ENVIRONMENTAL RESEARCH LETTERS

LETTER • OPEN ACCESS

Nitrogen and carbon limitation of planktonic primary production and phytoplankton–bacterioplankton coupling in ponds on the McMurdo Ice Shelf, Antarctica

To cite this article: Brian K Sorrell et al 2013 Environ. Res. Lett. 8 035043

View the article online for updates and enhancements.

You may also like

- <u>3D bioprinting of *E. coli* MG1655 biofilms on human lung epithelial cells for building complex *in vitro* infection models Samy Aliyazdi, Sarah Frisch, Alberto Hidalgo et al.
 </u>
- Planktonic foraminifera in the Arctic: potentials and issues regarding modern and quaternary populations
 Frédérique Eynaud
- <u>First occurrence of planktonic foraminiferal</u> <u>species Boliella adamsii as a marker for</u> <u>the Pleistocene-Holocene Boundary in the</u> <u>sea around Sumba Island</u> R D W Ardi, Aswan, K A Maryunani et al.



This content was downloaded from IP address 3.23.101.60 on 05/05/2024 at 06:47

IOP PUBLISHING

Environ. Res. Lett. 8 (2013) 035043 (10pp)

Nitrogen and carbon limitation of planktonic primary production and phytoplankton-bacterioplankton coupling in ponds on the McMurdo Ice Shelf, Antarctica

Brian K Sorrell^{1,3}, Ian Hawes² and Karl Safi¹

 ¹ National Institute of Water and Atmospheric Research, PO Box 11-115, Hillcrest, Hamilton 3216, New Zealand
 ² Gateway Antarctica, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand

E-mail: ian.hawes@canterbury.ac.nz

Received 13 April 2013 Accepted for publication 21 August 2013 Published 11 September 2013 Online at stacks.iop.org/ERL/8/035043

Abstract

We compared planktonic primary and secondary production across twenty meltwater ponds on the surface of the McMurdo Ice Shelf in January 2007, including some ponds with basal brines created by meromictic stratification. Primary production ranged from 1.07 to 65.72 mgC m⁻³ h⁻¹ in surface waters. In stratified ponds primary production was always more than ten times higher in basal brines than in the corresponding mixolimnion. Regression tree analysis ($r^2 = 0.80$) identified inorganic nitrogen (as NH₄⁺) as the main factor limiting planktonic primary production. However, there was also evidence of inorganic carbon co-limitation of photosynthesis in some of the more oligotrophic waters. Neither C nor N limited carbon fixation at [NH₄–N] > 50 mg m⁻³, with photoinhibition the factor most likely limiting photosynthesis under such conditions. Primary production was the only factor significantly correlated to bacterial production and the relationship ($r^2 = 0.56$) was non-linear. Nitrogen limitation and tight coupling of planktonic primary and bacterial production is surprising in these ponds, as all have large pools of dissolved organic carbon (1.2–260 g m⁻³) and organic nitrogen (all > 130 mg m⁻³). The dissolved pools of organic carbon and nitrogen appear to be recalcitrant and bacterial production to be constrained by limited release of labile organics from phytoplankton.

Keywords: nutrient limitation, photosynthesis, respiration, regression trees, thymidine, leucine

1. Introduction

The McMurdo Ice Shelf (MIS) is a small part of the Ross Ice Shelf, located in the WSW corner of the Ross Sea (figure 1). The MIS is an unusual ice shelf in that parts of its surface are covered with marine-derived sediments, in an undulating terrain that supports a variety of meltwater ponds. This property derives from adfreezing of seawater beneath the ice shelf (Debenham 1920), during which process marine sediments (and organisms) are incorporated into the ice, which then slowly migrate to the ice surface as the surface ice ablates (Swithinbank 1970, Gow and Epstein 1972). The density of debris varies, depending on the velocity of the ice, giving rise to two types of ice shelf surface topography, the 'pinnacle ice' and the 'undulating ice' (Howard-Williams *et al* 1989). Pinnacle ice is highly dynamic, with thin patches

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.

³ Present address: Department of Bioscience, Aarhus University, DK-8000 Aarhus C, Denmark.



Figure 1. Comparison of (a) primary production and (b) secondary production (measured as BPthy) between layers of meromictically stratified ponds. Mean values with standard deviations (n = 4).

of sediment, ephemeral meltwater channels and pools forming amongst ice mounds with a relief of 1-2 m. Undulating ice is, in contrast, slow moving, being topographically constrained between Bratina Island and Brown Peninsula, and has a rolling surface relief of approximately 10 m, with most of the surface covered by >100 mm of sediment. The undulating ice carries an array of sediment-lined, long-lived meltwater ponds of a few 10's to 100's of square metres, lying between rounded mounds of ice-cored sediment (Hawes *et al* 2008).

In an environment where the availability of liquid water is the major factor restricting life, the array of meltwater ponds on the MIS supports highly diverse microbial communities. Important to the high biodiversity value of these ponds are the wide range of size, degree of ice cover and chemistries that have evolved in different ponds (Howard-Williams et al 1989, Hawes et al 1993), and the seasonal changes in ambient conditions (Hawes et al 2011a, 2011b). In many cases habitat diversity is enhanced by persistent stratification with an upper, relatively fresh layer overlying basal brines established through freeze-concentration of salts during autumnal freezing (Wait et al 2006, Hawes et al 2011a). Across the spectrum of meltwater ponds on the MIS, well developed, species-rich microbial mats tend to dominate pond biogeochemical processes (Suren 1990, Hawes et al 1993, Quesada et al 2008, Hawes et al 2011b). Benthic dominance does not, however, preclude the development of a species-rich

plankton, dominated by bacteria, flagellates and ciliates, often associated with the saline basal layers (James *et al* 1995, Hawes *et al* 1997, Safi *et al* 2012).

