawes *et al.* (2011a, 2011b).

Ice transparency

Once ice had formed and could be walked on, spectral ice transparency was measured approximately weekly. This was accomplished by lowering a spherical irradiance collector connected by an optical fibre to an Ocean Optics S2000 spectrometer underneath the ice cover and recording three spectra at different locations. The light collecting element on the fibre optic cable was pushed down through a 50 mm hole in the ice. A small float attached to the cable just behind the collecting sphere brought the latter to the underside of the ice. It was pushed out away from the hole to minimize "hole effects". The under-ice spectra were combined with spectra taken above the ice to calculate ice transmissivity. Full methods are described in Hawes *et al.* (2011a, 2011b).

Water sampling

Water samples were collected at *c.* 10 cm intervals, using an acid rinsed, 5 mm internal diameter plastic hose inserted to the required depth and connected to a hand-driven peristaltic pump (Hawes *et al.* 2011b). Samples were analysed for pH, dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), nitrate-N (NO_3-N), ammonium-N (NH_4-N), dissolved reactive phosphorus (DRP) and chlorophyll *a* (chl *a*), either on site or after preservation and return to New Zealand. Full analytical methodology for these determinants is given in Hawes *et al.* (2011a, 2011b).

Water samples were also analysed for biological components: phytoplankton (abundance and speciation), microzooplankton (abundance and speciation), prokaryotic and eukaryotic picophytoplankton abundance, bacterial abundance and bacterial production. Additional samples were also taken for flagellate grazing experiments.

Phytoplankton and microzooplankton

Where possible live samples were examined to determine species present, but for routine estimation of abundance of phytoplankton ($>$ 2 μm diameter) and microzooplankton ($<$ 200 μm), 25–50 ml subsamples of water were also preserved in Lugol's iodine at a final concentration of 1% and stored in the dark. In the laboratory, subsamples were pipetted into a nannoplankton counting chamber (PhycoTech Inc) and/or gridded Sedgwick-Rafter Counting Cell (PhycoTech Inc) before being enumerated and examined for species composition using a Leitz compound microscope. The settling volumes and magnification used for counts were adjusted according to both changes in the concentration of populations over time and with depth, to allow numerically significant counts to be obtained.

Phytoplankton were identified to the lowest possible taxonomic level, usually species or genus. Some phytoflagellates were difficult to distinguish when preserved in Lugol's solution, but additional samples from grazing experiments (see below), aided in retrospective species identification. Microzooplankton were identified in the same samples, to genus or group level.

Picophytoplankton and bacterial numbers

Duplicate picophytoplankton samples were prefiltered through 2.0 μm Nuclepore filters, preserved with formaldehyde at a 1% final concentration and frozen until analysed. Bacteria were treated in the same way but were not prefiltered. The abundance of eukaryotic, prokaryotic picophytoplankton ($<$ 2 μm), and bacteria were all measured using flow cytometry on a FACSCalibur instrument (Becton Dickinson, Mountain View CA, USA). Picophytoplankton populations were counted using their natural fluorescence. Bacterial samples were stained with SYBRII stain (Molecular Probes Inc) at a concentration of 10^{-4} of stock solution and incubated in the dark for 10–15 min before being analysed (Lebaron *et al.* 1998). Analysed volumes for all counts were calculated using Trucount™ (Becton Dickinson) beads as a tracer.

Grazing experiments

Experiments were conducted to estimate flagellate grazing on bacterial and picophytoplankton populations. We used fluorescently labelled microsphere (beads; Polysciences Inc, Washington PA, USA) 0.5 μm and 1.0 μm beads to represent bacteria and picophytoplankton. Our experimental design followed that of Sherr *et al.* (1987) as modified by Safi & Hall (1999). A 250 ml quantity of pond water was poured gently into a 500 ml polycarbonate bottle and allowed to recover from handling for 30 min before the fluorescently labelled microspheres were added. Once beads were added the bottles were gently mixed to achieve a homogeneous distribution. Each feeding experiment was run in duplicate at ambient temperature. Duplicate subsamples of between 1 and 75 ml were taken after 30 min. Samples for flagellate enumeration were fixed 1:1 with ice cold glutaraldehyde (2% final concentration) for 1 hr, filtered onto 0.8 μm polycarbonate filters which were mounted on slides and analysed under florescence using a Leica compound microscope following the methods described in Safi & Hall (1999). Trials conducted at T0 during the study indicated that $<$ 5% of beads enumerated could be those trapped under cells rather than ingested.

The optimal concentration of labelled food particles is *c.* 30% of the total natural bacterial abundance (Sherr *et al.* 1987) and therefore an estimate of abundance of prey items

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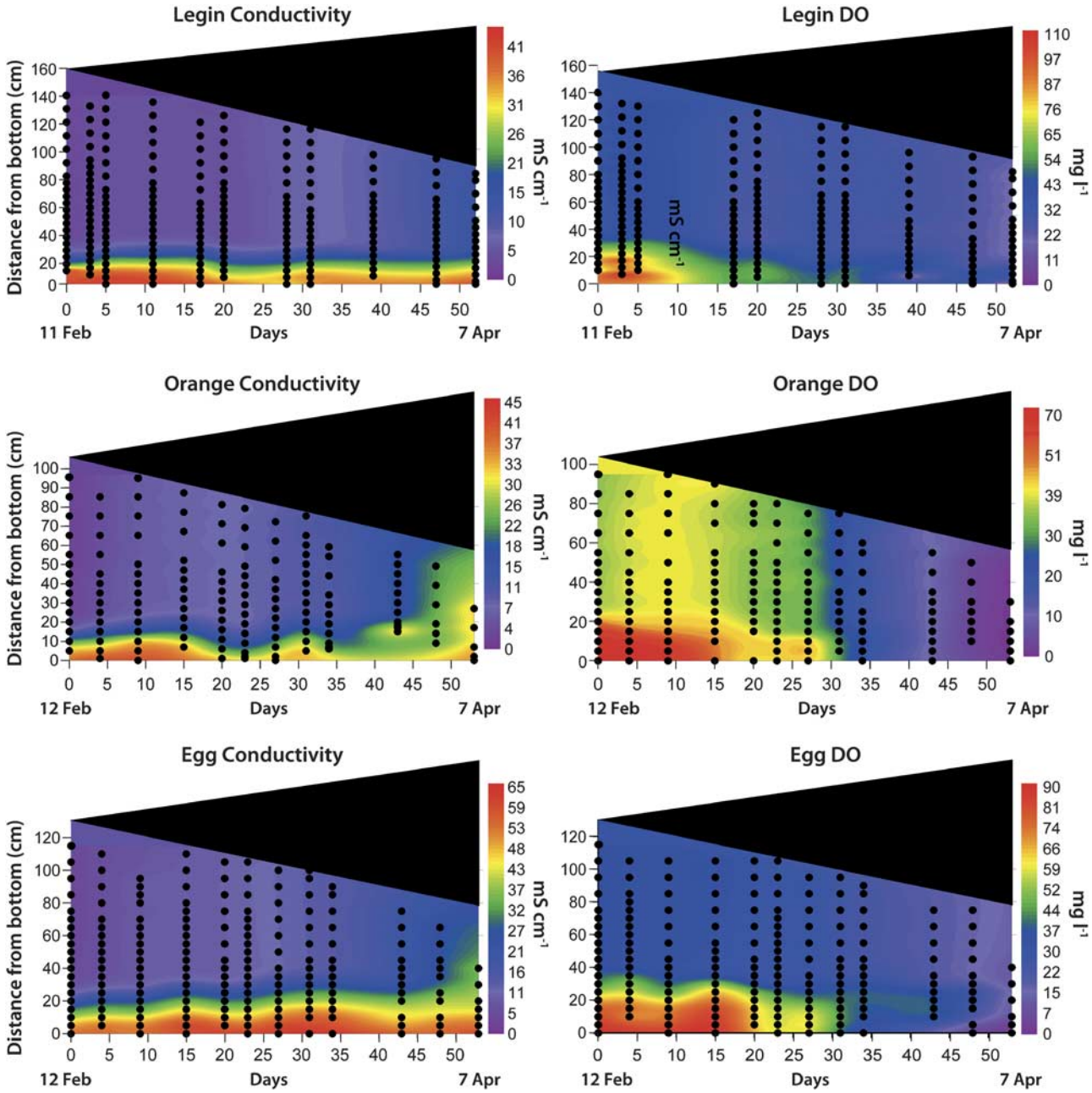

Fig. 2. Development of vertical profiles of conductivity and dissolved oxygen (DO) during progressive freezing in our three stratified ponds on the McMurdo Ice Shelf. The blacked out region represents ice growth with time. Ice both froze downward and expanded upward raising the level of the pond/ice surface while reducing the remaining water volume. ● = sampling points.

is required when planning experiments. For this previous publications from these ponds were used, however, the unexpectedly large range of concentrations of bacteria and picoplankton we observed lead to this 30% guideline being exceeded at times. Results are reported only where tracer concentrations were below 40% of ambient for bacteria grazing experiments. Similarly, we overestimated the picophytoplankton abundance at times which will have induced a prey saturated environment (Fenchel 1982). Picoplankton results are still presented, though provide only an indicative measure of grazing potential.

