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Biochemical composition and photosynthate distribution in sea ice microalgae of McMurdo Sound, Antarctica: evidence for nutrient stress during the spring bloom

MICHAEL P. LIZOTTE[1] and CORNELIUS W. SULLIVAN[2]

[1]*Present address: Biology Department, Montana State University, Bozeman, Montana 59717, USA*
[2]*Hancock Institute of Marine Studies and Department of Biological Sciences, University of Southern California, Los Angeles, California 90089-0371, USA*
[2]*Reprint requests*

Abstract: The nutrient status of microalgae inhabiting sea ice in McMurdo Sound, Antarctica was evaluated during the peak and decline of the spring bloom in November and December. Natural populations of microalgae were analysed for C, N, chlorophyll *a*, protein, lipid, polysaccharide, and low-molecular-weight carbohydrate content, and for the distribution of ^{14}C-labelled photosynthate into macromolecular fractions. Ratios of N:C and protein to carbohydrate (PR:CHO) were similar to values reported for nutrient-limited phytoplankton. Biochemical ratios and ^{14}C-photosynthate allocation patterns suggest that microalgae from congelation ice habitats may be more nutrient-stressed than those from underlying platelet ice habitats. This trend would be consistent with the presumed gradient of seawater nutrient influx through the platelet layer to the bottom congelation ice habitat. Microalgae from congelation ice subjected to an experimental depletion of nutrients (particularly nitrate) showed decreased N:C, PR:CHO, and allocation of ^{14}C-photosynthate to proteins. This evidence suggests that sea ice microalgae are nutrient-stressed during the peak and decline of the spring bloom in McMurdo Sound, which presumably begins when microalgal biomass concentrations and demands for growth reach or exceed the rate of nutrient supply from underlying seawater.

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Introduction

Microalgae bloom in several sea ice habitats in McMurdo Sound, including the bottom of congelation ice, within underlying platelet ice, and on surface ice infiltrated by seawater. The largest sea ice algal blooms occur in the austral spring, with biomass often exceeding 100 mg chlorophyll *a* m^{-2} (Palmisano & Sullivan 1983, Grossi *et al.* 1987, Palmisano *et al.* 1988). The overall productivity of bottom ice algae is limited by light availability (Bunt & Lee 1970, Grossi *et al.* 1987). However, recent time course studies have suggested that algae may become nutrient-stressed at the peak and demise of the spring bloom in McMurdo Sound in November and early December (Palmisano *et al.* 1988, Nichols *et al.* 1989, Cota & Sullivan 1990).

Nutrient limitation of growth in sea ice algae has received more study in the Arctic (Gosselin *et al.* 1985, Maestrini *et al.* 1986, Cota *et al.* 1987, Demers *et al.* 1989, Gosselin *et al.* 1990, Smith *et al.* 1990, Cota *et al.* 1990) than in the Antarctic, in part because it is presumably more important in the nutrient-poor Arctic seas (Sakshaug & Holm-Hansen 1984). However, the large standing crop of sea ice microalgae in McMurdo Sound (e.g. Palmisano & Sullivan 1983, Grossi *et al.* 1987, Palmisano *et al.* 1988), which can be up to an order of magnitude higher than reported from the Arctic (e.g. Gosselin *et al.* 1985, Smith *et al.* 1987), may have the potential to significantly deplete nutrients despite the relatively large nutrient stocks in underlying waters.

Various sea ice habitats where microalgae bloom in McMurdo Sound may differ substantially in rates of nutrient supply. Microalgae bloom in bottom congelation ice up to 20cm from the ice-seawater interface (Palmisano & Sullivan 1983), where seawater exchange is supplied by gravity drainage through a series of brine tubes and channels *c.* 0.4–10mm in diameter (Reeburg 1984). Beneath the congelation ice, algae also bloom on ice surfaces and in interstitial water within an unconsolidated layer of ice platelets (Grossi *et al.* 1987). The platelet ice layer has a water to ice ratio of *c.* 3:1, and thus may have relatively high exchange rates with underlying seawater. We propose that the platelet ice physically reduces the seawater flux to congelation ice because it acts as a thick unstirred layer, and any seawater passing through the platelet ice layer would probably contain less nutrients due to *in situ* algal uptake. The residence times of seawater in congelation ice and platelet ice are not known, and nutrient supply could be continuously excessive, continuously limiting, or periodic. The upper surface of sea ice where algae can grow is infiltrated by seawater intermittently, based on tidal cycles and ice deformation processes, and would presumably exhibit the effects of periodic nutrient supply.