Controls on the activity of the plankton are poorly understood. There is little evidence to suggest top-down control by grazing (Safi *et al* 2012) and limitation by low nitrogen flux to the ponds has been suggested (Howard-Williams and Hawes 2007). Phytoplankton also appears to suffer photoinhibition in the relatively bright, 24 h daylight environment, perhaps exacerbated by the effects of low temperature (Rae *et al* 2000). Controls on bacterial production are even less well understood. In ice-based systems allochthonous sources of labile organic carbon are minimal and we might expect planktonic photosynthesis and bacterial production tightly coupled, as in other freshwater communities where autochthonous production dominates (Kirchman 1993, Fouilland and Mostajir 2010).

The goal of this contribution is to determine the factors controlling planktonic populations of algae and bacteria in the meltwater ponds of the MIS. To do this we have adopted a comparative approach, measuring primary and secondary production across the salinity and nutrient ranges of meltwater ponds during summer, including the concentrated monimolimnetic brines. Specifically, the hypotheses that have guided this research are that:

- (i) rates of primary and secondary production are tightly coupled within the planktonic community,
- (ii) brines are important foci of primary and secondary planktonic production; and
- (iii) because of coupling between primary and secondary production, both will be limited by the supply of inorganic nutrients.

In order to test these hypotheses, we have compared classification and regression tree (CART) analysis and multiple linear regression to explore gradients in production along nutrient gradients in twenty discrete ponds and three brines. A secondary aim of the study was to compare the applicability of these two approaches in analysing datasets with the non-linear relationships and multiple interacting factors that are often a feature of field production studies.

2. Study area and experimental design

The McMurdo Ice Shelf is a 1500 km^2 ablation region, with a covering of moraine and marine sediment supporting extensive areas of melt water during summer (Swithinbank 1970). Immediately south of Bratina Island (78°00'S, 165°35'E) is an area of sediment-covered ice supporting numerous discrete, sediment-lined meltwater ponds with lifetimes of several decades, that vary in size from tens to hundreds of square metres (Howard-Williams *et al* 1989, Hawes *et al* 2011a). The site contains ponds with a wide variation in water chemistry, due to localized salt lenses, sea water intrusions and pond ages, allowing biological comparisons across environmental gradients untainted by significant geographical variation. Permanent daylight, mild temperatures and high

Table 1. Physicochemical variables of all ponds studied, and of the monimolimnion brines of the three meromictically stratified ponds.

Pond	Conductivity $(mS cm^{-1})$	DRP $(mg m^{-3})$	NH_4-N (mg m ⁻³)	NO_3-N (mg m ⁻³)	TDN (mg m ⁻³)	$\frac{\text{TDP}}{(\text{mg m}^{-3})}$	Chl a (mg m ⁻³)	DOC (g m ⁻³)	pН	DIC $(mol m^{-3})$
	. ,		(Surface mixol	imnion					. ,
Duet	0.1	41	<1	<1	136	49	0.9	1.2	9.8	0.2
Permanent ice	0.2	19	2	2	133	28	1.1	7.9	10.2	0.3
Fogghorne	0.7	0	8	<1	496	17	0.6	8.1	9.7	2.2
Skua	0.7	107	10	2	441	133	2.3	9.4	8.4	0.9
Sushi	0.9	<1	9	1	754	11	0.5	10.0	9.1	1.8
Fresh	1.1	81	4	6	381	106	1.0	6.4	7.6	0.9
K081	1.1	74	7	<1	517	102	1.3	7.4	7.9	0.9
Casten	1.4	183	11	2	743	233	4.3	9.8	9.0	2.3
Dosli	2.1	41	31	<1	1 520	116	1.8	17.6	9.8	0.4
Legin	3.1	3	15	2	899	33	1.6	13.3	9.0	1.6
Orange	3.3	7	55	7	2130	102	1.5	21.0	9.6	6.4
Pond 2	3.7	386	9	7	908	456	3.0	n.d.	8.4	2.8
Eggtimer	4.1	<1	5	<1	818	24	0.7	12.8	9.2	3.9
Howard	4.4	66	7	4	220	81	0.6	1.6	9.2	0.5
Huey	6.4	<1	59	40	2010	47	8.4	21.8	10.1	2.1
P70	6.4	13	5	<1	1950	73	1.0	26.2	9.6	2.7
Pond 3	7.0	21	49	1	3 580	147	4.7	61.1	9.7	11.1
Upper	8.4	76	41	1	3 3 4 0	258	8.4	23.7	9.9	7.4
P70E	9.3	<1	2	11	1 040	24	1.2	11.9	8.7	2.0
VXE6	10	<1	19	2	1 800	41	1.9	19.9	8.8	1.8
Brack	20	3	14	7	4430	230	7.3	20.1	10.0	2.9
Salt	66	26	111	5	18430	892	37.2	236.0	9.8	7.9
			Ν	Ionimolimnio	n brines					
Eggtimer brine	92.9	<1	270	<1	24 760	455	363.0	406.0	8.5	92.9
Orange brine	48.0	180	601	<1	38 660	1560	111.7	640.0	9.2	108.9
Sushi brine	14.2	23	260	<1	1 560	340	27.9	n.d.	9.3	55.6

photosynthetic activity result in high pH values in many ponds during the summer open water period.

The study was carried out in late January 2007, at the time of maximum ice melt and peak summer temperatures (5.0–8.5 °C). We selected twenty ponds ranging from dilute, fresh water (0.1 mS cm⁻¹) to highly saline (>60 mS cm⁻¹) for production comparisons (table 1). Most of these ponds (all local unofficial names) have been described and mapped in various previous studies (Howard-Williams et al 1989, Wait et al 2009, Hawes et al 2011a). Stratification was examined by profiling from the shore, using a combined temperature/conductivity probe suspended from a 4 m long telescopic aluminum probe. At the time of our study three ponds (Orange, Eggtimer and Sushi Ponds) showed meromictic stratification; the remainder had fully mixed water columns. The experimental design was therefore based on a comparison of the 20 ponds plus a comparison of surface and brine layers in the three stratified ponds. Two ponds (Duet Pond and Permanent Ice Pond) retained extensive surface ice with 1-2 m-wide thawed moats, but the remainder were fully ice-free at the time of sampling.