Bacterial productivity

Bacterial production was measured with the ^{3}H-thymidine incorporation method as described in Smith & Azam (1992) following the full methods as described in Hawes *et al.* (2011b).

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Results

Nocturnal ice cover was observed on all of the ponds in late January and persistent ice formed on 2 February 2008. The thickness of ice on the three ponds increased steadily at rates which averaged 1.4 cm day^{-1} (linear regression of time vs ice thickness). By the end of the study period each pond had between 92 and 97 cm of ice, with *c.* 32 and *c.* 34 cm of water remaining in Orange pond and Egg pond respectively, while the deepest pond, Legin pond, still retained over 80 cm of liquid water. In Legin pond this represented 15% of the pond's original water volume while only 10% of the sediment remained unfrozen. In Orange pond and Egg pond *c.* 8% of the ponds original water volume remained unfrozen.

Ice transparency

Immediately after ice formed it was clear and highly transparent, but transmission of irradiance declined over time as the ice became opaque through the inclusion of gas bubbles and its thickness increased. Maximum transmission was at *c.* 570 nm. Transmission of wavelength-integrated irradiance (440–700 nm) fell from 18, 20 and 38% in mid-February in Legin, Orange and Egg ponds respectively, to 3.6, 0.3 and 3.4% on 3 March and to 1.7, 0.1 and 0.5% by the end of March. In absolute terms, the combination of declining incident irradiance and ice transparency resulted in a fall of more than two orders of magnitude in daily average subsurface irradiance. Irradiance dropped from an average for the three ponds of 80 W m^{-2} in mid-February, to between 2 and 0.2 W m^{-2} in March.

Water column conductivity, temperature, pH, DIC, DOC

Profiles of conductivity and temperature taken immediately after the ice had formed showed that the three ponds all had vertical structure in their water column, primarily imposed by salinity. Each pond comprised an upper mixed layer (mixolimnion), overlying a density gradient (chemocline) in which conductivity increased steadily with depth from 15–35 cm above the pond floor to the base. In Legin and Orange ponds, conductivity increased from *c.* 3 mS cm^{-1} in the mixolimnion to over 40 mS cm^{-1} at the base of the pond (Fig. 2). Egg pond was higher in conductivity than the other two, ranging from *c.* 4 mS cm^{-1} to over 60 mS cm^{-1} (Fig. 2). As ice formation progressed, freeze exclusion of salts at the ice-water interface resulted in the mixolimnia steadily increasing in conductivity and gradually entraining the chemocline as density equilibration proceeded. By late March–early April, the entrainment of the higher salinity bottom waters meant Egg and Orange ponds were more or less completely mixed while Legin pond remained stratified (Fig. 2). Once fully mixed as their remaining volume was reduced Orange pond reached a maximum conductivity of *c.* 34 mS cm^{-1} and Egg 37 mS cm^{-1}. In Legin pond, which was never fully mixed, conductivity reached 14 mS cm^{-1} in the remaining mixolimnion by the end of the study period.

Initially, temperature in the surface waters of the three ponds ranged from 2°C in Egg pond to 4°C in Orange pond. Deep water temperature in all three ponds exceeded this with maximum temperature of 6, 7.8 and 8.4°C in Egg, Legin and Orange ponds. In Legin and Orange ponds the highest temperature was at the bottom, while Egg pond had its temperature peak within the chemocline *c.* 25 cm from the bottom. By early March, temperature profiles had reversed, with all ponds showing mixolimnion temperatures at or below zero, but with bottom temperatures even lower. By late March–early April, water temperatures had dropped to between -0.3°C and -2.7°C, with temperatures throughout appearing to be at or close to freezing for the salinity at each depth.

Changes in DO concentration profiles were complex. At the onset of freezing, DO in the mixed layers was close to atmospheric saturation (13–14 g m^{-3}), while the deeper layers were markedly supersaturated, with DO concentrations exceeding 60 g m^{-3}. Immediately after ice formation, DO concentrations increased rapidly in all three mixolimnion, and to a lesser extent in the chemoclines. Increase in the mixolimnion was probably a combination of freeze-concentration and photosynthesis, while that in the chemocline can be attributed to photosynthesis alone. Freeze-concentration describes the process that occurs when solutes such as DO are concentrated into the reducing liquid volume as it is reduced by ice formation. These increases were maintained until late February, after which time concentrations began to decline, with decline most rapid at the base of the water column. Declines can only be attributed to net oxygen consumption, since ongoing ice formation would otherwise have led to concentration increase. The decline in the mixed layer was clearly sufficient to offset ongoing freeze-concentration. Dissolved oxygen reached zero at the bases of Egg and Orange ponds by the end of the study period, although not in Legin pond. pH paralleled DO concentration, increasing initially and then declining through March and into April.

Dissolved inorganic carbon concentration was highest in the chemocline in all ponds. For example, on 7 March 2008, DIC increased from the surface to the maximum sampled depth in Egg from 84–150 g m^{-3}, in Orange pond from 127–562 g m^{-3} and in Legin pond from 22–55 g m^{-3}. Temporally, DIC behaved oppositely to DO and pH, declining immediately after ice formation then increasing from late February onwards, at all depths. Again, this is potentially attributable to net photosynthetic uptake initially exceeding the effects of freeze-concentration until irradiance declined, though the impact of precipitation cannot be excluded.

Dissolved organic carbon increased in the mixolimnion as pond volume declined, but was consistently highest in the chemocline, where no substantial changes in concentration were detected over the study period. In February the lowest

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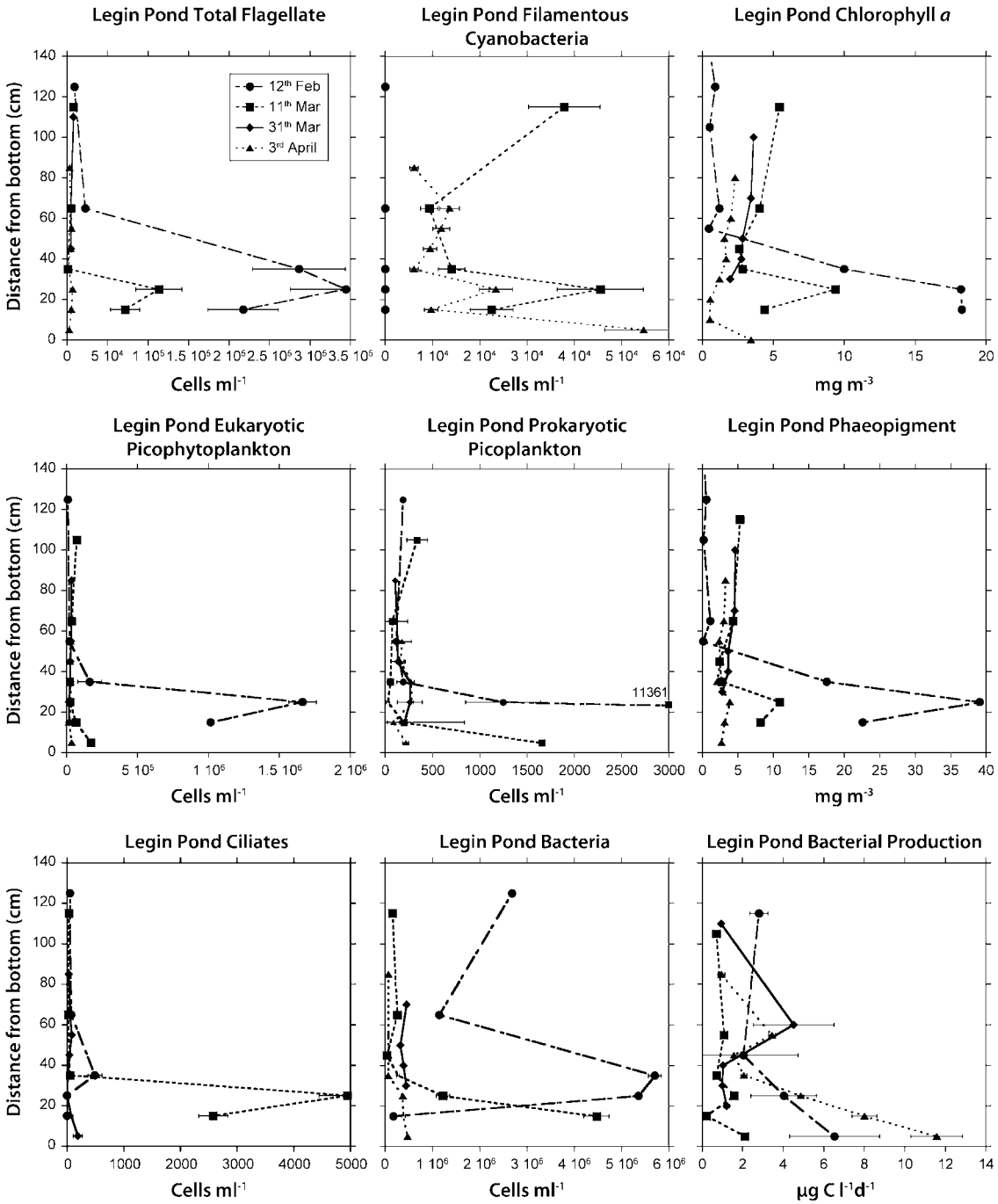

