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Changes in photosynthetic carbon assimilation in Antarctic sea-ice diatoms during spring bloom: variation in synthesis of lipid classes

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Abstract: Carbon assimilation into three lipid classes (neutral lipids, glycolipids, and phospholipids) as well as into protein, low-molecular weight metabolites, polysaccharides, and total lipids was determined during a spring bloom of sea-ice diatoms in McMurdo Sound, Antarctica. The most dramatic changes in incorporation of $NaH^{14}CO_3$ were found in the neutral-lipid fraction. During late November, when chl $a \cdot m^{-2}$ showed an overall increase, 7–11% of the total carbon assimilated was incorporated into neutral lipids. Incorporation dropped to 0.1% in early December as chl $a \cdot m^{-2}$ leveled off, then recovered to 13% in late December, as a secondary sea-ice diatom bloom became established. The dynamic changes in photosynthetic carbon assimilation into neutral lipids suggested that profound changes in physiological state occurred in sea-ice diatom communities during the spring bloom.

Key words: Antarctica; Diatom; Lipid; Photosynthesis

Introduction

Austral-spring blooms of microalgae, primarily diatoms, occur in the lower layers of annual sea ice in McMurdo Sound, Antarctica (Bunt & Wood, 1963; Bunt & Lee, 1970; Palmisano & Sullivan, 1983). Microalgae are found in the bottom of coarse-grained congelation ice as well as in the unconsolidated platelet ice below. In congelation ice, microalgae are found in minute (0.1–1 mm) brine pockets and channels that permeate sea ice. Diatoms have been found in sea ice as early as July (mid-winter) (Bunt & Lee, 1970). Ice-algal communities in McMurdo Sound develop through the spring (September–October), with a sharp increase in chl *a* in late October–November (Bunt & Lee, 1970; Grossi *et al.*, 1987). Grossi *et al.* (1987) found that the timing of the development of ice-algal communities was strongly affected by surface snow cover. In 1982, in McMurdo Sound, snow-free areas had an accelerated bloom that reached a peak in

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chl *a* in early November, while levels of chl *a* in communities with $\geqslant 25$ cm of surface snow continued to increase into December. Standing crops as high as 300 mg chl $a \cdot m^{-2}$ have been reported in congelation ice (Palmisano & Sullivan, 1983).

Microalgae living in the bottom of congelation ice provide an excellent system in which to examine changes in the physiological state associated with the progress of an algal bloom. First, microalgae are physically trapped in sea ice allowing the repetitive sampling of one community over time; thus, it is unnecessary to employ artificial enclosures that may have adverse effects on algal communities (Gieskes *et al.*, 1979). Secondly, ice algae are free from the short-term variations in light fields associated with mixing and horizontal advection of phytoplankton (Marra, 1980; Legendre *et al.*, 1986). Such variations in irradiance may affect patterns of carbon assimilation (Hitchcock *et al.*, 1986). Thirdly, variations in temperature in McMurdo Sound are small averaging -1.86 ± 0.09 °C (Littlepage, 1965). Finally, losses from sinking or grazing appear to be greatly reduced relative to other oceanographic systems. Although algae at the ice–sea water interface may be subject to grazing, those algae higher in the ice column are physically inaccessible to most common metazoan grazers, such as amphipods.

To examine changes in physiological state during the spring bloom of ice algae in McMurdo Sound, we determined the photosynthetic assimilation of carbon into proteins, polysaccharides, low-molecular weight metabolites (LMWM), and lipids (Li *et al.*, 1980). This technique has previously been used to evaluate physiological state in temperate phytoplankton (see Morris, 1980, for review), polar phytoplankton (Smith & Morris, 1980; Barlow & Henry, 1982; Li & Platt, 1982; Rivkin & Voytek, 1987), and sea-ice microalgae (McConville *et al.*, 1985; Palmisano & Sullivan, 1985; Smith *et al.*, 1987). It has been suggested that enhanced assimilation of carbon into carbohydrates at the expense of proteins is indicative of nutrient-limited algal populations (Hitchcock, 1978; Konopka, 1983; Lancelot, 1984). Near Davis Station in East Antarctica, McConville *et al.* (1985) described the progressive decline of an ice-algal bloom in early December; an algal sheet detached from the ice in suspended strands prior to release to the water column. The authors suggested that the pattern of carbon assimilation in these algal populations (high incorporation into carbohydrates but low incorporation into protein) indicated nutrient limitation. In an earlier study, Palmisano & Sullivan (1985) reported a similar pattern of carbon assimilation in sea-ice microalgae from congelation ice in early December and suggested possible nutrient limitation or stationary-growth phase at that time. They recognized the need for time-series information during the bloom period, which we present here. Furthermore, as lipids represent a class of compounds that serve such diverse cellular functions as carbon storage (e.g., triglycerides) and structural support (e.g., membrane phospholipids), we determined the assimilation of carbon into three classes of lipids: neutral lipids, glycolipids, and phospholipids. The assay assimilation of carbon into lipid classes provided more detailed information of physiological and biochemical changes occurring during a bloom of sea-ice microalgae.