Surface waters were collected by hand from the shore, as far from the edge as possible. For the stratified ponds we used a pulley attached to a line stretched across each pond to lower a weighted, acid rinsed, 5 mm diameter plastic hose into the brine, extracting the liquid with a hand-operated vacuum pump. Production assays involved incubations and were carried out under common conditions (see below) rather than *in situ* for in each pond, to exclude the effects of localized differences in light and temperature. During the summer melt period, the light and temperature regimes are in any case relatively similar in most ponds (see Hawes *et al* 2011a, 2011b, Safi *et al* 2012).

3. Methods

3.1. Water chemistry

All water for experiments and analysis was collected in acid-washed polyethylene bottles. The pH was measured immediately upon collection with a Mettler portable pH meter, calibrated using standard buffers, and temperature and conductivity measured with a C-90 conductivity–temperature meter (TPS Ltd, Springwood, Australia), calibrated using a two-point procedure against air and a conductivity standard.

We measured dissolved inorganic carbon (DIC) on 0.5 or 1.0 ml samples injected into pre-washed, partially evacuated 4 ml vacutainers containing 0.2 ml of 10% phosphoric acid. Headspace subsamples were subsequently analysed on a LiCor infrared gas analyser, calibrated with standard solutions of sodium bicarbonate. Samples for total dissolved organic carbon (DOC) were filtered through a pre-combusted GF/F filter and stored, unfrozen, in pre-combusted brown glass bottles for return to New Zealand. Analysis was as described by Hawes *et al* (2011b). For estimating planktonic chlorophyll a (chl a), we filtered samples onto Whatman GF/F filters which were stored frozen for return to New Zealand. Dissolved nitrogen and phosphorus species were determined on the filtrate, which was also frozen. Methods for nutrient and chlorophyll analysis were as described by Hawes *et al* (2011b).

3.2. Phytoplankton photosynthetic activity

Planktonic photosynthesis (PP) was measured on 10 ml of freshly collected pond water in glass vials (n = 4), to which 10 μ Ci of ¹⁴C-labelled bicarbonate was added (Amersham International Ltd). The vials were incubated for 24 h in a common outdoor water bath at 6 °C under ambient daylight, with dark controls for each wrapped in foil and incubated simultaneously. At the end of incubations, 0.2 ml of 10% HCl was added to preserve the sample and remove unfixed inorganic carbon. To test the efficacy of HCl, we also ran acidified control vials in parallel to our experimental vials, which confirmed complete removal of inorganic ¹⁴C. Less than 3% of the added ¹⁴C was assimilated in all incubations, confirming that DIC depletion was not an issue during these experiments. Vials were counted and photosynthetic rates calculated after return to New Zealand as described by Hawes *et al* (2011b).

3.3. Bacterial activity

Bacterial production (BP) was measured from parallel ³H-thymidine (BPthy) and ³H-leucine (BPleu) incorporations (Kirchman *et al* 1985, Kirchman and Ducklow 1993, Bell 1993) on 10 ml pond water samples. Quadruplicate samples and two killed controls (0.5 ml filtered (0.2 μ m) formalin, pH 7.0) were incubated at 6 °C with 24 nmol 1⁻¹ radiotracer over 2, 4, 12 and 20 h and stopped with 0.5 ml formalin. Time courses showed linearity until 12 h; hence all rates were based on the 4 h incubations. After return to New Zealand, vials were centrifuged at 16 000 g for 7 min, washed and counted in Optiphase HiSafe (Wallac) scintillation cocktail, as described by Hawes *et al* (2011b). Thymidine and leucine uptake were converted to carbon fixation rates using the equations and conversion factors of Kirchman (1993) and Kirchman and Ducklow (1993).

3.4. Data analysis

We compared multiple linear regression and regression tree analyses in exploring factors controlling variation in PP and BP in the pond environment. Correlations and multiple linear regression (using stepwise forward regression and a significance level for each variable of P = 0.05) were conducted with variables transformed to meet assumptions of normality. CART analysis is particularly suited to data with non-linear relationships and where there are many variables with large numbers of potential interactions (De'Ath and Fabricius 2000, Forbes *et al* 2008). They do not require data to be normally distributed or homoscedastic, as they rank continuous variables and partition data recursively into subsets that reveal the hierarchy of which factors are most important among interacting variables (Qian *et al* 2003, Scott *et al* 2008). Regression tree models were generated by cross-validation, running a series of 50, ten-fold cross-validations, and then pruning the tree to achieve the smallest tree size with an estimated error rate within one standard error of the minimum (Breiman *et al* 1984). All analyses were performed in JMP 10.0 for Windows 7 (SAS Institute Inc., Cary, NC).

4. Results

4.1. Pond chemistry and metabolic rates

Water chemistry parameters in the ponds varied up to three orders of magnitude, with the most saline ponds more than 60 times higher in conductivity, 135 times higher in TDN, and 200 times higher in TDP and DOC, than the most dilute (table 1). Both inorganic nitrogen and phosphorus values ranged from below detection limits to $>100 \text{ mg m}^{-3}$. Inorganic nitrogen was consistently low compared with TDN, reaching a maximum of 5%, and was mainly present as ammonium rather than nitrate. 70% of ponds had a chlorophyll biomass of $< 2 \text{ mg m}^{-3}$, with an overall range of 0.5-37.2 mg m⁻³. Conductivity and nutrients were also high in the monimolimnetic brines of the three stratified ponds, and these also had high chlorophyll biomass, and accumulated high concentrations of DOC and nutrients compared with the mixolimnions of the same ponds, or the mixed water column in any of the other ponds.