Fig. 3. Vertical profiles of nine biological water column components (total flagellates, filamentous cyanobacteria, chlorophyll *a*, phaeopigment, eukaryotic and prokaryotic picophytoplankton, ciliates, bacteria and bacterial production) in stratified Legin pond during four major samplings in autumn. Profiles are shorter as ice thickens. Error bars equal ± 2 standard errors. Legin pond is an unofficial name.

value of 11 mg m^{-3} was reported in the mixed layer of Legin pond, though by April this had increased to 38 mg m^{-3}. The magnitude of this increase is similar to that of conductivity and hence consistent with simple freeze-concentration. Orange pond had the highest reported DOC concentration *c.* 5 cm from the bottom of 380 mg m^{-3} in late March while at the same position Legin pond was 130 mg m^{-3}.

Nutrients

Nutrient dynamics in Egg and Orange ponds followed similar trajectories, while Legin pond was substantially different. At the onset of ice formation, the mixed layer of Legin pond contained undetectable concentrations of DRP, whereas Egg and Orange ponds contained 12 and 5 mg m^{-3}

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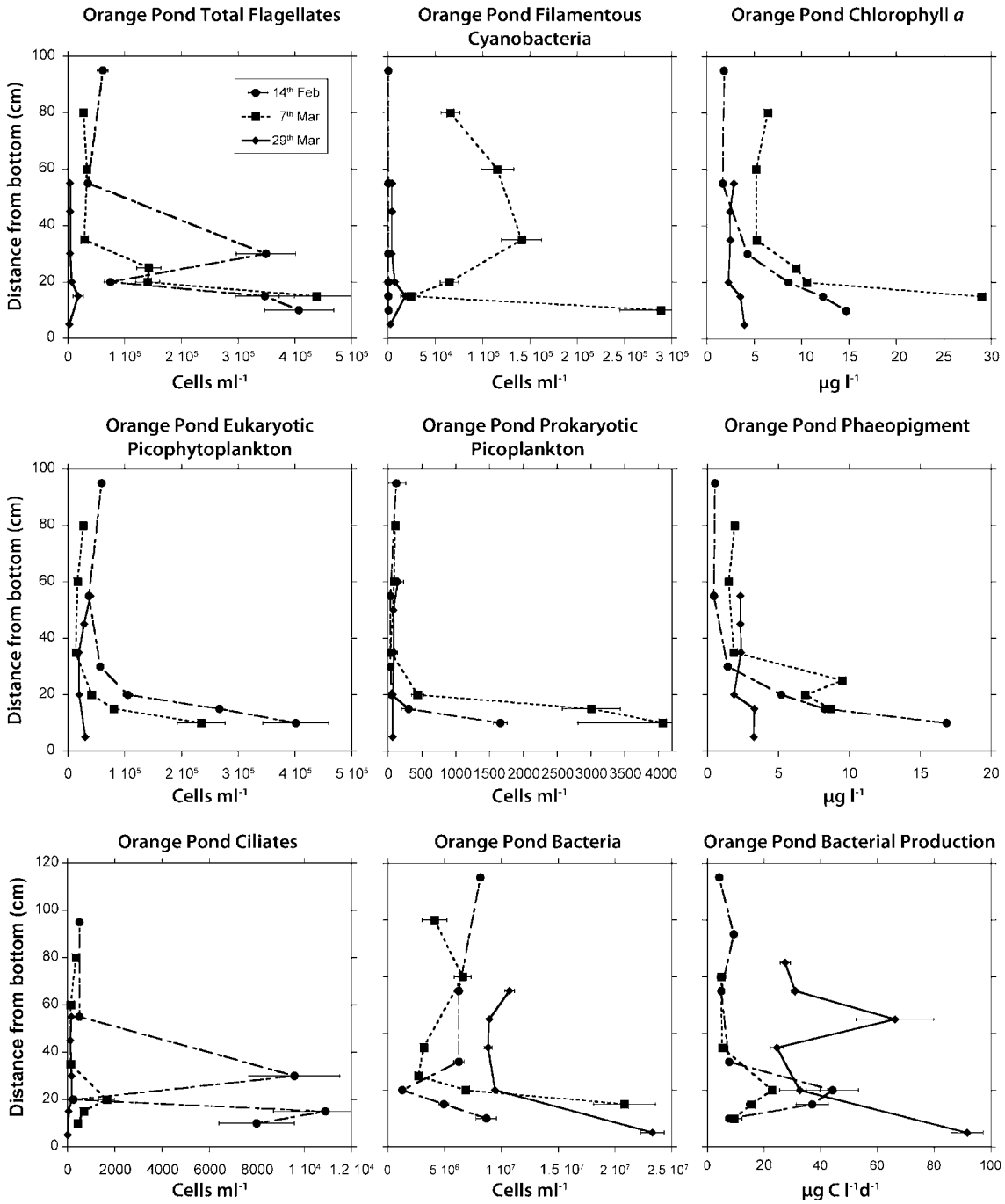

Fig. 4. Vertical profiles of nine biological water column components (total flagellates, filamentous cyanobacteria, chlorophyll *a*, phaeopigment, eukaryotic and prokaryotic picophytoplankton, ciliates, bacteria and bacterial production) in stratified Orange pond during three major samplings in autumn. Profiles are shorter as ice thickens. Error bars equal ± 2 standard errors. Orange pond is an unofficial name.

respectively. In all three ponds DRP concentration increased with depth into the chemocline, reaching maxima of 29, 65 and 21 mg m^{-3} in Legin, Orange and Egg ponds. In all three ponds DRP in the mixolimnion tended to increase steadily as the study progressed, at rates broadly consistent with freeze-concentration. In the chemocline of Legin pond DRP remained largely unchanged over time, but had decreased to undetectable levels in the other two ponds.

Nitrate was undetectable in the mixed layers of Orange and Egg ponds, whereas in Legin pond it was present at concentrations of 15 mg m^{-3} at the beginning of the study. In Legin and Orange ponds, nitrate was also undetectable in