METHODS

Two study sites were chosen on annual sea ice in McMurdo Sound, Antarctica, in November–December 1985. Site 1 was ≈200 m off Cape Armitage over a water column ≈20 m in depth (Fig. 1); this has been the site of 5 yr of previous studies on sea-ice microalgae. Site 2 was located ≈5 km north of Cape Armitage, ≈200 m south of the Erebus Ice Tongue over a water column 520 m in depth.

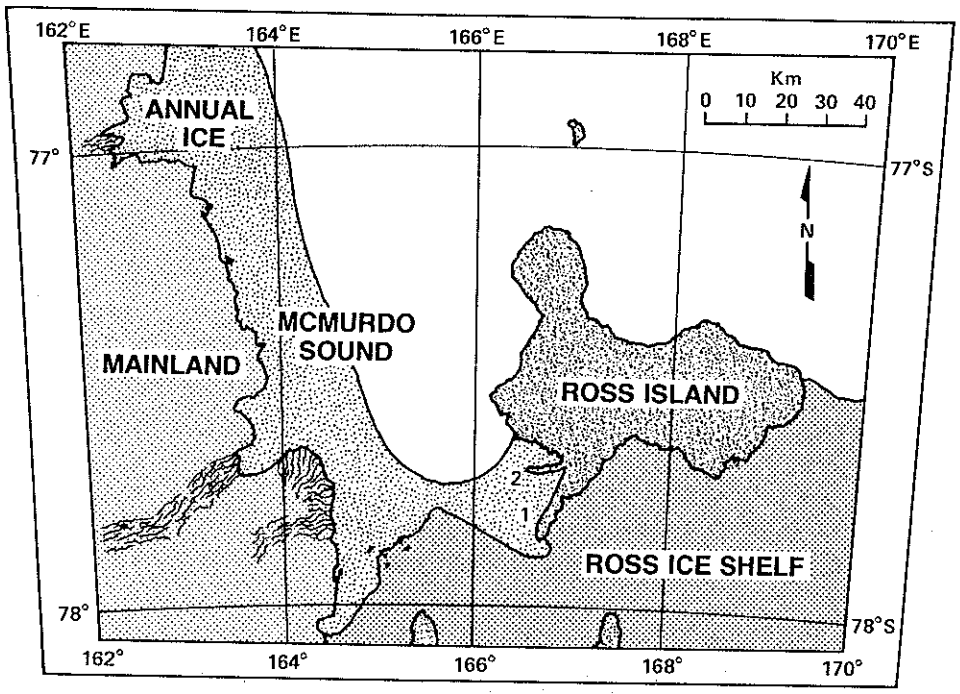

Fig. 1. Map of McMurdo Sound, Antarctica, showing study Sites 1 and 2 on annual sea ice.

Sea-ice cores from both sites were collected with a 7-cm diameter SIPRE ice auger through ≈1.7–2.5 m of annual sea ice. For physiological experiments, the lower 5 cm of the ice core containing the highest density of algae were placed in 400 ml of 0 °C filtered sea water (Whatman GF/C filters). The samples were promptly transported to the Eklund Biological Laboratory at McMurdo Station, where they were allowed to melt at 0 °C under 9 $\mu E \cdot m^{-2} \cdot s^{-1}$, a typical under-ice irradiance during the bloom (Palmisano *et al.*, 1987). Salinities were maintained at >30‰ during the melting process to avoid osmotic stress.

Chl *a* was used to estimate biomass. The bottom 20 cm of triplicate cores were melted in the dark at 0 °C into 1200 ml of filtered (Whatman GF/C) sea water. Duplicate samples for chl *a* analyses were collected on GF/C filters and extracted overnight in

90% acetone. Chl *a* concentration, corrected for phaeopigments, was determined on a Turner 111 fluorometer by the methods of Holm-Hansen *et al.* (1965).