All waters sampled had high pH (>8.4) with values over pH 10.0 in some cases, but in general pH did not differ greatly and pH of brines was similar to pH of mixed surface layers. In contrast, there was a wide range in DIC, from 0.2 mol m⁻³ in the more oligotrophic ponds, to high concentrations (up to 7.9 mol m⁻³) in some of the more nutrient-rich ponds. Brines were rich in inorganic carbon, with DIC in the brine in Orange Pond as high as 109 mol m⁻³, 17 times higher than its mixolimnion.

PP ranged from 1.07 mgC m⁻³ h⁻¹ in Duet Pond, to 65.72 mgC m⁻³ h⁻¹ in Salt Pond (table 2), but was even higher in the brines (figure 1(a)). PP rates in all three brines were higher than in any of the mixed pond waters, and were 61, 84 and 14 times higher in Eggtimer, Orange and Sushi Pond brines than in the corresponding mixolimnions. Specific assimilation rates of phytoplankton were more uniform, with mean values ranging from 0.83 to 5.97 mgC mg⁻¹ chl h⁻¹, most of the variation in volumetric rates therefore resulting from differences in algal biomass between ponds. Notably, the specific assimilation rates of the brines were well within the range of the surface waters (0.99, 1.15 and 5.97 mgC mg⁻¹ chl h⁻¹ in Sushi, Orange and Eggtimer brines).

BP rates also differed between mixed surface waters of ponds (table 2), but rates were lower and within a narrower range (0.08–1.66 mgC m⁻³ h⁻¹ for BPthy, 0.03–3.14 mgC m⁻³ h⁻¹ for BPleu) than photosynthesis.

Pond	Photosynthesis $(mgC m^{-3} h^{-1})$	Photosynthesis $(mgC mg^{-1} chl h^{-1})$	Bacterial production thymidine method $(mgC m^{-3} h^{-1})$	Bacterial production leucine method $(mgC m^{-3} h^{-1})$
Duet	1.07 (0.43)	1.18 (0.48)	0.08 (0.01)	0.03 (0.01)
Permanent ice	1.30 (0.31)	1.18 (0.29)	0.11 (0.01)	0.28 (0.05)
Fogghorne	2.59 (0.85)	4.32 (1.41)	0.46 (0.02)	1.27 (0.21)
Skua	3.64 (1.18)	1.58 (0.51)	0.68 (0.03)	1.00 (0.66)
Fresh	2.44 (0.99)	2.44 (0.99)	0.60 (0.02)	1.96 (0.36)
K081	2.85 (0.42)	2.19 (0.32)	0.57 (0.07)	1.13 (0.14)
Casten	11.49 (3.75)	2.67 (0.87)	1.53 (0.12)	1.48 (0.10)
Dosli	1.53 (1.13)	0.85 (0.63)	0.17 (0.03)	0.54 (0.16)
Legin	2.13 (1.28)	1.33 (0.80)	0.46 (0.03)	0.92 (0.39)
Orange	8.71 (1.76)	5.81 (3.87)	1.15 (0.18)	1.60 (0.50)
Pond 2	8.66 (2.57)	2.89 (0.86)	0.48 (0.03)	1.66 (0.17)
Howard	1.71 (0.21)	2.85 (0.36)	0.28 (0.01)	1.06 (0.07)
Huey	15.90 (2.40)	1.89 (0.29)	0.60 (0.04)	1.50 (0.03)
P70	2.09 (0.54)	2.09 (0.54)	0.30 (0.01)	0.87 (0.13)
Pond 3	5.79 (1.40)	1.23 (0.29)	0.75 (0.06)	1.39 (0.13)
Upper	16.08 (6.01)	1.92 (0.72)	1.07 (0.40)	2.22 (0.24)
P70E	2.76 (0.87)	2.30 (0.73)	0.36 (0.02)	0.85 (0.13)
VXE6	4.38 (3.43)	2.30 (1.81)	0.74 (0.05)	2.13 (0.16)
Brack	6.05 (2.80)	0.83 (0.38)	1.42 (0.06)	2.08 (0.38)
Salt	65.72 (29.47)	1.77 (0.79)	1.66 (0.17)	3.14 (0.43)

Table 2. Photosynthetic and bacterial production rates of 20 study ponds. Mean values (n = 4) with SD in brackets.

Table 3. Pearson correlation coefficients of log-transformed data for all variables. Correlations >0.55 and significant at P < 0.05 shown in bold. Cond is conductivity, PP is phytoplankton production, BPthy and BPleu are bacterial production measured with the thymidine and leucine methods.

	Cond	DRP	NH ₄ –N	NO ₃ –N	TDN	TDP	Chl a	DOC	pН	DIC	РР	BPthy	BPleu
Cond	1	-0.214	0.754	0.109	0.890	0.603	0.687	0.783	0.027	0.785	0.766	0.596	0.523
DRP		1	-0.003	-0.138	-0.137	0.568	0.052	-0.087	-0.167	-0.103	-0.013	0.113	0.156
NH ₄ –N			1	-0.042	0.833	0.659	0.788	0.771	0.127	0.841	0.856	0.743	0.587
NO ₃ –N				1	-0.024	-0.026	-0.023	-0.063	-0.008	-0.164	-0.039	0.060	0.258
TDN					1	0.663	0.754	0.936	0.137	0.829	0.804	0.585	0.444
TDP						1	0.754	0.626	-0.084	0.604	0.724	0.580	0.410
Chl a							1	0.731	0.015	0.744	0.837	0.571	0.271
DOC								1	0.060	0.784	0.752	0.463	0.370
pН									1	0.021	0.031	-0.059	-0.087
DIC										1	0.917	0.759	0.499
PP											1	0.730	0.430
BPthy												1	0.829
BPleu													1

Thymidine and leucine methods correlated well ($r^2 = 0.64, P < 0.0001$), but BPleu rates were slightly higher than BPthy with an overall Leu:*T* ratio of 1.26 (*t*-test t = -6.87, P < 0.0001). BPthy in the brines was more than twice as high as any of the surface waters and within-pond comparisons (figure 1(b)) showed large differences between layers in individual ponds, although not as large as differences in photosynthesis.