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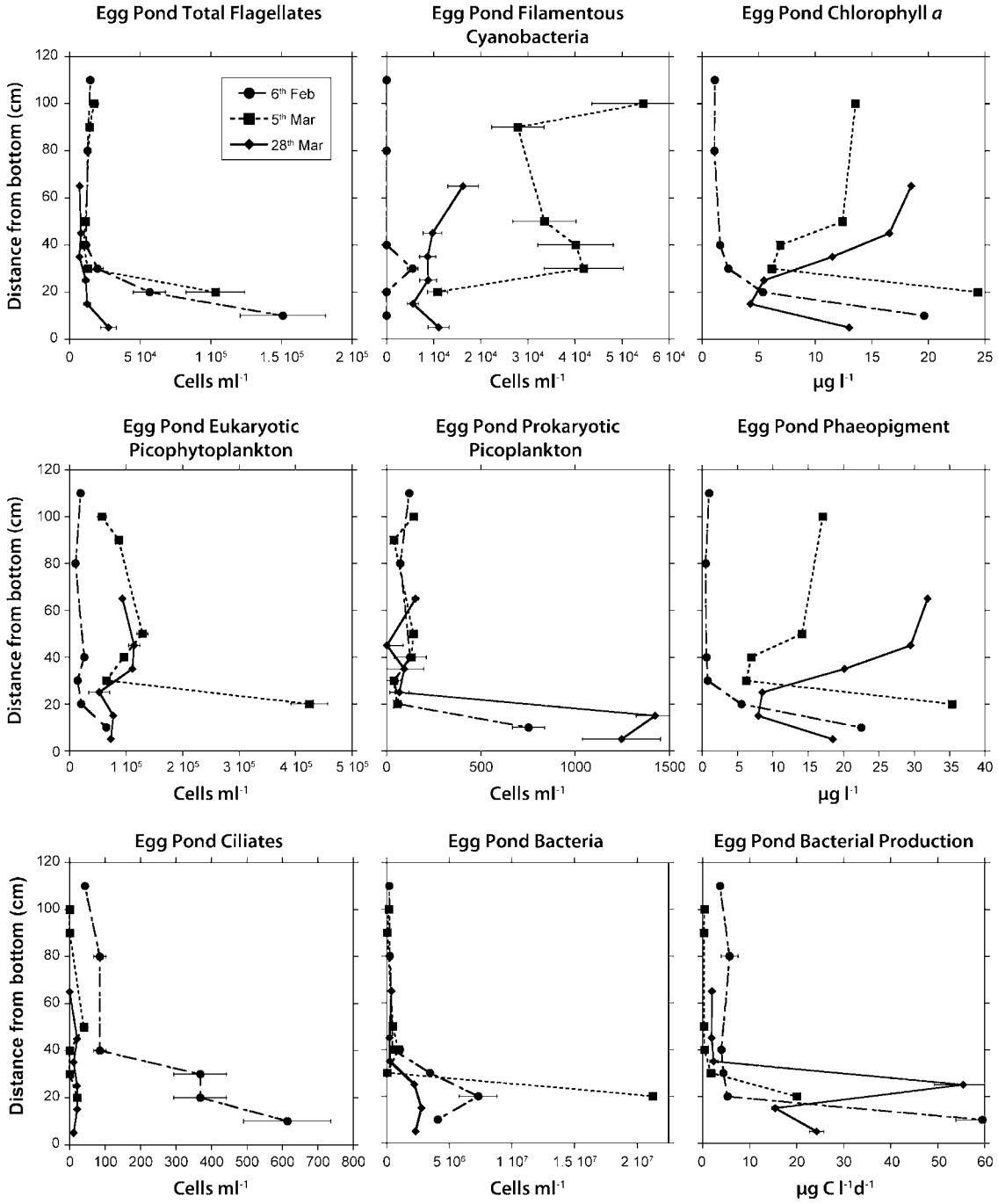

Fig. 5. Vertical profiles of nine biological water column components (total flagellates, filamentous cyanobacteria, chlorophyll *a*, phaeopigment, eukaryotic and prokaryotic picophytoplankton, ciliates, bacteria and bacterial production) in stratified Egg pond during three major samplings in autumn. Profiles are shorter as ice thickens. Error bars equal ± 2 standard errors. Egg pond is an unofficial name.

the chemocline while in Egg pond concentrations of 20–30 mg m^{-3} were recorded. There was little consistent change in nitrate concentrations over time and by the end of the study vertical distributions and absolute concentrations were similar to those at the beginning in all ponds.

In all ponds NH_4-N was the most abundant form of nitrogen. In the mixed layers it was initially present at *c.* 10 mg m^{-3} in Legin and *c.* 20 mg m^{-3} in Egg and Orange ponds and increased gradually over the study, at rates consistent with freeze-concentration. In all ponds NH_4-N increased to $>$ 50 mg m^{-3} and within the chemocline a substantial increase in NH_4-N was seen over time at the deepest part of Egg and Orange ponds, but not in Legin pond.

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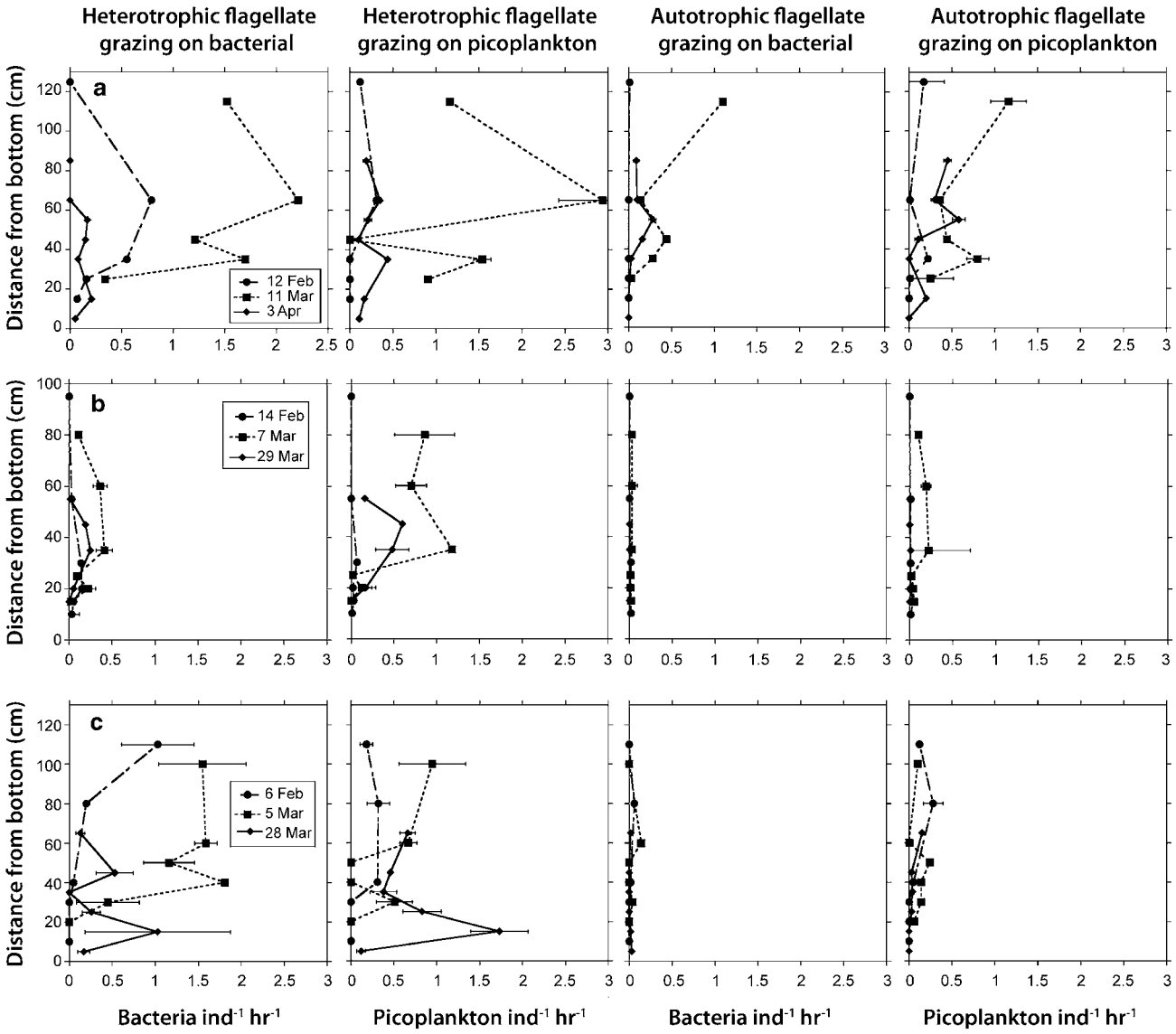

Fig. 6. Mean grazing rates on bacterial and picophytoplankton sized populations by heterotrophic and mixotrophic flagellates in our three stratified ponds on the McMurdo Ice Shelf. **a.** Legin pond, **b.** Orange pond, and **c.** Egg pond. Error bars equal ± 2 standard errors from duplicate trials.

Phytoplankton structure, abundance and biomass

Chlorophyll *a*

Chlorophyll *a*, a proxy for total autotrophic biomass, behaved similarly in all three ponds, initially with low concentrations in the mixolimnion and peaking at 15–20 mg m^{-3} in the chemoclines. For the first month after ice formation chl *a* increased in all ponds in both the mixolimnion and chemocline regions, and the highest concentrations (24–30 mg m^{-3}) were observed within the chemoclines of Egg and Orange ponds in early March (Figs 3–5). Chlorophyll *a* concentration continued to increase in Egg pond, but declined in both Orange and Legin pond in the second month after ice formation (Figs 3–5). Phaeopigment was also measured and usually exceeded chl *a* concentrations where chl *a* was elevated (Figs 3–5). Phaeopigment increased proportionally to chl *a* between February and late March–early April.