Our understanding of the ecology of sea ice algae is limited both by our inability to study sea ice algae *in situ*, and a lack

of techniques to measure the physicochemical conditions in the varied microenvironments of the sea ice (Kottmeier & Sullivan 1990). An indirect approach for assessing these conditions is to determine the physiological state of algae experimentally exposed to conditions representing the range of physicochemical conditions probable within sea ice. Used in studies of photosynthate allocation to various macromolecular end-products (McConville *et al.* 1985, Palmisano & Sullivan 1985, Smith *et al.* 1987, Palmisano *et al.* 1988) and gross biochemical composition (Nichols *et al.* 1989, Gosselin *et al.* 1990) the results of this approach suggest that sea ice algae may be nutrient-stressed during the latter stages of a bloom. Preliminary studies of the distribution of ^{14}C-labelled photosynthate among macromolecular fractions also suggest that algae from congelation ice are nutrient-stressed compared with algae from platelet ice (Palmisano & Sullivan 1985). We examined the physiological characteristics of algae from a variety of sea ice habitats to extend the work of Palmisano & Sullivan (1985) by including analysis of carbon(^{14}C) allocation to various classes of lipids and analyses of gross biochemical composition. Additional laboratory experiments focused on algae from bottom congelation ice to determine the time course of their response to decreased nutrient supply.

Methods

Sampling

Sea ice was sampled from nine sites in McMurdo Sound (Fig.1) between October 25 and December 27 in 1984, 1985 and 1986. Cores of first-year congelation ice were collected with a SIPRE ice coring device (7 cm core barrel) and the bottom 1–10cm (discoloured by algae) was removed and placed in -1°C filtered (Whatman GF/C) seawater (FSW). Surface ice samples were collected from overflow regions near coastal tide cracks (sites T and W) or from melt pools (sites D and I) and placed in FSW. Ice samples were transported to McMurdo Station and allowed to melt at 0°C in the dark. More FSW was added, if necessary, to maintain salinity (measured with a refractometer) between 30 and 35ppt. Samples from the platelet ice layer (*c.*20–100cm thick) were collected by SCUBA divers using 4 l polyethylene jars. Interstitial seawater containing algae was immediately decanted into another container; algae firmly attached to ice were not included in experimental platelet ice samples. The salinity of platelet interstitial water was 34–35ppt, approximately the same as that of the underlying seawater. Large aggregates of tube dwelling diatoms (*Berkeleyi* sp.) occasionally present in ice core samples, were removed by passing the sample through a 400 μm mesh size Nitex screen.

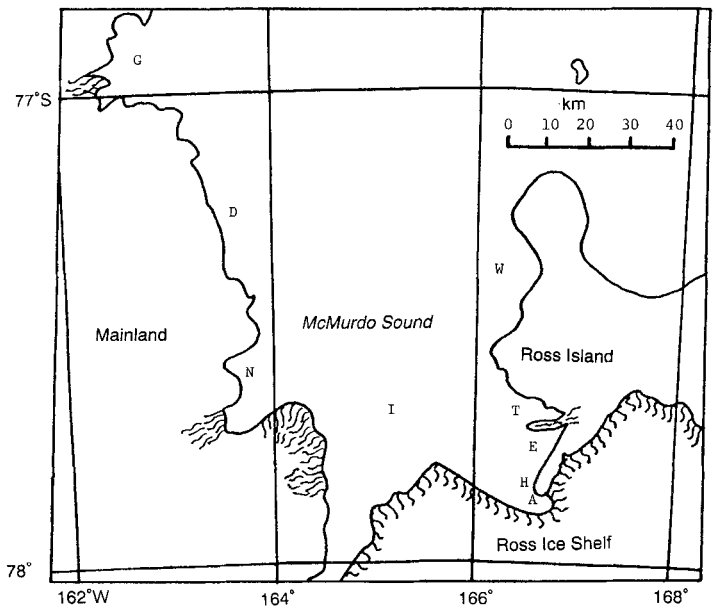

Fig. 1. Map of McMurdo Sound, Antarctica showing sites where sea ice was collected. Abbreviations: G-Granite Harbour; N-New Harbour; D-Daily Islands; I-ice edge; W-Wohlschlag Bay; T-Tent Island; E-Erebus Bay; H-Hut Point; and A-Cape Armitage.

Chemical composition

Subsamples for chl *a* analysis were concentrated on Whatman filters (GF/C) and stored at-20°C. Filtered material was extracted in 90% acetone for 8–12h at 4°C in the dark. Chlorophyll *a* concentration in the extract was determined with a Turner 111 fluorometer calibrated with standard amounts of chl *a* (Sigma Chemical), and corrected for phaeopigment fluorescence (Strickland & Parsons 1972). The data were also corrected for any dilution with FSW.