The samples were mixed and an aliquot was collected for incubation with final concentration of 0.5 $\mu Ci \cdot ml^{-1}$ of $NaH^{14}CO_3$. To allow for intracellular equilibrium of the radiolabel (Lizotte, unpubl. data), samples were incubated for 24 h at $-2\,^{\circ}C$ and 9 $\mu E \cdot m^{-2} \cdot s^{-1}$ under cool-white fluorescent bulbs to mimic in situ under-ice conditions. At this irradiance, photosynthesis by Antarctic sea-ice microalgae is light-limited (Palmisano *et al.*, 1985). A parallel sample from the original suspension of collected ice algae was also incubated in the dark. Total dark uptake of $NaH^{14}CO_3$ was typically $<2\%$ of uptake by cells incubated in the light.

Upon completion of the incubation period, aliquots were collected for total activity of the solution, total uptake activity, incorporation into end products of photosynthesis (EPPS), and incorporation into lipid classes. Radioactivity was assayed in 10 ml of Aquasol II using a Beckman LS100 or LS6800 scintillation counter. Quench corrections were made by the external standard ratios method. Total uptake by cells was determined by filtering (thrice) 5 ml onto Whatman GF/C filters, rinsing twice in 5 ml 0 °C filtered sea water, and counting with 10 ml of Aquasol II. Duplicate 25-ml samples for radioactively labeled lipids and EPPS were also filtered onto Whatman GF/C filters, rinsed with sea water, and frozen at $-20\,^{\circ}C$ for future extraction.

Assimilation of $NaH^{14}CO_3$ into EPPS was determined by the methods of Li *et al.* (1980). These methods result in a crude fractionation of cellular constituents into protein (TCA-insoluble), polysaccharide (TCA-soluble), lipid (chloroform-soluble), and LMWM (methanol-water soluble). An average of 94% of the total label was recovered during these extractions; label recovered in each fraction is reported as a percentage of the sum total of the four fractions. Replication of individual fractions was generally within 5%.

Radioactively labeled lipids were extracted using the modified Bligh & Dyer (1959) technique described in detail elsewhere (White *et al.*, 1979; Guckert *et al.*, 1985). The dried lipid was then dissolved in 1 ml of chloroform, a 200-μl aliquot was transferred to a scintillation vial, and radioactivity was estimated for total lipid incorporation. The remaining 800 μl was transferred with (two) 500-μl chloroform washes to a 0.4-g column of activated silicic acid (Clarkson Chemical, Williamsport, Pennsylvania, U.S.) prepared in a glass disposable Pasteur pipet containing a glass-wool plug. The total lipid was then separated into neutral lipid, glycolipid, and phospholipid fractions by consecutive solutions with 5 ml chloroform, 10 ml acetone, and 5 ml methanol (Guckert *et al.*, 1985). The fractions were evaporated to dryness and counted with 10 ml of Aquasol II. Disintegrations $\cdot$ min^{-1} were always a minimum of 300 dpm above instrument background.

RESULTS

Ice thickness (Table I) increased at Site 1 in November levelling off at ≈250 cm from 21 November 1985 to 13 December 1985, then decreased during ice ablation. At Site 2, ice thickness generally increased until 7 December 1985.

TABLE I

Congelation ice thickness in McMurdo Sound, Antarctica, in austral spring of 1985 ($\bar{x}$ ± SD of three to eight cores).

Cape Armitage		Erebus Ice Tongue	
Date	Thickness (cm)	Date	Thickness (cm)
17 November 1985	237 ± 6	16 November 1985	172 ± 1
19 November 1985	240 ± 2	19 November 1985	174 ± 2
21 November 1985	250 ± 4	22 November 1985	169 ± 2
27 November 1985	251 ± 3	27 November 1985	173 ± 1
6 December 1985	249 ± 1	30 November 1985	176 ± 2
10 December 1985	250 ± 0	3 December 1985	178 ± 2
13 December 1985	250 ± 0	7 December 1985	178 ± 2
18 December 1985	250 ± 0	10 December 1985	161 ± 1
27 December 1985	242 ± 1		

Site 1 was dominated by *Amphiprora* sp. and *Nitzschia stellata*, with lesser amounts of *Berkeleya* sp. present. Site 2 was dominated by *N. stellata* with some *Amphiprora*, *Pleurosigma*, *Nitzschia kerguelensis*, and smaller (<30 μm) centrics; by 28 December 1985, *Berkeleya* were also common at this site.

In November–December 1985, changes in ice-microalgal biomass, as estimated by chl $a \cdot m^{-2}$, showed similar patterns at both study sites (Fig. 2A, B). Algal biomass generally increased at both sites through November reaching a maximum standing crop of ≈150–170 mg chl $a \cdot m^{-2}$ in early December. Subsequently, algal biomass declined as the community was released from the ice to the water column during ice ablation. The wide error bars on several points reflect the natural heterogeneity of the ice-microalgal communities at these sites; however, overall trends in algal biomass were similar at both sites.