Picophytoplankton

Picophytoplankton distribution showed a strong effect of water column structure, with peaks embedded in the chemoclines. The highest eukaryotic picophytoplankton (picoE) numbers were found in Legin pond in early February, at 35 cm above bottom at *c.* 1.6 x 10^6 cells ml^{-1}. Orange pond had the lowest numbers overall with a peak at 10 cm above the pond bottom of 4.0 x 10^5 cells ml^{-1} in early February (Figs 3, 5 & 6). Prokaryotic picophytoplankton (picoP) numbers were usually very low at < 400 cells ml^{-1}

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Table I. Presence of phytoplankton in three stratified Antarctic ponds. + = present, - = absent.

	Orange pond	Egg pond	Legin pond
Phytoplankton			
Cyanophyta			
Anabaena			
Aphanothece sp.	-	+	-
Geitlerinema sp. Anagnostidis & Komárek (West & West)	+	+	+
Leptolyngbya antarctica Anagnostidis & Komárek (West & West)	+	+	+
Leptolyngbya augustissima West & West	+	+	+
Leptolyngbya spp.	+	+	+
Oscillatoria limosa Agardh	+	-	+
Phormidium spp.	+	-	+
*Phormidium autumnale (*Agardh) Gomont	+	+	+
Pseudanabaena spp.	-	+	+
Prokaryotic picophytoplankton (*Synechococcus* sp.)	+	+	+
Eukaryotic picophytoplankton	+	+	+
Chlorophyta			
Brachiomonas submarina Boh.	+	+	+
Chlamydomonas spp.	+	+	+
Monoraphidium sp.	+	-	-
Dinophyta			
Gymnodinium sp.	-	+	+
Chrysophyceae			
Mallomonas sp.	+	-	+
Synura sp.	+	+	-
Cryptophyta			
Chroomonas spp.	+	+	+
Ochromonas spp.	+	+	+
Prasinophyceae			
Pyramimonas sp.	-	+	+
Unidentified autotrophic flagellates (inc. cf. *Hillea* sp., *Chromulina* sp.)	+	+	+
Miscellaneous diatoms			
Amphiprora sp.	+	-	+
Navicula spp.	+	-	+
Unidentified	+	+	+

throughout the study in the mixolimnions, but peaked in the chemoclines with the highest number in Legin pond at 1.1×10^4 cells ml^{-1} at 35 cm in early February, and the lowest numbers overall peaking at 10 cm above the pond bottom at 1425 cells ml^{-1} in late March (Figs 3–5).

Flagellates

Like picophytoplankton, the distributions of flagellates were strongly affected by stratification. Flagellate numbers in the mixolimnions were always less than 7×10^4 ml^{-1} with the highest flagellate numbers, both autotrophic flagellates (AF) and heterotrophic flagellates (HF), within the chemoclines (Figs 3–5). Throughout the study we consistently observed low numbers of flagellates that appeared to be either encysted or becoming encysted. However, cyst numbers were variable, with no consistent temporal pattern observed.

Orange pond was initially dominated by small AFs (including species tentatively ascribed to *Hillea*, *Chromulina* and *Chilomonas*) with an *Ochromonas* sp., a *Chroomonas* sp., *Brachiomonas submarina* (chemocline only) and a *Chlamydomonas* sp. (mixolimnion only) also present. This assemblage was maintained throughout, though after March a decline in abundance of all species was observed.

In Egg and Legin ponds, the large quadriflagellate *Pyramimonas* dominated, initially mostly located within the chemocline (Table I, Figs 3 & 5). *Ochromonas* and *Chroomonas* spp. were found throughout the water column, but both peaked within the chemocline. Less abundant taxa in Egg and Legin ponds included the group of small flagellates seen in Orange pond. By early March a *Pyramimonas* sp. was present throughout the water column, though the highest concentrations remained within the chemocline. By mid-March *Ochromonas* spp. and small unidentified flagellates had become equally numerous to *Pyramimonas* in the chemocline. In Legin pond in late March, the *Pyramimonas* abundance peak was just below the ice.

By late March, AF numbers were low and consistent across depths in all ponds. In Egg and Legin ponds the small flagellates were dominated by heterotrophs. The HF genera we observed included species of *Chilomonas*,

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Table II. Presence of microzooplankton taxa in three stratified Antarctic ponds. + = present, - = absent.

	Orange pond	Egg pond	Legin pond
Unidentified heterotrophic flagellates (inc. *Telenema* cf. *Chilomonas*, *Bodo*)	+	+	+
Protozoa			
Phylum Ciliophora			
Postciliodesmatophora			
Class Spirotrichea			
Subclass Choreotrichia			
Strombidium	+	+	+
Halteria	+	-	+
Small oligotrichs	+	+	+
Subclass Stichotrichia			
Stichotrichs < 80 um			
Rhabdophora			
Class Prostomatea			
Urotricha	+	+	+
Pseudobalanion	+	+	+
Bursellopsis	+	+	+
Class Litostomatea			
Subclass Haptorida			
Trachelophyllum	+	+	+
Didinium	+	+	+
Actinobolina	+	+	+
Class Nassophorea			
Subclass Nassophorea			
Chilodonella	-	+	-
Nassula	-	+	-
Subclass Hypotrichea			
Euplotes	-	+	+
Class Oligohymenophorea			
Subclass Hymenostomia			
Cinetnchilum	+	+	+
Scuticociliates	+	+	+
Subclass Peritrichia			
Vorticella	+	+	+
Astylozoon			
Rotifera	-	+	-

Telenoma and cf. *Bodo,* as well a number of small unidentified taxa and heterotrophic *Ochromonas* spp. (Table II). An increase in ratio of heterotrophic to autotrophic *Ochromonas* spp., the latter distinguished by the presence of a fluorescing chloroplast, was observed in all three ponds as our study progressed, while no clear trends were observed in the other taxa. Heterotrophic flagellates declined less rapidly over time than AF and thus gradually increased as a proportion of the total flagellate population. The average ratio of AF to HF of 10:1 in February and early March had declined to only 3.5:1 in late March–early April.

Cyanobacteria

Cyanobacteria, dominated by filamentous forms, appeared in the water column in late February–early March, before declining in abundance into late March. They were more evenly distributed throughout the water column than the other groups, but peaks in abundance were also seen within the chemocline (Figs 3–5). The cyanobacteria in Orange pond were dominated by small *Geitlerinema* and *Leptolyngbya* spp. with lower numbers of a *Phormidium* spp. In Egg pond, filamentous cyanobacteria peaks contained a mix of *Leptolyngbya* and *Geitlerinema* spp. with low numbers of a *Pseudanabaena* sp. In Legin pond, when cyanobacteria appeared in mid-March, a filamentous cyanobacteria peak near the underside of the ice in the mixolimnion was dominated by *Leptolyngbya* sp., while a deeper peak at 25 cm from the bottom contained this genus with significant numbers of a *Geitlerinema* sp. and, in later samples, *Phormidium autumnale*.

Microzooplankton

The microzooplankton community in all ponds consisted of ciliates and HF (Table II). At the beginning of the study these groups had a mean HF:ciliate ratio (by numbers) of *c.* 12:1. This however, represented an approximately equal ratio by cell volume due to the larger size of ciliates. By late February and early March, the steadily declining number of ciliates allowed this ratio to rise to 21:1, though by late March and early April the decline in flagellate numbers reduced the HF:ciliate ratio to only 3:1, at which time ciliates dominated the remaining biomass.

The ciliate assemblages varied between ponds and over time, not always is a consistent manner. In Egg pond, small oligiotrichs dominated in February, with *Gymnodinium*, *Vorticella* and cf. *Astylozoon* spp. also important. Species of *Uroticha* and cf. *Pseudobalanion* became common in early March. In contrast, the *Urotricha* and cf. *Pseudobalanion* spp. dominated Orange pond in February but rapidly decreased by March, with *Trachelophyllum* sp. becoming dominant. In Legin pond during February a mix of ciliates were important including *Urotricha* sp. cf. *Pseudobalanion*, *Gymnodinium* sp., and small oligiotrichs. In March and through into April a mix of *Urotricha* sp., cf. *Pseudobalanion* and *Halteria* sp. dominated in Legin pond, though by late March numbers of all microzooplankton taxa were low in all ponds. Carnivorous species large enough to be capable of consuming flagellates and other ciliates were relatively rare but included the larger choreotrichs *Trachelophyllum* sp., very low numbers of *Strombidium*, and *Didinium*, which was more commonly observed. Ciliates dominated by *Trachelophyllum* sp. and *Didinium* sp. peaked at *c.* 800 cell ml^{-1} in Orange pond in early March, though even their cumulative abundance still only produced a maximum ratio of *c.* one potential predator per 100 flagellates.