Subsamples for particulate organic carbon, nitrogen, protein, lipid, and carbohydrate analysis were filtered onto precombusted GF/C (500°C for 6h) and stored at-20°C (see Table I for abbreviations). For C and N, filtered material was dried at 60°C and duplicate samples were analysed on a Control Equipment Corporation 240XA CHN analyser by the Marine Science Institute Analytical Laboratory at the University of California, Santa Barbara. Values from blank

Table I. Biochemical components quantified for sea ice microalgae, with abbreviations used throughout text.

chl *a*	chlorophyll *a*
C	organic carbon
N	nitrogen
PR	protein
LP	lipid
LMWC	low-molecular-weight carbohydrate
PS	polysaccharide
CHO	total carbohydrate (LMWC + PS)
^{14}C-PR	^{14}C-labelled protein
^{14}C-LP	^{14}C-labelled lipid
^{14}C-LMWM	^{14}C-labelled low-molecular-weight metabolites
^{14}C-PS	^{14}C-labelled polysaccharide
^{14}C-NLP	^{14}C-labelled neutral lipid
^{14}C-GLP	^{14}C-labelled glycolipid
^{14}C-PLP	^{14}C-labelled phospholipid

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precombusted filters were subtracted from C and N values. For protein, triplicate samples were extracted in boiling 0.1N NaOH for 10 min, passed through a precombusted GF/C, and assayed by a modified Lowry method (Schleif & Wensink 1981) calibrated against bovine serum albumen standards. For lipid, low-molecular-weight carbohydrate, and polysaccharide, another triplicate set of samples was sequentially processed: 1) extraction with methanol:chloroform (2:1 by volume) for 12 h; 2) collection of the extract passing through a precombusted GF/C; 3) extraction of the residue in boiling 0.1N NaOH for 10min; and 4) collection of the NaOH-extract passing through a precombusted GF/C. Deionized water was added to the methanol:chloroform extract to give a final methanol:water:chloroform ratio of 2:1:1, which partitioned into chloroform and methanol:water fractions. The chloroform extract was dried under nitrogen and assayed for lipid content using a charring technique (Marsh & Weinstein 1966) calibrated against safflower oil standards (Shifrin & Chisholm 1981). The methanol:water fraction (LMWC) and the NaOH extract (PS) were assayed for carbohydrate content using a phenol-sulphuric acid method (Kochert 1978) calibrated against glucose standards dissolved in methanol:water (2:1) and 0.1N NaOH, respectively.

PR, LP, LMWC, and PS (μg standard) were normalized to μg C for comparison between samples. To assess the proportion of C accounted for by the fractions, we multiplied PR:C by 0.53 (Ditullio & Laws 1983), LP:C by 0.77 (Vollenweider 1985), and LMWC:C and PS:C by 0.40 (Vollenweider 1985); the sum (PR+LP+LMWC+PS) ranged from 69 to 112% of total C (mean =85%, sd =14%, n=13).

Bacterial numbers and biovolumes were determined by direct counts of DAPI (4', 6-diamidino-2-phenylindole 2 HCl) stained cells and bacterial carbon was estimated using a conversion factor of 220 fg C μm^{-3} (Bratbak & Dundas 1984).

Distribution of ^{14}C-photosynthate among end-products

Suspensions of ice algae were placed in borosilicate glass containers and inoculated with $NaH^{14}CO_3$ solution (ICN Pharmaceuticals) to a final activity of 18.5–37KBq ml^{-1}. Samples from congelation ice and platelet ice were incubated at -1°C under cool-white fluorescent lamps at 6–9 $\mu E\ m^{-2}\ s^{-1}$ of light (measured with a Biospherical Instruments QSI-140 4Π steradian scalar radiometer) typical of the under ice environment during the spring bloom period and presumably limiting for photosynthesis (Palmisano *et al.* 1985). After 24–26 h, aliquots were filtered onto Whatman GF/C filters, rinsed twice with FSW, and stored at-20°C; this incubation period was sufficient to reach intracellular equilibrium of the radiolabel (Lizotte 1989).

Duplicate filters were thawed and extracted using the procedures of Li *et al.* (1980). This technique separates four crude fractions dominated by low-molecular-weight metabolites (methanol:water soluble), lipids (chloroform soluble), polysaccharides (hot trichloroacetic acid soluble) and proteins (insoluble). For two-thirds of these samples, a second set of duplicate filters was available for determining the distribution of ^{14}C-photosynthate among lipid fractions. ^{14}C-LP was fractionated using the silicic acid column chromatography method of Guckert *et al.* (1985) as described in Palmisano *et al.* (1988). This technique yields three fractions (see Table I for abbreviations) dominated by neutral lipids (eluted by chloroform), glycolipids (eluted by acetone), and phospholipids (eluted by methanol). All fractions were dried at 60°C, and ^{14}C-PS samples were redissolved in 1 ml deionized water. Ten ml of Aquasol-II scintillation cocktail was added and radioactivity was determined on a Beckman model LSC100, model LS3801, or model LS6800 liquid scintillation counter. Counts per minute were corrected to disintegrations per minute based on sample quench determined by the external standards ratio.