Several chemical and biological variables were strongly correlated (table 3). The conductivity gradient that guided pond selection was reflected in other chemical variables, with many significant positive correlations between conductivity, TDN and TDP, ammonium, DOC and DIC as shown in table 3. Conductivity, TDN, TDP, ammonium and DIC were all strongly associated with chlorophyll, which in turn was well correlated to PP. DIC was the single variable most strongly correlated to PP (r = 0.917). BPthy was associated with ammonium, DIC and PP, whereas BPleu correlated only

to BPthy and not to other variables. Three variables (pH, DRP and nitrate) were not significantly correlated to any other variables.

4.2. Multiple linear regression

The stepwise forward multiple linear regressions modelled the production rates by selecting the best predictors on the basis of those describing most of the variance; models and equation parameters are shown in table 4. For each of PP and BPthy multiple regression models selected two significant variables; PP was best predicted from DIC and chlorophyll, and the DIC–PP relationship was apparent over the whole range of the DIC concentrations present in pond waters (figure 2(a)). The overall r^2 for the PP model was 0.88 (adjusted $r^2 = 0.87$). DIC also made the greatest contribution to the multiple regression model for BPthy (figure 2(a)), with ammonium as a second parameter (figure 2(b)); the strong collinearity

Table 4. Multiple linear regression statistics describing dependence of phytoplankton production and bacterial production rates on predictor variables.

Variable	Parameter estimate	P > F	Partial r^2 (adjusted r^2)				
Phytoplankton production model							
Log DIC	0.625	< 0.006	0.825				
Log Chl a	0.290	< 0.0001	0.057				
Model summar		0.882 (0.870)					
Bacterial production model (BPthy)							
Log DIC	0.305	< 0.0001	0.510				
Log NH ₄ –N	0.218	< 0.05	0.051				
Model summa	0.561 (0.517)						
Bacterial production model (BPleu)							
Log NH ₄ -N	0.538	< 0.001	0.531				
Model summa	ry		0.531 (0.502)				

between variables in table 3 suggests caution in interpreting this as a direct causal limitation. The overall r^2 for the BPthy model was 0.56 (adjusted $r^2 = 0.52$). The multiple linear regression model for BPleu identified a single significant parameter, ammonium, with r^2 of 0.53 (adjusted $r^2 = 0.50$).

4.3. Regression tree models

The regression tree model for PP (figure 3) selected two significant variables, NH_4^+ and DIC (table 5). These two variables together explained 80% of the variation in PP, with NH_4^+ accounting for most of the variation in the model (partial $r^2 = 0.683$). The NH_4^+ threshold was 50 mg m⁻³, with the high- NH_4^+ group including the brines and the four ponds with highest surface NH_4^+ ; these sites had the highest mean production rates ($1.78 \pm 0.54 \text{ mgC m}^{-3} \text{ h}^{-1}$). Sites with $NH_4-N < 50 \text{ mg m}^{-3}$ were further split into two groups across a DIC threshold of 2.29 mol m⁻³ (partial $r^2 = 0.119$). The lowest mean production rates ($0.34\pm0.18 \text{ mgC m}^{-3} \text{ h}^{-1}$) occurred in the group of sites with both low DIC and low NH_4^+ .

The regression tree analyses identified NH_4^+ availability as the main factor limiting PP (table 5). Figure 3 indicates that the NH₄–N threshold of 50 mg m⁻³ is the saturation concentration on a NH₄⁺-PP response curve, above which PP is not nutrient-limited. DIC co-limitation is seen in the linear response of PP to DIC (figure 2) and in the stepwise regression model, although it exaggerates the overall importance of DIC relative to NH_4^+ because the DIC-PP response is linear, whereas the NH_4^+ -PP response is non-linear. PP at $NH_4\text{--}N$ < 50 mg m^{-3} was notably related to inorganic carbon availability, with a group of low-N, high DIC $(>2.29 \text{ mol } \text{m}^{-3})$ sites that is solely N-limited and not C-limited (mean PP = $0.89 \pm 0.18 \text{ mgC m}^{-3} \text{ h}^{-1}$), and the oligotrophic group in which N and C are both low and, according to the model, co-limiting. The next split, which was pruned from the tree, was a chl *a* split with $r^2 = 0.009$ that did not contribute explanatory power to the model.



Figure 2. The relationship of significant predictors from multiple linear regression of (a) inorganic carbon limitation and (b) ammonium limitation on primary and secondary production.

 Table 5. Regression tree threshold values, uncertainty estimates, and P-values for phytoplankton production and bacterial production.

Variable	Threshold (95% CI)	Partial r^2
Phytoplan	kton production model	
NH ₄ -N	54.95	0.683
DIC	2.29	0.119
Model summary		0.802
Bacterial pro	oduction model (BPthy)	
PP (first threshold)	6.03	0.426
PP (second threshold)	2.14	0.134
Model summary		0.560
Bacterial pro	oduction model (BPleu)	
РР	2.45	0.451
Model summary		0.451

In the regression tree model for BP, PP was the only significant variable (figure 4), with the pruned regression tree explaining 56% of the variation in BPthy and 45% of the variation in BPleu (table 5). The BPthy model identified three significantly distinct groups of BPthy rates, a consequence of the highly non-linear relationship between



Figure 3. Results from CART analysis of phytoplankton production rates (mgC m⁻³ h⁻¹). Threshold values of predictors are shown below each split, and variance explained for predictors is shown above each split. Mean values ± 1 SD and the number of samples (*n*) are shown for each subset of data, with the three terminal groups identified by numbers in **bold**. The total variation explained by this CART model was 80%. Scatterplot illustrates the relationship between PP and NH₄–N, with datapoints identified into the three CART groups. The vertical dashed line identifies the NH₄–N value that best explained variation in PP, and the horizontal line is the PP threshold between the N-limited sites that are limited or not limited by inorganic C.