Photosynthetic carbon assimilation into crude fractions of lipids, protein, LMWM, and polysaccharides are shown in Fig. 3A, B. The peak in algal biomass in early December was followed by a small transient increase in LMWM and proteins and by a decrease in lipids and polysaccharides. Overall, changes in the relative proportions of ^{14}C assimilated into these four major biochemical constitutents were relatively small in magnitude. During the study period (27 November–27 December), photosynthetic performance (mg C · mg chl $a^{-1} \cdot h^{-1}$) changed very little, averaging 0.037 ± SE 0.001 at Site 1 and 0.035 ± SE 0.002 at Site 2 (Lizotte, unpubl. data).

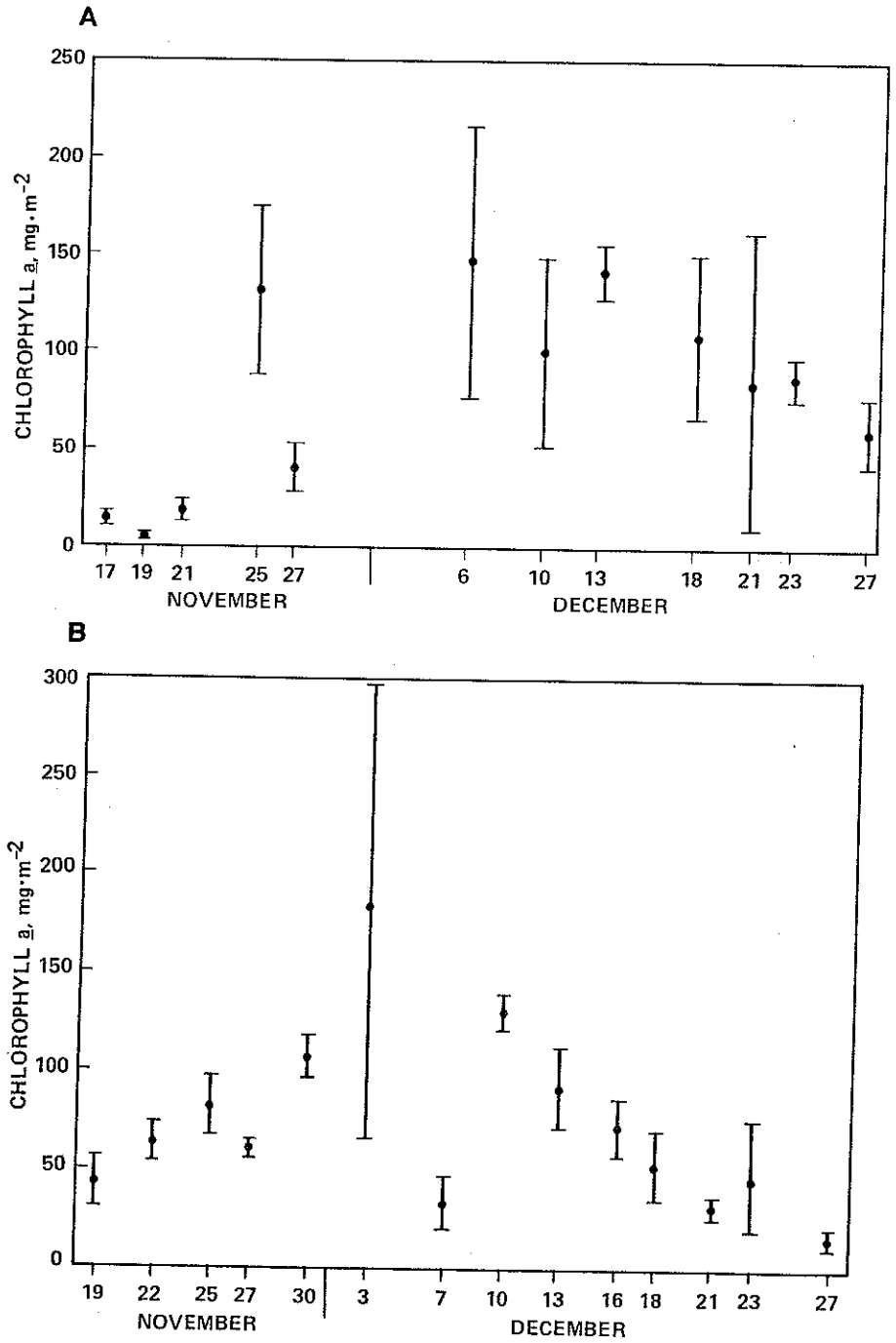

Fig. 2. Changes in standing stock of sea-ice microalgae (chl $a \cdot m^{-2}$) during spring bloom in McMurdo Sound, Antarctica. Error bars show range of three replicate samples. A, Site 1; B, Site 2.