Nutrient depletion experiment

The bottom 5 cm of congelation ice was collected at site T on 28 November, 1986 and melted in FSW to produce a salinity of 31ppt. Initial subsamples were withdrawn for chl *a*, C, N, PR, LP, LMWC and PS as described above. One litre polycarbonate flasks were filled with 1 l of the cell suspension and treated with the following daily nutrient additions:

1) Control A: NO_3^-, PO_4^{-3}, and H_2SiO_4;
2) Control B: no amendment;
3) Minus N : PO_4^{-3} and H_2SiO_4;
4) Minus P : NO_3^- and H_2SiO_4; and
5) Minus Si : NO_3^- and PO_4^{-3}

Additions of 20 μM NO_3^-, 0.6 μM PO_4^{-3}, and 8.6 μM H_2SiO_4 were calculated to replace the demands of the algae assuming a growth rate of $0.2d^{-1}$. All treatments except Control B were inoculated initially with micronutrients and vitamins equivalent to F/10 culture media (Carlucci 1973). Incubations were conducted at -1.5°C under 7.5 $\mu E\ m^{-2}\ s^{-1}$ light.

Control A, Minus N, Minus P, and Minus Si were subsampled daily for chl *a* and nutrients before the daily nutrient addition. After nutrients were added, a subsample was incubated with ^{14}C-bicarbonate (55.5 KBq ml^{-1}) to determine carbon fixation rates and the distribution of ^{14}C-photosynthate to end-products. Control B and a duplicate flask for Control A were maintained for 10 d then filtered for biochemical composition.

Samples for dissolved nutrient analysis were passed through a GF/C filter and frozen in acid-rinsed polyethylene bottles. Concentrations of PO_4^{-3}, H_2SiO_4, NO_2^-, and $NO_3^- + NO_2^-$ were measured on a 5-channel AlpKem RFA-300 continuous flow analyser (Whitledge *et al.* 1981). Nitrate concentrations were estimated by subtracting NO_2^- (which was always <0.08 μM) from "$NO_3 + NO_2^-$".

Results

Biochemical composition of algae was determined for four

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Table II. Microalgal species composition in sea ice from McMurdo Sound. Numbers in parentheses are percent of total cell count. See Fig.1 for site locations.

ice type	site	date	species*
surface	W	14-12-85	A(99.9%), O(0.1%)
	T	27-12-85	A(99.9%), O(0.1%)
	D	05-12-86	A(98.9%), O(1.1%)
	I	05-12-86	B(63%), C(27%)
congelation	E	16-11-86	C(26%), D(21%), E(21%), O(32%)
	E	24-11-86	C(52%), D(11%),E(10%), O(27%)
	E	28-11-86	C(40%), D(17%),E(15%), O(28%)
	E	03-12-86	C(56%), E(13%), D(2%), O(29%)
	E	07-12-86	C(41%), D(19%),E(9%), O(31%)
	T	28-11-86	F(40%), D(29%), O(31%)
	T	11-12-86	F(35%), D(34%), O(31%)
	W	18-11-86	F(80%), O(20%)
	W	25-11-86	F(92%), O(8%)
platelet	E	07-12-85	G(52%), H(4%), O(42%)
	E	12-18-85	G(60%), H(17%), O(23%)

*A - *Navicula glaciei*; B - *Nitzschia* sp.; C - *Chaetoceros* sp.; D -*Amphiprora kufferathii*; E - *Biddulphia* sp.; F - *Nitzschia stellata*; G - *Porosira pseudodenticulata*; H - *Pleurosigma* sp.; and O-other diatom species.

samples from surface ice, nine samples from bottom congelation ice, and two samples from platelet ice. All samples were dominated by diatoms and several were virtually unialgal (Table II). Species composition was notably different for each habitat. Surface ice communities were most often dominated by *Navicula glaciei* van Huerck. Congelation ice communities were dominated by either *Nitzschia stellata* Manguin or *Chaetoceros* sp., with the larger *Amphiprora kufferathii* Manguin an important second in most samples. Platelet ice was dominated by *Porosira pseudodenticulata* (Hustedt) Jouse in both samples collected for chemical composition and in 5 of 11 samples used for studies of ^{14}C-bicarbonate assimilation; the remaining samples were dominated by species listed above for congelation ice.

Little detrital material and few empty diatom frustules were observed microscopically. Bacterial carbon was 0.1–3.5% of total C (mean=1.0%) but its actual contribution was probably smaller because bacterial cells were counted on 0.2 μm filters whereas all other samples were collected on Whatman GF/C filters (effective retention size *c.*1 μm. Furthermore, a regression of C on chl *a* (C=58.7 · chl-1.5, n=15, r=0.904, P<0.01) had an intercept that was not significantly different from zero (sd = 9.8).