Bacterial numbers and activity

All ponds had their greatest concentration of bacteria within the chemocline, and in all ponds bacterial numbers increased in the first month of ice formation and maximum abundance was attained in March, though the exact date

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Table III. Results of Pearson's correlation analysis between chemical and physical variables and biological populations for combined data from Legin, Orange and Egg ponds. Chl *a* = chlorophyll *a*, ns = not significant, n/a = not applicable, * = significant at $P<0.05$, ** = significant at $P<0.01$, *** = significant at $P<0.001$, df = degrees of freedom. DOC = dissolved organic carbon, TDP = total dissolved phosphorus, DIC = dissolved inorganic carbon, PN = particulate nitrogen, NH_4-N = ammonium-N, DRP = dissolved reactive phosphorus, DO = dissolved oxygen.

	Bacteria (ml^{-1})	Bacterial production	Chl *a* ($\mu g\ l^{-1}$)	Eukaryotic picophytoplankton	Prokaryotic picophytoplankton	Flagellates (ml^{-1})	Ciliates (ml^{-1})	Filamentous cyanobacteria
DOC	0.82*** df = 39	0.76*** df = 39	ns	ns	0.38* df = 39	ns	ns	ns
TDP	0.73*** df = 64	0.62*** df = 58	0.37** df = 69	ns	0.31* df=60	0.45*** df = 60	0.27* df = 60	ns
DIC	0.61*** df = 44	0.33* df = 39	0.59*** df = 39	0.77*** df = 40	0.36* df = 40	0.69*** df = 40	0.33* df = 40	0.50*** df = 40
PN	0.57*** df = 56	0.53*** df = 56	0.56*** df = 56	0.38** df = 56	0.38** df = 56	0.50*** df = 56	0.28* df = 56	ns
NH_4-N	ns	0.35* df = 59	ns	ns	ns	0.49*** df = 59	ns	ns
DRP	ns	ns	0.42*** df = 69	0.38** df = 59	ns	0.58*** df = 59	0.59*** df = 59	ns
DO	ns	ns	0.37** df = 71	0.48*** df = 59	0.39** df = 59	ns	0.37** df = 59	ns
Cond	ns	ns	0.52*** df = 71	0.29** df = 59	ns	ns	ns	
Temp	ns	ns	ns	0.46*** df = 59	0.26* df = 59	0.54*** df = 58	0.44*** df = 59	(-) 0.30* df = 59
Bacterial production	0.53*** df = 48	n/a	ns	ns	ns	ns	ns	ns
Chl *a* ($ug\ l^{-1}$)	0.40*** df = 48	ns	n/a	ns	ns	ns	ns	ns
Flagellates	0.38** df = 59	ns	0.61*** df = 61	0.56*** df = 56	0.42** df = 56	n/a	0.82*** df = 61	ns
Ochromonas/ *Chroomonas* sp.	0.31* df = 48	ns	0.47*** df = 61	0.32* df = 56	0.31* df = 56	0.91*** df = 56	0.77*** df = 61	0.23* df = 61
Prokaryotic picophytoplankton	ns	ns	0.47*** df = 56	0.54** df = 56	n/a	ns	ns	ns
Eukaryotic picophytoplankton	ns	ns	0.54*** df = 56	n/a	ns	ns	ns	ns
Ciliates	ns	ns	0.23* df = 59	ns	ns	0.72*** df = 61	n/a	ns
Filamentous cyanophytes	0.28* df = 61	ns	0.39** df = 59	ns	ns	0.26* df = 6	ns	n/a

varied (Figs 3–5). The highest bacterial numbers were measured in Orange pond, immediately above the sediment, in late March at 23×10^6 cells ml^{-1}. In Legin pond bacterial numbers peaked at 6×10^6 cells ml^{-1} in mid-March at 15 cm just above the sediment. In Egg pond bacterial numbers also peaked in March at 20 cm (21×10^6) and then declined at all depths in April (Figs 3–5). Like bacterial numbers, bacterial production tended to be highest in the chemocline. Production per bacterium declined across all three ponds between samplings with a drop of 37% in production per cell between February and early March and a drop of 74% by late March–early April.

Flagellate grazing activity

Grazing rates in the three ponds showed similar trajectories over time. Grazing rates were low in February and the maximum rates were in the mixolimnion rather than the chemocline (Fig. 6). By early March, grazing rates per cell were consistently higher but still peaked in the mixolimnion. By April grazing rates were in decline, as were flagellate abundances. These patterns were similar for both HF and nominally AF, though the latter were clearly acting as mixotrophs.

Bacterial standing stock removal indicated between 2 and 14% of bacteria could be grazed with high rates in occurring in early March within the chemocline. The median standing stock removal rate for picophytoplankton was 10% per day while average removal was 31%. These high rates were driven by values of up to 300% at some depths largely due to very low picophytoplankton cell concentrations. Bacterial grazing was also assessed in terms of the percentage of daily production grazed. This increased significantly with 34% of bacterial production grazed, on average in February while in early March, 15 times the daily production was being grazed (excluding one atypical surface sample in which 150 times the measured production was grazed). By late March four times the daily production was still potentially being grazed, suggesting that bacterial populations were under considerable grazing stress. These results varied between the mixolimnion and the chemocline, with rates consistently higher in the mixolimnion. When considered separately in the mixolimnion, 26 times the average bacterial production was grazed in late February–early March while in the chemocline only 7.5 times the average production was grazed.

Mixotrophic flagellate grazing also increased in importance over time. In February the HF flagellates grazing was four times that of AF, but by early March HF were responsible for only 35% of the grazing and by late March only 4% of grazing, due to increases in grazing rates of nominally AF.

Relationships among variables

Pearson's correlation analysis (Microsoft Excel) showed that the declining physical and environmental conditions observed during autumn correlated to declines observed

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in our microbial populations (Table III). Specifically phytoplankton and microzooplankton populations correlated with declining temperatures (Table III). There were, however, exceptions with chl *a* and filamentous cyanobacteria overall negatively correlated with temperature (Table III). A number of microbial populations also correlated with the higher nutrients and DIC concentrations found within the chemocline (Table III).

Bacterial numbers correlated to bacterial production, chl *a* and flagellates but not ciliate populations, while ciliates were correlated to flagellate populations (Table III). Specifically total flagellates were most strongly correlated with the ciliates *Trachelophyllum* sp., $r = 0.56$ ($P = < 0.01$, df = 32) and *Urotricha/Pseudobalanion*, $r = 0.62$ ($P = < 0.001$, df = 28). Flagellates populations although correlated with bacteria were not significantly correlated with picoE and picoP (Table III).

Higher AF grazing rates in the mixolimnion lead grazing to correlate with the distance from the pond bottom for AF grazing on 1.0 μm beads ($r = 0.36$, $P = < 0.001$, df = 48) and on 0.5 μm beads ($r = 0.43$, $P = < 0.05$, df = 29). Heterotrophic flagellates grazing rates on 0.5 μm beads were negatively correlated to both bacterial numbers and production ($r = 0.38$, $P = < 0.001$, df = 48, and $r = 0.31$, $P = < 0.001$, df = 48, respectively).

Discussion

Ice formation plays a fundamental role in defining the physical characteristics of Antarctic ponds. It divides the year into liquid and non-liquid phases, exacerbates the effect of annual irradiance cycles and, through the freeze-concentration of solutes, leads to temporal variation in osmolarity (Wait *et al.* 2006, Hawes *et al.* 2011a, 2011b). In the case of the three ponds investigated, ice formation during the winter prior to our sampling left a legacy of a stratified water column through the 2007–08 summer, which persisted into the beginning of the next round of freezing. This density-derived stratification divided the ponds into upper mixed layers (mixolimnion) overlying steep density gradients (chemocline). The chemocline layers were initially super-saturated with dissolved oxygen, had elevated concentrations of DOC, DIC, DRP and NH_4-N, though not NO_3-N, and were warmer than the mixolimnion. Planktonic communities were initially stratified within this structure and remained so during the process of freezing as the irradiance and temperature declined, the ice cover thickened and the mixolimnion became increasingly saline. Phytoplankton populations occurred in defined layers within the stratified chemocline initially appearing to correspond to a light, temperature, chemistry optimum for each of the different groups. It appears this structure remained largely intact even when light was lost with the migration of populations being observed only in filamentous cyanobacteria as light declined and *Pyramimonas* sp. only in Legin pond at the end of the study. All phytoplankton populations, however, clearly declined once light became limiting in late February while temperature correlated with microbial population abundances throughout the study reflecting the impact of reduced irradiance. Elevated planktonic communities also remained associated with elevated concentrations of DOC, DIC, DRP and NH_4-N found in the chemocline layers, even as their numbers declined, whether through active selection or through the physical constraints. This stratification of planktonic components only broke down when the chemocline became fully mixed into the increasingly saline mixolimnion. This had occurred by the end of the study in the smaller Egg and Orange ponds, where most of the pond had frozen, but not in the larger Legin pond which retained a larger volume of liquid water.