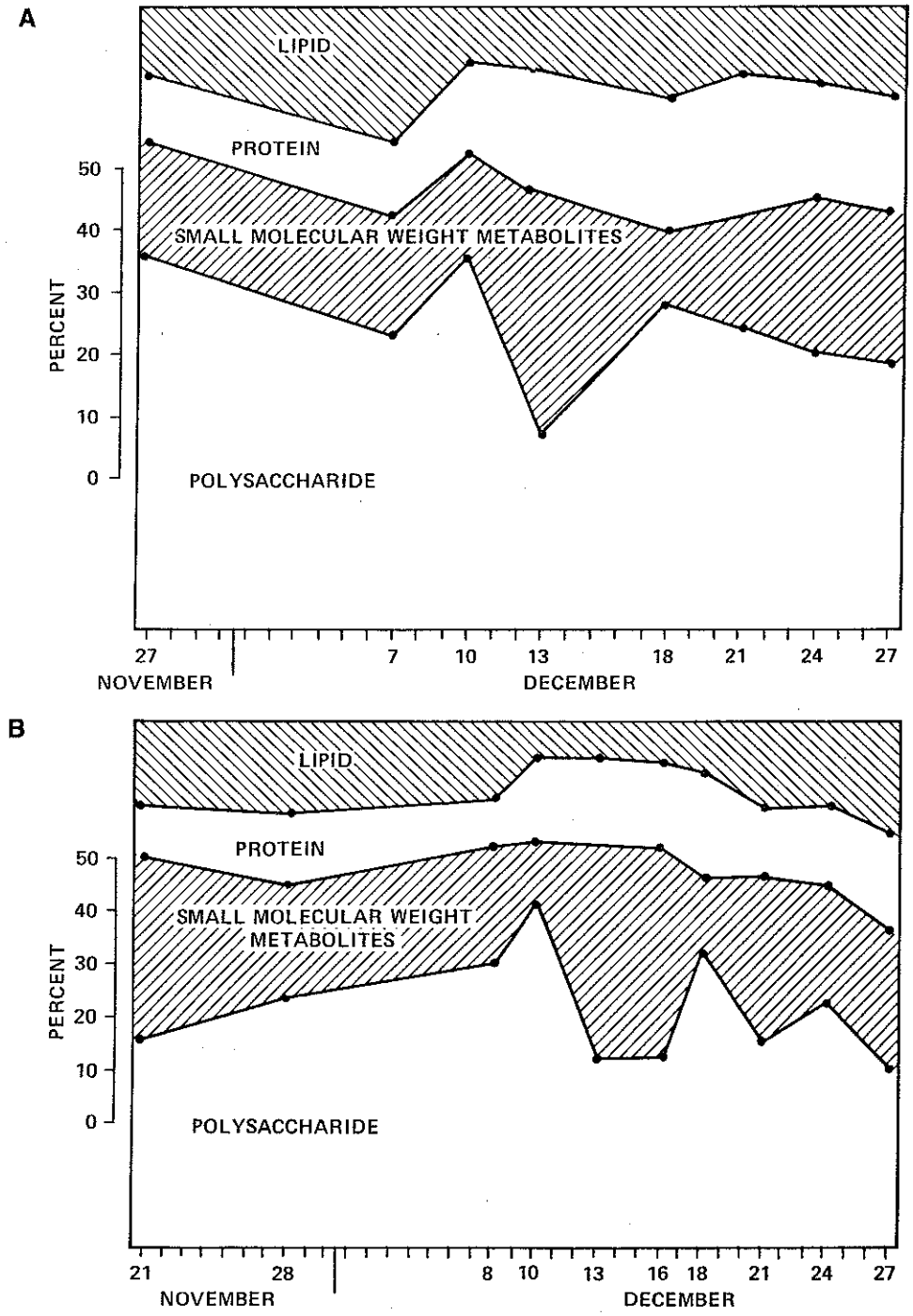

Fig. 3. Percentage of photosynthetic carbon assimilation into lipids, proteins, polysaccharides, and low-molecular weight metabolites. A, Site 1; B, Site 2.