All samples examined for biochemical composition had dense concentrations of algae, with chl *a* concentrations 0.14–10.1 mg l^{-1} (Table III). The densest chl *a* concentrations were found in thin layers at the base of congelation ice (1 cm at W and 5 cm at T). Concentrations could not be determined for two samples because the sea ice was not sampled quantitatively; however, visual observations suggested that chl *a* concentrations in surface ice from T was similar to the other sites and that algal concentration in congelation ice from W was comparable for both dates. Actual biomass concentrations within the microenvironments of congelation and surface ice must be greater since these values include the volume of ice as well as interstitial fluid. Ratios (per weight) of chl *a*:C ranged from 0.011–0.043, while N:C ranged from 0.08–0.22 (Table III). Biochemical constituents normalized

Table III. Biochemical composition of particulate material in sea ice of McMurdo Sound during the spring ice algal bloom. Ratios are based on mass concentrations. ND is not determined.

ice type	site	date	chl *a* (mg·l^{-1})	chl *a*: C	N: C	LMWC: C	PS: C	LP: C	PR: C	PR: CHO
surface	W	14-12-85	0.52	0.027	0.17					
	T	27-12-85	ND	0.013	0.11	0.40	0.35	0.18	0.45	0.60
	D	05-12-86	0.27	0.022	0.12	0.46	0.38	0.34	0.89	1.05
	I	05-12-86	0.73	0.012	0.11	0.51	0.40	0.22	0.60	0.66
	mean			0.018	0.13	0.45	0.38	0.25	0.65	0.77
congelation	E	16-11-86	0.14	0.016	0.15	0.41	0.28	0.26	0.73	1.06
	E	24-11-86	0.51	0.019	0.13	0.48	0.40	0.39	0.69	0.78
	E	28-11-86	0.49	0.013	0.12	0.32	0.31	0.25	0.66	1.05
	E	03-12-86	0.31	0.011	0.08	0.31	0.27	0.20	0.58	1.00
	E	07-12-86	0.39	0.011	0.09	0.41	0.33	0.24	0.52	0.70
	T	28-11-86	2.9	0.024	0.11	0.26	0.39	0.32	0.48	0.74
	T	11-12-86	2.7	0.021	0.09	0.31	0.33	0.31	0.46	0.72
	W	18-11-86	10.1	0.012	ND	0.17	0.25	0.46	0.39	0.93
	W	25-11-86	ND	0.020	0.08	0.09	0.39	0.51	0.41	0.85
	mean			0.016	0.11	0.31	0.33	0.33	0.55	0.87
platelet	E	07-12-85	0.43	0.043	0.20					
	E	18-12-85	0.38	0.040	0.22	0.35	0.26	0.38	0.73	1.20
	mean			0.041	0.21					
	grand mean			0.020	0.13	0.34	0.33	0.31	0.58	0.87

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to C showed the least variation in polysaccharide (0.25–0.40) and the most variation in protein (0.39–0.89). LMWC:C and LP:C were also highly variable (0.09–0.51 and 0.18–0.51, respectively). Ratios of proteins to total carbohydrates averaged 0.87, and were generally <1.

The biochemical ratios of microalgae from different ice habitats generally had overlapping ranges and means, but there were some notable exceptions and trends. Platelet ice algae had chl *a*:C and N:C ratios that were significantly higher (two-group *t*-tests, $P<0.01$) than algae from either congelation or surface ice. A platelet ice sample also showed the highest PR:CHO, which was 38% higher than the overall mean value. Congelation ice algae collected from siteW, which were predominantly *N. stellata*, had the highest ratios for LP:C and the lowest ratios for LMWC:C. At site E, congelation ice algae decreased chl *a*:C, N: C and PR: C over the 21 d period studied.

Platelet ice microalgae also differed significantly from congelation ice microalgae in the distribution of ^{14}C-photosynthate. On average, platelet ice microalgae showed lower ^{14}C-photosynthate allocation to ^{14}C-PS (11%less) and ^{14}C-NLP (2.3%less), and greater allocation to ^{14}C-PR (6% more), ^{14}C-LMWC (6% more), and ^{14}C-PLP (1.8% more), than congelation ice algae (Table IV). The ratio of ^{14}C-PR to ^{14}C-PS was always <1, from 0.04–0.77 in congelation ice algae (mean = 0.33) and from 0.27–0.91 in platelet ice algae (mean=0.62). There were no seasonal trends in the distribution of ^{14}C-photosynthate during the spring bloom period. The percent ^{14}C-photosynthate distributed to particular end-products did not show a significant linear relationship with the % of total C in nominally equivalent biochemical fractions, but this may have been due to different extraction methodologies.