Previously we have shown, from considerations of oxygen dynamics in other ponds on the McMurdo Ice Shelf, a shift from net autotrophy to net heterotrophy in late February as irradiance declines (Hawes *et al.* 2011b). Likewise in this study, all three of the ponds showed DO concentration maxima in late February–early March followed by declines. Phytoplankton populations also declined in all ponds from this time after they had showed some initial increases during early February probably through continuing growth and freeze-concentration in the reducing pond volume. The start of these declines was also coincident with planktonic photosynthesis becoming undetectable in Legin pond as reported in Hawes *et al.* (2011b), and supports the supposition that this is the time when the ponds became net heterotrophic. The measured integrated irradiances in Orange and Egg ponds were similar or lower than that reported in Legin pond in both early and late March. These low irradiances again support a heterotrophic onset in Orange and Egg ponds occurring around the same time as Legin pond. We can thus postulate that two temporal domains and two spatial domains occur in these ponds, temporally separated by the autotrophic-heterotrophic shift at the end of February, and spatially separated into the mixed and stratified parts of the water columns.

Composition and trends in microbial populations

We hypothesized that pond microbial populations would change over time as conditions deteriorated. However, we found that rather than the introduction of new species, in both the mixolimnion and chemocline, population changes largely occurred in relative and absolute abundances.

At the beginning of this study composition and abundance showed general conformity among the three ponds with previous reports from the McMurdo Ice Shelf (James *et al.* 1995, Hawes *et al.* 1997) and with other Antarctic freshwater systems (Butler 1999, Roberts & Laybourn-Parry 1999). In particular, the dominance by phytoflagellates appears to be ubiquitous in Antarctic ponds and lakes, as does the

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appearance of strata of specific taxa of flagellates within density gradients (in this study *Brachiomonas* and/or *Pyramimonas*). *Pyramimonas* layers have previously been reported in McMurdo Ice Shelf ponds (Hawes *et al.* 1997) and this genus has been found in high salinity layers in other Antarctic systems (Laybourn-Parry *et al.* 2004) including nearby Lake Fryxell in the McMurdo Dry Valleys (Vincent 1981). All of the other flagellated genera, cyanobacteria, ciliates and diatoms have also been reported at the McMurdo Ice Shelf (James *et al.* 1995, Hawes *et al.* 1997) or elsewhere in Antarctica (Butler 1999). The filamentous cyanobacteria, which were only reported after the first sampling, were again similar to those previously reported in this area either in benthic mats or in the water columns of ponds (James *et al.* 1995, Jungblut *et al.* 2005, although a number of taxonomic reclassifications have occurred).

Specific inter-pond differences in assemblage and composition are difficult to interpret from existing data. Whether the absence of *Pyramimonas* from Orange pond reflects genuine habitat differences, stochastic or other effects is not clear, though we do note that Orange pond has been found to be fully mixed in some summers (authors' unpublished data) and this may preclude persistence of a long-term population of what may be an obligatory brackish species.

As hypothesized, changes during the autumnal shift did lead to population declines favouring species suited to the more heterotrophic conditions, this consistently occurred once light levels dropped to below those required for photosynthesis in late February. The ratio of heterotrophic species to autotrophic species did change with the onset of heterotrophic conditions but only by difference, with AF declining more rapidly than HF. Heterotrophic flagellates gradually increased as a proportion of the total flagellate population, and at least part of this appeared to be due to some AFs becoming HFs through loss of chloroplasts (notably *Ochromonas* spp.). Ciliate succession was again determined by their different rates of decline under deteriorating environmental conditions. Ciliates overall declined rapidly, with a fivefold drop between February and the onset of heterotrophy in early March. This suggested this group was more susceptible to changing conditions than HF, a similar pattern to trends reported, with depth, in oceans (Sohrin *et al.* 2010). Ciliates however, became proportionally more important under fully heterotrophic conditions in late March due to the comparatively faster decline of HF in the final stages of freezing. The rapid decline of ciliates has implications for their ability to cope with physical and chemical changes in this system as unlike other marine polar systems ciliates are the highest predators in these ponds and therefore not limited by top-down grazing pressure.

The only exception to the general trend of decline around the onset of heterotrophy was observed amongst the filamentous cyanobacteria. Filamentous cyanobacteria became a major component of the phytoplankton, as conditions transitioned to heterotrophy. Benthos cyanobacteria microbial mat communities are known to dominate pond biomass in this area (Hawes *et al.* 1997) and it appears that some of this community were brought into suspension. Density currents caused by salt exclusion from the freezing ice are one way benthic cyanobacteria are reported to enter the water column (Garneau *et al.* 2008). However, the density structure in the ponds did appear to remain largely intact until late in the study suggesting other processes such as active movement in response to declining light may have brought the filamentous cyanobacteria into suspension. Migratory responses of benthic cyanobacteria in response to reduced irradiance are widely reported but are usually limited to migration within the benthic mat itself (Castenholz 1992). Regardless of the mechanism, the movement of this group will have brought organic carbon from the benthos into the water column, potentially prolonging water column bacterial production once autochthonous production was impaired by light availability. This occurred in an analogous way, albeit at a much smaller scale, to that described by Garneau *et al.* (2008) in a coastal polar system. Accompanying the filamentous cyanobacteria we also saw the movement of other organisms from the pond floor into the water column including several of the larger ciliates, *Chilodonella* and *Nassula* (Dillon & Bierle 1980), which are typically 'benthic' taxa, known to feed on filamentous cyanobacteria and diatoms (Patterson & Hedley 1992).

Bacterial numbers increased in the first month of ice formation, probably due to reducing pond volumes and lower grazing pressure resulting in maximum abundances in early March. Throughout this study bacterial populations were significantly correlated with DIC and DOC as well as chl *a*, flagellates and filamentous cyanobacteria suggesting carbon sources were important. Garneau *et al.* (2008) reported that in Arctic seawaters bacterial production rates, although reduced, remained steady through winter when light was absent and temperatures were reduced to below freezing. This is thought to be due to the increased carbon inputs and the slow breakdown of larger molecular weight DOC. It is probable that similar processes may have sustained later bacterial production in this study and potentially may sustain bacterial populations over winter in the ponds. Production per bacterium however, declined across all three ponds with a drop to 63% of February production per cell by early March and a drop to 24% of February's production by late March–early April indicating a continuing decline in pond bacterial activity. It is possible that bacterial production during summer was linked to phytoplankton photosynthesis which declined rapidly to below detection in late February (Hawes *et al.* 2011b), or to photooxidation of DOC (Laybourn-Parry *et al.* 2004), which will also have declined rapidly with declining light. The onset of anoxic conditions, only seen late in our study, in two of the three ponds is again likely to have influenced

bacterial activity inducing denitrification and then reducing sulphate, however, the extent to which these processes had progressed is unknown. Bacterial production rates per cell even at the end of this study were still comparable to those observed both in Antarctica (Laybourn-Parry *et al.* 2004) and other cold regions including the Arctic (Garneau *et al.* 2008).

Grazing and food web processes

Heterotrophic flagellates and AF grazing rates varied but were comparable to other studies in Antarctica (Roberts & Laybourn-Parry 1999). Autotrophic flagellates (mixotrophic flagellate) grazing rates were on average half that of HF, similar to grazing ratios between these groups reported elsewhere. Consistent with the shift from a dominance of autotrophy to heterotrophy in late February and early March, in these ponds (Hawes *et al.* 2011b), and in late autumn around Antarctica (Butler 1999, Roberts & Laybourn-Parry 1999), many of the AF observed became weakly pigmented or lost chloroplasts altogether towards the end of our study, indicating a possible change in nutritional mode. The highest flagellate grazing rates per cell, on both picophytoplankton and bacteria were reported in late February and early March, again reflecting the shift to heterotrophy with bacteria the preferred prey of HF as reported elsewhere (Jones *et al.* 1993). We probably overestimated picophytoplankton grazing rates where low picophytoplankton numbers occurred especially in the mixed layer (see Methods) making an overall assessment of their impact difficult. The amount of grazing we observed on picophytoplankton and bacteria indicate they are a significant source of nutrient for cells during the autumn. The grazing impact also increased as a percentage of bacterial production through the period of transition to heterotrophic conditions in early March and beyond (an overall 5.5 fold increase). Initial grazing by flagellates would not have controlled production alone, while by early March grazing by flagellates alone, should have been reducing standing stocks. This was however, not reflected in our counts probably due to the freeze-concentration of bacterial populations by ice exclusion countering grazing effects. If freeze-concentration of bacteria is occurring, it may be enhancing the potential for heterotrophic nutrition by increasing prey density even while overall numbers and productivity are declining. The impact of grazing by nominally AF also became more prominent as the study wore on. Even though AF numbers declined more rapidly than HF, increases in the AF grazing rate allowed them to become proportionally more important in grazing bacterial populations.