Examination of three lipids classes (neutral lipids, glycolipids, and phospholipids) however, revealed dramatic changes in the percent of total ^{14}C assimilated into neutral lipids during the course of the spring bloom; similar patterns were observed at both study sites (Fig. 4A,B). Neutral-lipid fractions were predominant during late

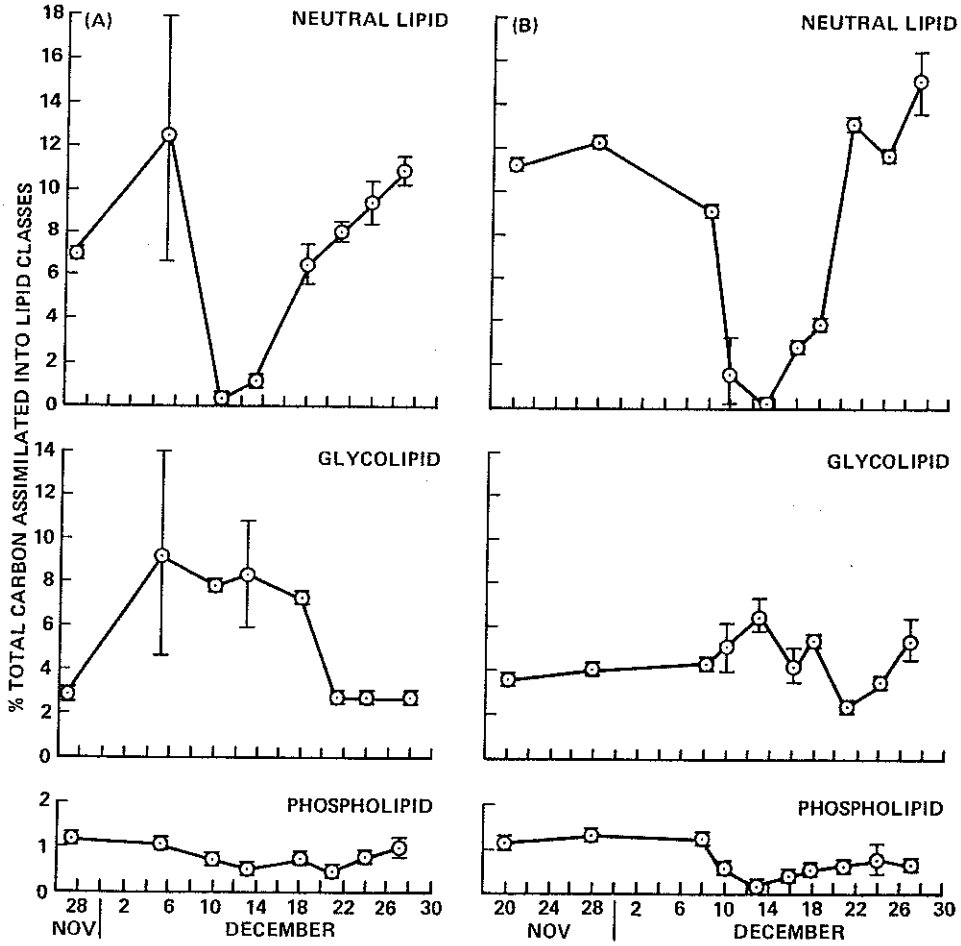

Fig. 4. Percentage of photosynthetic carbon assimilation into neutral-lipid, glycolipid, and phospholipid fractions. Error bars show range of two replicate samples. A, Site 1; B, Site 2.

November–early December, representing 11% of total carbon assimilated; however, carbon assimilation into neutral lipid declined sharply in mid-December to 0.1% of total carbon assimilated. The decrease in neutral lipids was coincident with the decrease in total lipid fraction in the EPPS analysis (Fig. 3A, B). Percent of total carbon assimilation into neutral lipids increased subsequently in late December, levelling off at $\approx 13\%$