Reduced concentrations of nutrients produced changes in the biochemical composition of congelation ice algae in batch culture (Table V). Nutrients withheld from specific treatments were decreased considerably in concentration after 9–10 d: nitrate by 94–96%, phosphate by 80–90%, and silicic acid by 55–75%. Nutrient additions led to increased concentrations, indicating that daily additions exceeded uptake by the algae; by the fifth day, NO_3^- exceeded the upper limit of detection (110 μM). Control A, Minus N and Minus P treatments had similar chl *a*:C ratios while treatments with lower silicate concentrations (Minus Si and Control B) were slightly lower. Algae that received nitrate additions (Control A, Minus P and Minus Si) increased N:C whereas those that depleted nitrate (Minus N and Control B) had N:C ratios significantly lower than Control A ($P<0.05$ by paired *t*-tests). PR:CHO increased 27% in algae given daily additions of all three macronutrients (Control A) and decreased slightly (7%) in algae given no nutrients (Control B).

Only the Minus N treatment showed any significant changes in the distribution of ^{14}C-photosynthate, primarily as a decrease in ^{14}C-PR after 5 d relative to the control (Fig. 2). ^{14}C-PR decreased from 18% to 13% by day 9, and was accompanied by a reciprocal increase in ^{14}C-LP from 14% on day 5 to 20% by day 9; these changes coincided with the decrease in nitrate to <2 μM. ^{14}C-PR and ^{14}C-LP in the Minus P and Minus Si treatments were consistent with the control, and there were no consistent differences between treatments in ^{14}C-PS, ^{14}C-LMWM or the rate of carbon fixation (data presented in Lizotte 1989).

Table IV. Percent of ^{14}C-photosynthate distributed to end-products (^{14}C-PR, ^{14}C-LP, ^{14}C-PS, and ^{14}C-LMWM) by microalgae collected from congelation ice (n=46) and platelet ice (n=11) during the spring blooms of 1984, 1985 and 1986. Photosynthate distribution into different lipid fractions (^{14}C-NLP, ^{14}C-GLP, and ^{14}C-PLP) was determined for a subset of samples from congelation ice (n=30) and platelet ice (n=7). Congelation ice data includes values (n =18) published previously (Palmisano *et al.* 1988). Means and 95% confidence intervals (in parentheses) are based on the mean, standard deviation, and sample number assuming a normal distribution of values.

	congelation ice	platelet ice
proteins	15 (13–17)	21 (18–24)
polysaccharides	45 (41–49)	34 (31–37)
LMW-metabolites	26 (23–29)	32 (27–37)
lipids	14 (13–15)	13 (10–16)
neutral lipids	5.9 (4.6–7.1)	3.6 (2.5–4.8)
glycolipids	6.9 (6.4–7.3)	6.2 (5.5–7.0)
phospholipids	1.3 (1.0–1.5)	3.1 (1.9–4.3)

Table V. Initial and final nutrient concentrations and the biochemical composition of particulate material in experimental treatments containing microalgae from congelation ice. Daily additions of NO_3^-, PO_4^{-3}, and H_2SiO_4 were made to Control A and two of three nutrients were added to each 'Minus' treatment (allowing the designated nutrient to be depleted); no additions were made to Control B. Values are averages of duplicate samples from each treatment flask.

	Day	NO_3^- (μM)	PO_4^{-3} (μM)	H_2SiO_4 (μM)	C (mg l^{-1})	Chl *a*: C	N: C	LMWC: C	PS: C	LP: C	PR: C	PR: CHO
INITIAL		31	3.9	76	12.7	0.024	0.11	0.26	0.39	0.32	0.48	0.74
Control A	9	>110	9.8	86	16.3	0.026	0.13					
	10	>110	6.3	82	14.3	0.025	0.14	0.35	0.40	0.38	0.71	0.94
Control B	10	2	0.8	34	18.5	0.021	0.09	0.30	0.31	0.28	0.42	0.69
Minus N	9	1	10.1	83	15.7	0.025	0.11					
Minus P	9	>110	0.4	87	14.6	0.028	0.13					
Minus Si	9	>110	5.7	19	13.8	0.021	0.13					

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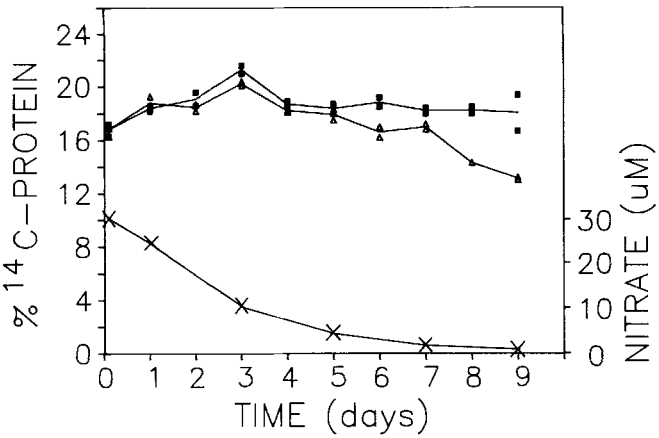

Fig. 2. Time course of allocation to ^{14} C-PR for congelation ice algae in the Minus N (triangles) and Control A (squares) treatments, and of nitrate concentration (X) in the Minus N treatment. Points are replicate measurements and lines connect average values.