PP and BPthy (figure 4). At PP < 2.1 mgC m⁻³ h⁻¹, PP is strongly limiting BPthy, and this threshold is the onset of saturation of the PP–BPthy relationship. It is almost identical to the PP threshold identified in the BPleu model (PP = $2.45 \text{ mgC m}^{-3} \text{ h}^{-1}$), confirming that both bacterial production methods were responding similarly to PP. The additional threshold at PP = $6.03 \text{ mgC m}^{-3} \text{ h}^{-1}$ in the BPthy model is driven by the high rates in the brines, which were not studied with the BPleu method.

The PP and BP models together thus implicate a causal chain from strong inorganic N limitation of PP (with a secondary effect of inorganic carbon), with PP in turn primarily limiting BP (table 5). The models exclude other



Figure 4. Results from CART analysis of bacterial production rates (mgC m⁻³ h⁻¹, thymidine method). Threshold values of predictors are shown below each split, and variance explained for predictors is shown above each split. Mean values ± 1 SD and the number of samples (*n*) are shown for each subset of data, with the three terminal groups identified by numbers in **bold**. The total variation explained by this CART model was 56%. Scatterplot of relationships of BPthy and BPleu to PP, the only significant parameter selected by regression tree analysis for both methods (CART for BPleu not shown). The vertical lines identify the values of PP that best explained variation in BPthy (dashed lines) and BPleu (**bold** dotted line).

variables that correlate strongly with PP and BP (table 3), and suggest that the significant linear regressions on BP of NH_4^+ and DIC (table 4) are merely indirect consequences of their importance for PP. The response of BP to PP in figure 4 is a saturation curve, with higher rates of BP sustained at high PP in the brines than in the mixolimnions.

5. Discussion

The range of carbon fixation rates found in this study for phytoplankton of meltwater ponds of the MIS is similar to rates measured in many fresh and brackish waters in small and/or shallow lakes in temperate latitudes (Kalff 2002, p 337). This is true of both volumetric rates and of assimilation numbers (photosynthesis expressed per chlorophyll biomass). MIS pond assimilation numbers are lower than in most tropical and warm-climate waters, but similar to or even higher than many temperate lakes. During the brief summer melt period, temperatures and light intensities in the ponds are comparable to those at many temperate locations, and the TDN and TDP concentrations are also comparable to those found in temperate lakes and ponds. In this regard the MIS ponds are unusual for Antarctic inland waters, when compared against the permanently ice-covered large lakes where light attenuation and very low nutrient concentrations lead to assimilation numbers that are more than an order of magnitude lower (e.g. Lizotte and Priscu 1992). Phytoplankton biomass in waters further inland and in supraglacial ponds is also much lower than on the MIS (Webster-Brown et al 2010).

The predominant limitation of carbon fixation in the MIS ponds by nitrogen availability in our study is consistent with earlier work at the site (Howard-Williams *et al* 1989). Both stepwise regression and CART identified N, and never P, as important in nutrient limitation. Inorganic N:P ratios also identified N limitation, with 15 of the ponds having an inorganic N:P < 7.2, but again there was no relation between N:P and PP or the CART output.

The large number of ponds sampled in this study has allowed us to explore and model the ancillary factors affecting and limiting primary production more fully. Inorganic carbon limitation or co-limitation of photosynthesis is often thought to be rare in marine and freshwater phytoplankton (Hein and Sand-Jensen 1997, Clark and Flynn 2000), especially as most phytoplankton species have carbon-concentrating mechanisms, particularly bicarbonate usage (Raven et al 2008). At the prevailing summer temperatures, in the putatively C-limited (low-C, low-N) group identified in the CART analysis, bicarbonate still forms about 50% of the inorganic carbon (Stumm and Morgan 1970). However, DIC itself is also low, leading to bicarbonate concentrations <0.2 mol m⁻³, which are low enough to reduce carbon fixation due to trade-offs between investment in carbon-concentration and RuBisCo specificity, limitations of uptake of micronutrients, and competition (Morel et al 1994, Clark and Flynn 2000, Tortell 2000, Wirtz 2011). Based on these studies, we believe it is likely that there is an inorganic C co-limitation of photosynthesis and growth with N (e.g. Saito et al 2008) in this group of ponds. Nitrogen limitation may exacerbate such trade-offs in investment in N-intensive protein-based enzymatic systems.

The high-N, high-C group, which includes the meromictic brines, has the highest carbon fixation rates and is unlikely to be directly limited by either N or C. Instead, severe photoinhibition (Rae *et al* 2000) is probably the limiting factor here, as it appears phytoplankton in this group are unable to fully exploit the available inorganic N and C. Phytoplankton of the brines may also suffer photorespiration and photooxidative stress, as these are super-saturated with dissolved oxygen (>60 mgO₂ 1⁻¹, Safi *et al* 2012). Nevertheless, the brines are by far the most productive of all the waters of the MIS, and given that meromictic stratification is a prolonged feature during summer for most MIS ponds (Wait *et al* 2006, Howard-Williams and Hawes 2007), may previously have been underestimated as a contributor to both algal and bacterial production.

Strong nitrogen limitation of carbon fixation may initially appear counter-intuitive given the relatively high TDN concentrations in almost all the MIS ponds studied here. It is likely that most of this DON and the DOC is recalcitrant, as commonly observed in other aquatic ecosystems (Wetzel 1990, Berman and Bronk 2003, Willett *et al* 2004).