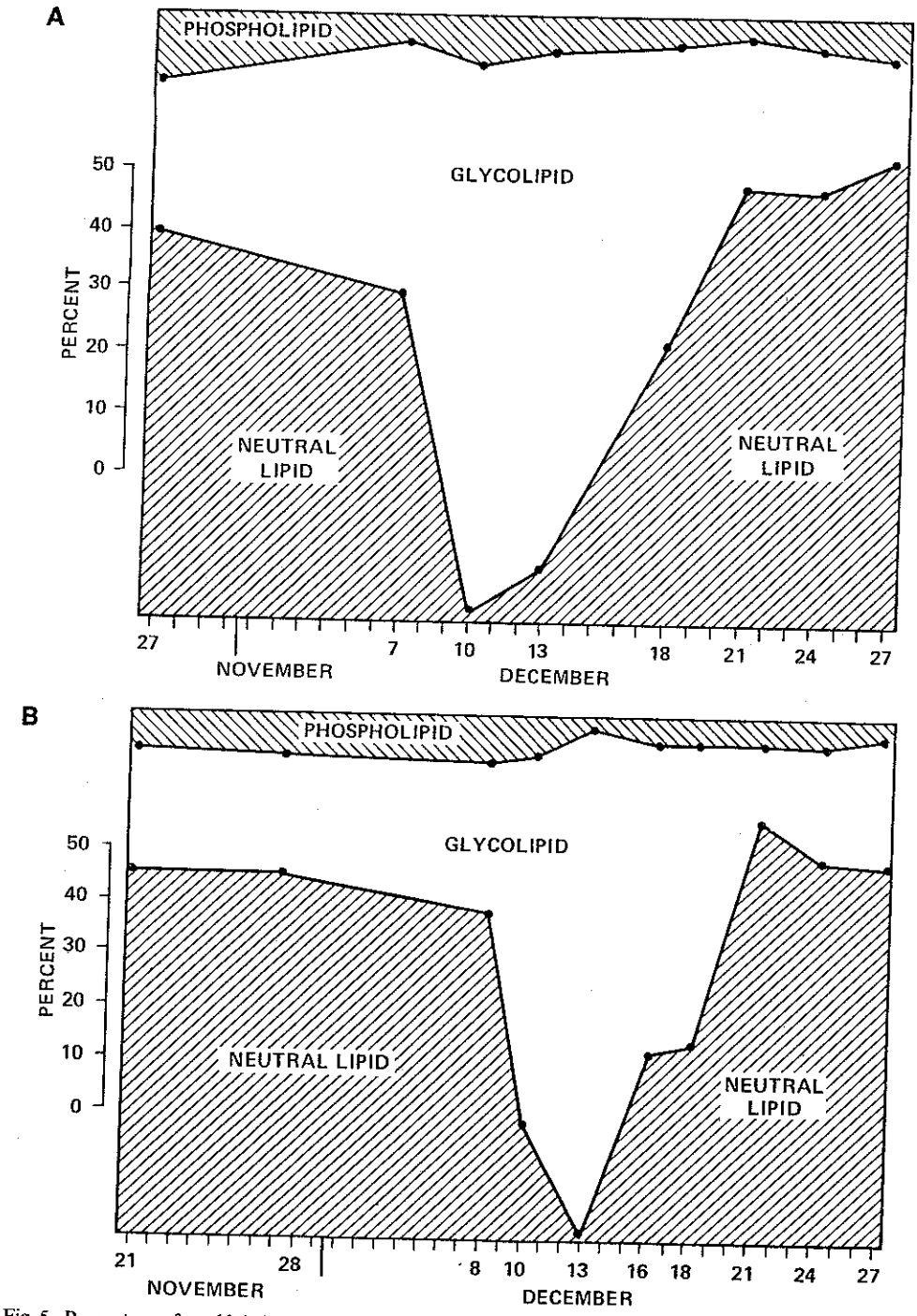

Fig. 5. Percentage of total labeled lipid in neutral-lipid, glycolipid, and phospholipid fractions. A, Site 1; B, Site 2.

of the total carbon fixed. By contrast, carbon assimilation into glycolipids and phospholipids remained relatively constant during the bloom at ≈4 and 1%, respectively, of total carbon fixed.

Fig. 5 (A,B) shows the relative proportions of the neutral lipid, phospholipid, and glycolipid fractions that comprised the total radiolabeled-lipid fraction. Assimilation of ^{14}C into neutral lipids accounted for ≈54–77% of the total lipids early in the bloom, but decreased to < 10% of total lipids between 10–13 December 1985. Glycolipids then became the dominant component (63–65%) of the total labeled-lipid fraction primarily due to the decline of the neutral-lipid fraction and also due to a small increase in net glycolipid synthesis. Assimilation of ^{14}C into phospholipids remained a fairly constant proportion (3–10%) of the total radiolabeled-lipid fraction.