Discussion

Changes in the physiological state of sea ice microalgae over the course of the spring bloom have been interpreted as both photo-acclimation and nutrient deficiency (Palmisano *et al.* 1988, Gosselin *et al.* 1990, Smith *et al.* 1990, Cota & Sullivan 1990). However, congelation ice algae in McMurdo Sound do not show consistent trends in photosynthesis-irradiance characteristics over the course of the spring bloom (Cota & Sullivan 1990) as incident irradiance increases. Coincident studies of photosynthate distribution (Palmisano *et al.* 1988) and lipid composition (Nichols *et al.* 1989) showed little change before the platelet ice disappeared and the underside of the congelation ice began to deteriorate (observed both by SCUBA divers and as a decrease in chl *a* m^{-2}). Furthermore, we did not detect any differences in biochemical composition of ice algae related to irradiance regime; for example, congelation ice algae had attributes similar to surface ice algae. Although previous studies have clearly shown that microalgae in the bottom ice of McMurdo Sound exhibit extreme acclimation to low irradiance (e.g. Palmisano *et al.* 1985, Cota & Sullivan 1990), we propose that there is little persuasive evidence that variations in biochemical composition or photosynthate allocation are related to photo-acclimation during the peak of the spring bloom (November and December). Therefore, our results will be interpreted in terms of a response to nutrient stress.

Sea ice algae from McMurdo Sound resemble nutrient-limited algae. Low N:C (<0.13) and PR:CHO (<1.2) correspond to values suggested as indicators of nutrient deficiency in temperate microalgae (reviewed by Healey 1975; see also Myklestad & Haug 1972, Myklestad 1974, Goldman 1980, Barlow 1982). Under low nutrient conditions, photosynthesis continues and diatoms have been shown to increase cellular content of both storage carbohydrates (Myklestad & Haug, 1972) and storage lipids (Shifrin & Chisholm 1981, Parrish & Wangersky 1987). The same trends have been noted for phytoplankton blooms as they peak (Haug *et al.* 1973, Conover 1975, Barlow 1984). Thus, low N:C may be due to accumulation of either storage product, but low PR:CHO suggests significant carbohydrate storage. Patterns of ^{14}C-photosynthate distribution also indicate that sea ice algae were nutrient-stressed. For instance, the ratio of ^{14}C-PR to ^{14}C-PS is low in sea ice algae from McMurdo Sound, ranging from 0.03–0.90 (Palmisano & Sullivan 1985, Palmisano *et al.* 1988, this study). These results are consistent with photosynthate distribution patterns reported for N-limited phytoplankton blooms in temperate waters (Hitchcock 1978, Barlow 1982, Hama & Honjo 1987, Hama *et al.* 1988).

Studies of nutrient status of sea ice algae from McMurdo Sound are distinctive because comparisons can be made between two easily distinguished ice strata, each with different nutrient regimes. Platelet ice lies below the congelation ice and typically ranges from 0.5–3 m in thickness; this relatively thick and presumably "unstirred" layer may physically reduce seawater exchange rates. In addition, algae associated with platelet ice are potentially capable of stripping nutrients from the seawater that reaches the congelation ice. Grossi *et al.* (1987) observed that peak algal biomass in the bottom congelation ice was an order of magnitude lower in years when a platelet layer was present compared to a year when platelet ice was absent. We found that microalgae in congelation ice differed from those in platelet ice in having lower chl *a*:C, N:C, PR:CHO, ^{14}C-PR and ^{14}C-PLP, and higher ^{14}C-PS and ^{14}C-NLP. In contrast, under-ice phytoplankton in McMurdo Sound may have even higher ^{14}C-PR (39%) and lower ^{14}C-PS (15%) (Rivken & Voytek 1987). Congelation ice algae produce less cellular material that requires N or P (e.g. proteins, chl *a*, phospholipids) and may be shunting photosynthate to C storage compounds (e.g. glucan or triglycerides). These physiological characteristics indicate that microalgae in congelation ice are subjected to a greater degree of nutrient stress than those in platelet layers, which is consistent with the presumed gradient of seawater nutrient influx through the platelet layer to the congelation ice.