The role of ciliates could not be directly assessed as observations of ciliate grazing were not made. However, Sorokin (1999) proposed that relative size is an indicator as to which particles can be ingested by ciliates. The majority of ciliates observed were $< 30\,\mu m$ and were likely to mainly feed on picophytoplankton and bacteria. Based on sizes and known feeding strategies (Foissner *et al.* 1991, 1992, 1994) between 45 and 93% of the observed ciliates were probably bacterivores but ciliate populations were only significantly correlated to flagellates rather than picophytoplankton and bacteria. This suggests the linkage between ciliates and bacteria was highly variable and probably affected by the changing flagellate population. Ciliate grazing rates on bacteria and picophytoplankton can also be highly variable, but can be up to 40 times higher than flagellates (Barcina *et al.* 1991). Even given high ciliate grazing rates flagellate grazing probably dominated ciliate grazing, at times, due to their substantial numerical dominance. This is most likely to have occurred during the transition period in early March when the ratio of HF to ciliates was 21:1.

Carnivorous and herbivores ciliates were generally rare or associated with the benthos, although *Trachelophyllum* sp. and *Didinium* and cf. *Pseudobalanion* were abundant at times and were correlated with flagellates. These ciliates are reported to be capable of grazing flagellates (Müller *et al.* 1991, Foissner *et al.* 1991, 1992, 1994) suggesting top down grazing was occurring, at times, on flagellate populations.

The mixolimnion

The low nutrient, low biomass sparse upper water column populations in the mixolimnion layers showed little consistent change as freezing progressed. In effect, no change in numbers in the mixolimnion infers that loss processes, such as grazing, sedimentation and incorporation into the ice cover, exceeded the concentrating effect of declining water volume over time. The exception was the filamentous cyanobacteria that became a major component of the phytoplankton of the mixolimnion, but only during the onset of heterotrophic conditions in late February–early March. This lead to increased mixolimnion biomass in early March measured as chl *a*, but abundances were again low in late March. Low numbers of the dominant planktonic groups, in the mixolimnion at the beginning of the study, together with the chronically low yield of planktonic photosynthesis in Legin pond reported by Hawes *et al.* (2011b) point towards an unproductive late summer water column. This is consistent with earlier observations (e.g. Howard-Williams *et al.* 1989, Hawes *et al.* 1997, Howard-Williams & Hawes 2007) that planktonic productivity in mixed ponds of the McMurdo Ice Shelf is low, possibly nutrient limited, with overall pond carbon accrual being primarily benthic. Rae *et al.* (2000) attributed the low activity of mixed ponds plankton to chronic photoinhibition due to prolonged exposure to high irradiance (including UV) at low temperature. The increase in planktonic photosynthesis reported in Legin pond and three other ponds immediately after ice formation reported in the companion study (Hawes *et al.* 2011b) may reflect a reduction in photoinhibition during this period. After 20 February however, all ponds carbon

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uptake rates were below detection limits (Hawes *et al.* 2011b) reflecting the decline in available light.

We also observed that the increase in flagellate heterotrophy accompanying the shift from light to dark was also most pronounced in the mixolimnion. Here increased grazing rates by both heterotrophs and mixotrophs was evident. Increased grazing rates may also help to explain the absence of any significant increase in abundance in the mixed layer. These waters consistently had low nutrient and low carbon supplies which are identified as drivers of mixotrophy (Bird & Kalff 1989, Jones *et al.* 1993). Flagellate populations were also significantly correlated to bacteria throughout the study. In early March a small increase was found in bacterial production in the mixolimnion of two of our three ponds but at this time, although highly variable, the percentage of bacterial production being grazed on average increased to 26 times the average production in the mixolimnion. During this period grazing rates were consistently highest in the mixolimnion. At the same time flagellate grazing of bacteria was dominated by AF due to their significantly larger biomass, rather than their grazing rate. This indicates as we predicted that AF were at least partially changing their nutritional mode becoming more heterotrophic.

The chemocline (*lower domain*)

Flagellates, bacteria, picoE and picoP, and ciliates were all concentrated within the chemocline, as were peaks in bacterial production. This co-occurrence is probably linked to trophic interaction amongst these groups of organisms, which involves consumption of bacteria and picophytoplankton by heterotrophic and, to a lesser extent, mixotrophic flagellates. Filamentous cyanobacteria became a major component of the phytoplankton in the chemocline both in the middle and latter parts of the summer-winter transition during and after the onset of heterotrophic conditions. Larger filaments, such as *Phormidium*, were only ever observed near the pond bottom in the chemocline and their presence increased with time. Movement of larger benthic organisms from the pond floor to the water column was also largely limited to within the chemocline.

Dissolved organic carbon concentration was also highest in the chemocline, and appeared to increase with freeze-concentration in a conservative way (Hawes *et al.* 2011b) consistent with previous suggestions that the high concentrations of DOC in Antarctic ponds are largely refractory and unavailable masking the dynamics of any small labile pool that may be present (Howard-Williams & Hawes 2007, Hawes *et al.* 2008).

The higher abundance and productivity results observed in the chemocline are also not inconsistent with the views of Rae *et al.* (2000) and suggest the chemocline may represent a balance between adequate light penetration, allowing photosynthesis without excessive photoinhibition, and adequate available nutrients (see also Hawes *et al.* 1997).

Within the chemocline, as observed in the mixed layer bacterial numbers and activity, numbers of flagellates, picophytoplankton and ciliates all tended to respond to declining irradiance, temperature and the shift to heterotrophy with a decline in abundance. The highest flagellate grazing rates on both picophytoplankton and bacteria were again reported in late February–early March reflecting the shift to heterotrophy. At this time high removal rates occurred around the chemocline where substantial picophytoplankton populations occurred. Here high rates were not induced by prey saturation with bead to picophytoplankton ratios well within accepted ranges in contrast to parts of the mixed layer. In early March we saw a decrease in bacterial production in the chemocline while the percentage of bacterial production being grazed increased to 7.5 times the average production indicating significant pressure on bacterial populations. At the same time flagellate grazing pressure was again dominated by AF indicating a change in their nutritional mode was being brought about by the onset of heterotrophic conditions at depth as in the mixolimnion.

Conclusions

This study investigated how planktonic microbial populations, in three stratified Antarctic ponds, respond to the rapidly changing environmental conditions that occur in autumn before total winter freezing. Our working hypothesis was that as conditions change after ice formation there would be a corresponding decline in autotrophic numbers, shifts in species composition, and a change in nutritional strategies. We found pond conditions remained favourable for autotrophic growth only until late February and the shifts we observed in the dynamics of the planktonic microbial community were largely driven by the physical changes in the environment. Once light levels became limiting and heterotrophic conditions dominated all populations declined. Our results indicated that the predicted phytoplankton species succession did not occur with variation in the microbial populations involving changes in relative and absolute abundance rather than the appearance of new species. During the transition from autotrophy to heterotrophy however, filamentous cyanobacteria did briefly become important in the water column, although the mechanism behind their appearance remains unclear. It also appears that some species are sustained during the transition period by a combination of physical buffering in the chemocline, and/or changing nutritional modes from phototrophic to mixotrophic, with mixotrophy clearly increasing throughout the study. It is also clear a number of cells do move to resting forms but the rate at which this occurs could not be measured. Bacteria populations may also have been maintained by an increase in liable carbon sources through freeze-concentration changing water chemistry or migration of filamentous cyanobacteria and associated benthic components into the water column. Bacterial production however, was still

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declining per cell at the end of the study period. At this time, larger planktonic organisms not already in a state of stasis were probably metabolically limited by the extreme conditions, and preparing for the final freeze. Overall these results have implications for the autumn transition period both in our ponds and similar polar systems elsewhere, indicating a mix of strategies may be used during this period for long-term survival.

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