Discussion

Changes in photosynthetic carbon assimilation in Antarctic sea-ice microalgae occurred in late November–December 1985 in McMurdo Sound; in particular, profound changes were observed in carbon assimilation into neutral lipids. To discuss these physiological changes, it is useful to divide the study period into three phases. During phase I (15–30 November), an overall increase was observed in biomass (chl *a*); this increase was probably the result, in part, of in situ growth of microalgae (Grossi *et al.*, 1987; Kottmeier, unpubl. data). Assimilation of carbon into neutral lipids was 7 and 11% at Sites 1 and 2, respectively. During Phase II (1–15 December), maximal values of chl *a* (150 and 180 $mg \cdot m^{-2}$ at Sites 1 and 2) were observed. It was also in Phase II that a sharp drop in carbon assimilation into neutral lipids occurred from 12 to 0.1% of total assimilated carbon at Site 1, and from 9 to 0.1% at Site 2. Subsequently, in Phase III (15–30 December), an overall decrease in mean algal biomass was observed at both sites, probably due primarily to an increase in ice ablation, sloughing, and metazoan grazing of the algal communities (Grossi *et al.*, 1987; Palmisano *et al.*, 1987). During Phase III, carbon assimilation into neutral lipids increased from the previous low value of 0.1% to 11 and 15% at Sites 1 and 2. Also during Phase III, a secondary ice-algal bloom was observed at the ice–sea water interface; this algal community was distinct from the major bloom and accounted for a relatively small biomass.

While carbon assimilation into the neutral-lipid fraction showed sharp changes during the study period, assimilation into the glycolipid and phospholipid fractions was fairly constant. Lipids serve diverse functions in the cell, and as such, knowledge of carbon assimilation into individual lipid classes may reveal important physiological information. Using gas chromatography and gas chromatography–mass spectrometry, Nichols *et al.* (1986) performed detailed analyses of lipids of a log-phase pure culture of the ice diatom *Nitzschia cylindrus* isolated from McMurdo Sound. The neutral-lipid fraction was dominated by triglycerides; neutral lipids in diatoms may also include hydrocarbons, free sterols, B-carotene, and free fatty acids. The operationally defined

"glycolipid" fraction described here includes lipids with a carbohydrate moiety, such as monogalactosyl diacylglyceride – an important component of chloroplast membranes, as well as chl *a*, most carotenoids, and sulphonolipids. The phospholipid fraction contains lipids associated with both cell and chloroplast membranes (Harwood & Russell, 1984).

The widely used EPPS assay has, in practice, suffered from procedural and interpretative problems. The EPPS fractionation provides only a crude separation of biochemical constituents, including lipids (Hitchcock, 1978). McConville *et al.* (1985) found a significant proportion of polysaccharide (glucan) extracted in the methanol-water fraction generally assumed to contain LMWM. Smith & Morris (1980) reported an extraordinarily high (up to 80%) assimilation of fixed carbon into lipids in Southern Ocean phytoplankton, and they suggested that low-light and low-temperature conditions favored lipid synthesis. These findings have not been confirmed in Arctic phytoplankton (Li & Platt, 1982), Antarctic phytoplankton (Rivkin & Voytek, 1987), or sea-ice microalgae (McConville *et al.*, 1985; Palmisano & Sullivan, 1985; this study). This discrepancy may result from procedural problems inherent in a crude fractionation of lipids; refinement of the assay into individual lipid classes, may help to resolve such observed differences.

Moreover, some investigators using an EPPS-type fractionation have assumed that the increased assimilation into the total lipid fraction reflects an increase in carbon storage, i.e., neutral lipids (Priscu & Goldman, 1983; Hitchcock *et al.*, 1986; Smith *et al.*, 1987). However, we found that storage components, such as neutral lipids, and membrane-associated structural components, such as glycolipids and phospholipids, varied independently. Our results indicate that it is important to examine individual lipid classes to better understand their significance in physiological ecology. For example, measuring total lipids during Phase II of our study period would provide little information about carbon storage because 90% of total lipid was present as glycolipids (Fig. 5A,B). Using thin-layer chromatography, Sargent *et al.* (1985) found that the majority of radioactive carbon assimilated into lipids by exponentially growing phytoplankton, diatoms and *Phaeocystis pouchetii*, was found in polar lipids.

The dramatic changes in carbon assimilation into neutral lipids that occurred during December may result from one or more factors. At this time, the community biomass was declining as warmer temperatures led to the melting of surface snow and ice and to the eventual release of the community to the water column. One hypothesis is that the algal community may be entering a "stationary phase" of zero net growth due to light limitation (by self-shading), nutrient limitation (by depletion or by dilution with low-salinity meltwater), or other factors. Alternatively, components of this community may be responding favorably to their release from the ice, i.e., to the increased availability of nutrients as cells are being released from brine cells. Because of the multitude of factors affecting this natural community in a dynamic environment, it is evident that controlled experiments with pure cultures of ice diatoms are needed to determine the primary factors effecting the observed changes in carbon assimilation.

However, our results clearly show that the photosynthetic assimilation of carbon into lipid classes in sea-ice microalgae can be very dynamic during the bloom period.

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