Microalgae from congelation ice at the peak of the spring bloom decreased N:C, PR:C and chl *a*: C over time, similar to the response of microalgal cultures in stationary and senescent growth phases (Fogg 1965, Myklestad & Haug, 1972, Myklestad 1974, Shifrin & Chisholm 1981, Parrish & Wangersky 1987). For the same period in 1985, the distribution of photosynthate changed little as the bloom peaked, with low ^{14}C-PR (mean=16%) and ^{14}C-NLP as the dominant lipid product (Palmisano *et al.* 1988), but the proportion of triglycerides (used for carbon storage by diatoms) increased (Nichols *et al.* 1989). Together, our studies suggest that as the bloom in congelation ice peaks in late spring (late November to early December) algae produce and accumulate carbon storage products, which may indicate a metabolic shift to a stationary or senescent phase of growth.

Our comparisons rest on an assumption of common biochemical composition for diatom species in Antarctic sea

ice. However, some differences may be species-specific. For instance, the centric diatom (*P. pseudodenticulata*) that dominated in platelet ice samples analysed for biochemical composition may have a lipid composition distinct from pennate sea ice diatoms (Nichols *et al.* 1989). Congelation ice samples dominated by *N. stellata* had the highest LP:C ratios and this species may contain more neutral lipid than other sea ice algae (Priscu *et al.* 1990). However, surface ice blooms dominated by the same species (*N. glaciei*) in the Antarctic Peninsula region contained relatively more lipid than our samples (LP:C=0.58 to 0.70 in Whitaker & Richardson 1980). We have no evidence that substantial species-specific differences exist for compounds other than lipids.

In situ nutrient conditions are difficult to measure precisely for sea ice, but some general inferences can be made. First, the seawater enclosed during ice formation does not contain sufficient nutrients to produce the high algal standing stocks observed in McMurdo Sound (Cota & Sullivan 1990). At the underside of sea ice, the most likely source for an influx of nutrients is by advection and diffusion from seawater. Seawater exchange rates may depend on under-ice current velocities which can vary over periods based on diurnal tide, fortnightly tidal height or atmospheric pressure cycles (Cota *et al.* 1987). The physiological state of bottom ice algae varies fortnightly with tidal height in the Arctic (Gosselin *et al.* 1985, Cota *et al.* 1987, Demers *et al.* 1989), but not in McMurdo Sound (Cota & Sullivan 1990) where nutrient supply may vary over shorter periods dependent on diurnal tides or local atmospheric phenomena (3–6 d). In our study, an experimental decrease in NO_3^- concentrations did not effect the carbon fixation rate of congelation ice algae over 4 d (days 5 to 9 in Fig. 2; Lizotte 1989). Algal production may continue at the expense of the large intracellular supplies of dissolved nutrients recently observed in sea ice algae from the Arctic (Smith *et al.* 1990, Cota *et al.* 1990) and from McMurdo Sound (J.C.Priscu, personal communication). Thus, sea ice algae in McMurdo Sound may reach higher biomass and production levels than those from the Arctic because the length or frequency of nutrient-limited episodes is reduced by 1) higher seawater nutrient concentrations, 2) shorter cycles for seawater exchange, and 3) a large capacity for intracellular nutrient storage.

If seawater is the sole source of nutrients, our results suggest that N is more likely than P or Si to limit growth of sea ice algae in McMurdo Sound. They depleted NO_3^- more rapidly than PO_4^{-3} or H_2SiO_4 from unamended seawater and seawater amended with two of these three nutrients. Microalgae in nitrate-depleted treatments also showed the earliest and most distinct changes in their biochemistry and physiology, in a manner consistent with nitrogen-stress. Nitrogen limitation has been proposed for sea ice algae in the Arctic (Maestrini *et al.* 1986, Demers *et al.* 1989). However, biological regeneration may also provide N to algae within sea ice (Sullivan & Palmisano 1984, Maestrini *et al.* 1986, Arrigo *et al.* 1991); this may explain recent evidence that H_2SiO_4, which is only slowly remineralized, may be the nutrient in shortest supply (Gosselin *et al.* 1990, Smith *et al.* 1990, Cota *et al.* 1990, Cota & Sullivan 1990). Our results do not eliminate the possibility of silicic acid limitation *in situ*.

Microalgal blooms frequently end when biomass concentrations reach a level where the demand for nutrients exceeds the supply. Our evidence indicates that algae in McMurdo Sound are nutrient-stressed during the peak and decline of the spring bloom. A more specific analysis of the effect of nutrients on the productivity of these sea ice algal communities will require measurements of the nutrient uptake kinetics and assimilation rates of ice algae and estimates of *in situ* nutrient concentrations.

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