The predominance of recalcitrant DOC also apparently holds for the bacterial production of the MIS ponds, which was related only to PP in the CART analysis and not to any of the organic or inorganic nutrient pools. Such close coupling of BP to PP argues that release of immediately labile DOM from the phytoplankton is the predominant C (and perhaps N) source for the bacteria, with the large DOC and DON pools largely unavailable to them. Correlations between primary and bacterial productivity in aquatic ecosystems are common, as bacteria are strongly dependent on labile organic carbon release from phytoplankton (Cole et al 1988). There is nevertheless considerable variability in the PP:BP relationship between different types of aquatic ecosystems, with the strongest relationships occurring in smaller water bodies where PP and BP respond more rapidly to changes in nutrient inputs (Fouilland and Mostajir 2010). In many aquatic ecosystems BP can greatly exceed PP due to allochthonous organic inputs, but the much lower BP than PP at the MIS is consistent with predominantly closed pond basins receiving no external nutrient inputs from barren catchments. The total bacterial carbon demand includes both that incorporated in biomass (as measured by the thymidine and leucine methods) plus bacterial respiration (Rochelle-Newall et al 2008), and in oligotrophic systems bacterial growth efficiencies are low (del Giorgio et al 1997), but even applying the highest growth efficiencies of ca. 25% suggested by del Giorgio et al (1997) to our data suggests that total bacterial carbon demand is much less than PP. The two bacterial production methods gave similar results, especially given uncertainties in converting label uptake to C and the tendency for the thymidine method, which predominantly labels nucleic acids, to underestimate BP relative to leucine, which labels proteins (van Looij and Riemann 1993, Tuominen 1995).

Our study shows that the regression tree method can be a valuable approach for describing algal and bacterial production in aquatic ecosystems along an environmental gradient. Tree models are most useful when there is a hierarchical structure to the predictor–response relationships and relationships are non-linear. Such patterns are common in ecology and were certainly evident in the PP models on the MIS. In contrast, multiple linear models fit all parameters simultaneously and can underestimate the importance of predictors with a non-linear fit to the response. For example, the exaggerated effect of DIC over NH_4^+ on PP in the multiple regression analysis is a function of PP increasing linearly with DIC but non-linearly with NH_4^+ . The wide range of nutrient availabilities and other physicochemical parameters that are available on the MIS, unconfounded by other factors due to the limited geographical range of the site and simplicity of systems, provided a powerful comparison of these approaches, and CART allowed us to extract the important features of this unusual site from the many strongly correlated parameters that are driven by the range in size of the ponds on the shelf.

In conclusion, this study has supported the three hypotheses proposed for planktonic production in ice shelf ponds. It has highlighted the very close coupling of primary and secondary production in these waters during the summer melt phase, and that this is a function of strong inorganic nutrient, especially N, limitation. It has also for the first time identified a secondary inorganic C limitation in some ponds, which is a function of the high pH and low inorganic C concentrations. Finally it has highlighted the basal brines as zones of high activity, although biomass-specific productivity in brines is similar to the surface waters suggesting that this reflects high biomass concentration rather than specific activity.

Acknowledgments

This study was funded by the New Zealand Foundation for Research, Science and Technology (Contract C01X306) with logistical support provided by Antarctica New Zealand (Project K081).

References

- Bell R T 1993 Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine *Handbook of Methods in Aquatic Microbial Ecology* ed P F Kemp, B F Sherr, E B Sherr and J J Cole (Ann Arbor, MI: Lewis) pp 495–504
- Berman T and Bronk D A 2003 Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems *Aquat. Microb. Ecol.* **31** 279–305
- Breiman L, Friedman J H, Olshen R A and Stone C J 1984 *Classification and Regression Trees* (Belmont, CA: Wadsworth International Group) p 358
- Clark D R and Flynn K J 2000 The relationship between the dissolved inorganic carbon concentration and growth rate in marine phytoplankton *Proc. R. Soc.* B **267** 953–9
- Cole J J, Findlay S and Pace M L 1988 Bacterial production in fresh and saltwater ecosystems: a cross-system overview *Mar. Ecol. Prog. Ser.* **43** 1–10
- De'Ath G and Fabricius K E 2000 Classification and regression trees: a powerful yet simple technique for ecological data analysis *Ecology* **81** 3178–92
- Debenham F 1920 A new mode of transportation by ice: the raised marine muds of South Victoria Land (Antarctica) *Q. J. Geol. Soc. London* **75** 51–76
- del Giorgio P A, Cole J J and Cimbleris A 1997 Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems *Nature* 385 148–51
- Forbes M G, Doyle R D, Scott J T, Stanley J K, Huang H and Brooks B W 2008 Physical factors control phytoplankton production and nitrogen fixation in eight Texas reservoirs *Ecosystems* **11** 1181–97
- Fouilland E and Mostajir B 2010 Revisited phytoplanktonic carbon dependency of heterotrophic bacteria in freshwaters, transitional, coastal and oceanic waters *FEMS Microbiol. Ecol.* 73 419–29

- Gow A J and Epstein S 1972 On the use of stable isotopes to trace the origins of ice in a floating ice tongue *J. Geophys. Res.* 77 6552–7
- Hawes I, Howard-Williams C, Downes M T and Schwarz A-M 1997
 Environment and microbial communities in a tidal lagoon at Bratina Island, McMurdo Ice Shelf, Antarctica Antarctic Communities: Species, Structure and Survival ed B Battaglia, J Valencia and D W H Walton (Cambridge: Cambridge University Press) pp 170–7
- Hawes I, Howard-Williams C and Fountain A 2008 Ice-based freshwater ecosystems *Polar Lakes and Rivers—Arctic and Antarctic Aquatic Ecosystems* ed W F Vincent and J Laybourn-Parry (Oxford: Oxford University Press) pp 103–18
- Hawes I, Howard-Williams C and Pridmore R D 1993
 Environmental controls on microbial biomass in the ponds of the McMurdo Ice Shelf, Antarctica Arch. Hydrobiol. 127 271–87
- Hawes I, Safi K, Sorrell B K, Webster-Brown J and Arscott D 2011a Summer–winter transitions in Antarctic ponds: I. The physical environment Antarct. Sci. 23 243–54
- Hawes I, Safi K, Webster-Brown J, Sorrell B K and Arscott D 2011b Summer–winter transitions in Antarctic ponds: II. Biological responses Antarct. Sci. 23 235–42
- Hein M and Sand-Jensen K 1997 CO₂ increases oceanic primary production *Nature* 388 526–7
- Howard-Williams C and Hawes I 2007 Ecological processes in Antarctic inland waters: interactions between physical processes and the nitrogen cycle *Antarct. Sci.* **19** 205–17
- Howard-Williams C, Pridmore R, Downes M T and Vincent W F 1989 Microbial biomass, photosynthesis and chlorophyll a related pigments in the ponds of the McMurdo Ice Shelf, Antarctica Antarct. Sci. 1 125–31
- James M R, Pridmore R D and Cummings V J 1995 Planktonic communities of melt ponds on the McMurdo Ice Shelf, Antarctica *Polar Biol.* 15 555–67

Kalff J 2002 Limnology (Englewood Cliffs, NJ: Prentice-Hall) p 592

- Kirchman D 1993 Leucine incorporation as a measure of biomass production by heterotrophic bacteria *Handbook of Methods in Aquatic Microbial Ecology* ed P F Kemp, B F Sherr,
 E B Sherr and J J Cole (Ann Arbor, MI: Lewis) pp 509–12
- Kirchman D and Ducklow H W 1993 Estimating conversion factors for the thymidine and leucine methods for measuring bacterial production *Handbook of Methods in Aquatic Microbial Ecology* ed P F Kemp, B F Sherr, E B Sherr and J J Cole (Ann Arbor, MI: Lewis) pp 513–8
- Kirchman D, Knees E and Hodson R 1985 Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic ecosystems *Appl. Environ. Microbiol.* 49 599–607
- Lizotte M P and Priscu J C 1992 Photosynthesis–irradiance relationships in phytoplankton from the physically stable water column of a perennially ice-covered lake (Lake Bonney, Antarctica) J. Phycol. 28 179–85
- Morel F M M, Reinfelder J R, Roberts S B, Chamberlain C P, Lee J G and Yee D 1994 Zinc and carbon co-limitation of marine phytoplankton *Nature* **369** 740–2
- Qian S S, King R S and Richardson C J 2003 Two statistical methods for the detection of environmental thresholds *Ecol. Modelling* **166** 87–97
- Quesada A, Fernandez-Valiente E, Hawes I and Howard-Williams C 2008 Benthic primary production in polar lakes and rivers *Polar Lakes and Rivers* ed W F Vincent and J Laybourn-Parry (Oxford: Oxford University Press) pp 179–96
- Rae R, Howard-Williams C, Hawes I and Vincent W F 2000
 Temperature dependence of photosynthetic recovery from solar damage in Antarctic phytoplankton Antarctic Ecosystems: Models for Wider Ecological Understanding: Proc. VII SCAR

Biology Symp. ed W Davison, C Howard-Williams and P Broady (Christchurch: Caxton Press) pp 182–9

- Raven J A, Cockell C S and De La Rocha C L 2008 The evolution of inorganic carbon concentrating mechanisms in photosynthesis *Phil. Trans. R. Soc.* B 363 2641–50
- Rochelle-Newall E J, Torréton J-P, Mari X and Pringault O 2008 Phytoplankton–bacterioplankton coupling in a subtropical South Pacific coral reef lagoon *Aquat. Microb. Ecol.* **50** 221–9
- Safi K, Hawes I and Sorrell B K 2012 Microbial population responses in three stratified Antarctic meltwater ponds during the autumn freeze Antarct. Sci. 24 571–88
- Saito M A, Goepfert T J and Ritt J T 2008 Some thoughts on the concept of colimitation: three definitions and the importance of bioavailability *Limnol. Oceanogr.* **53** 276–90
- Scott J T, Doyle R D, Prochnow S J and White J D 2008 Are watersheds and lacustrine controls on planktonic N-2 fixation hierarchically structured? *Ecol. Appl.* 18 805–19
- Stumm W and Morgan J J 1970 Aquatic Chemistry (New York: Wiley–Interscience) p 780
- Suren A 1990 Microfauna associated with algal mats in melt ponds of the Ross Ice Shelf *Polar Biol.* **10** 329–35
- Swithinbank C 1970 Ice movement in the McMurdo Sound area of Antarctica Int. Symp. on Antarctic Glaciological Exploration, 1968 (International Association of Scientific Hydrology and Scientific Committee on Antarctic Research Publication vol 86) pp 472–87

- Tortell P D 2000 Evolutionary and ecological perspectives on carbon acquisition in phytoplankton *Limnol. Oceanogr.* **45** 744–50
- Tuominen L 1995 Comparison of leucine uptake methods and a thymidine incorporation method for measuring bacterial activity in sediment *J. Microbiol. Methods* **24** 125–34
- van Looij A and Riemann B 1993 Measurements of bacterial production in coastal marine environments using leucine—application of a kinetic approach to correct for isotope dilution *Mar. Ecol. Prog. Ser.* **102** 97–104
- Wait B R, Webster-Brown J G, Brown K R, Healy M and Hawes I 2006 Chemistry and stratification of Antarctic meltwater ponds I: coastal ponds near Bratina Island, McMurdo Ice Shelf Antarct. Sci. 18 515–24
- Webster-Brown J, Gall M, Gibson J, Wood S and Hawes I 2010 The biogeochemistry of meltwater habitats in the Darwin Glacier region (Lat 80°S), Victoria Land, Antarctica Antarct. Sci. 21 646–61
- Wetzel R G 1990 Land–water interfaces: metabolic and limnological regulators Verh. Int. Verein. Theor. Angew. Limnol. 24 6–24
- Willett V B, Reynolds B A, Stevens P A, Ormerod S J and Jones D L 2004 Dissolved organic nitrogen regulation in freshwaters J. Environ. Qual. 33 201–9
- Wirtz K W 2011 Non-uniform scaling in phytoplankton growth rate due to intracellular light and CO₂ decline *J. Plankton Res.* 33 1325–41