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Growth and lipid composition of high Arctic ice algae during the spring bloom at Resolute, Northwest Territories, Canada

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ABSTRACT: The concentration and composition of particulate organic matter, with special reference to lipids, was measured throughout the spring bloom of bottom ice algae in 1989 at a site in the Canadian high Arctic. By comparing areas of differing snow cover, and thus light history, we showed that light was initially limiting to algal production. Where snow cover was relatively thin (0 to 5 cm), light apparently ceased to be limiting to biomass accumulation in the ice as the bloom neared its peak. Compositional ratios, such as C : chlorophyll *a* and C : N, were consistent with a physiological response of the algae to light-sufficient and, possibly, nutrient-limited conditions following the peak of the bloom. The transition from early to late bloom conditions was accompanied by a shift in lipid composition, from a predominance of polar lipids (glycolipids and phospholipids) and pigments to a predominance of neutral lipids (triacylglycerols and free fatty acids). Neutral lipids varied directly, as a proportion of total lipid, with the light available to the algae under the different snow covers. Similar changes of lipid composition were only partially reproduced in a short-term (≤2 wk) manipulation of light availability to the natural communities, however, indicating that factors other than the immediate availability of light were important to lipid synthesis by the ice algae.

INTRODUCTION

Ice algae are important primary producers in polar oceans (Horner 1985). They constitute a unique spatial and temporal concentration of algal biomass, and elicit specially directed exploitation strategies from consumers (Stretch et al. 1988, Conover & Huntley 1991). Ice algae may also constitute a unique community in terms of their biochemical composition (McConville 1985, Cota & Smith 1991a). Algae living in the heavily shaded bottom ice environment might be expected to have a high protein content and small quantities of storage polymers such as carbohydrates and triacylglycerols (Morris 1981, Smith et al. 1989). The limited data available indicate a surprisingly low protein content and a high lipid content for high Arctic ice algae (Smith et al. 1989, Cota & Smith 1991a). The lipid-rich composition has implications for the nutrition of consumers and the transport of hydrophobic contaminants (Sargent & Whittle 1981, Hargrave et al. 1992). It may also reflect the factors controlling algal productivity in the bottom ice.

Numerous studies at Arctic, Antarctic and sub-Arctic sites have shown that light is the principal factor limiting the onset and early development of bottom ice algal blooms (Cota & Smith 1991b and references therein). A simple model of ice algal production indicated that the concentrations of ice algal chlorophyll *a* (chl *a*) in the present study area, in the Canadian high Arctic, may attain biomass limits set by self-shading (Smith et al. 1988), further suggesting light to be the dominant control. However, the model explicitly assumed that a major proportion of the algal community would not be physiologically light-limited even when crop size was at the theoretical maximum, and it was further shown that algae failed to attain the light-limited maximum where snow cover was very

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thin (Smith et al. 1988). The concentrations of algae and inorganic nutrients (e.g. Cota et al. 1987), patterns of photosynthate allocation and biochemical composition (e.g. Palmisano & Sullivan 1985, Smith et al. 1987, Gosselin et al. 1990, Lizotte & Sullivan 1992) and nutrient enrichment bioassays (Maestrini et al. 1986) have provided evidence of nutrient limitation in the later stages of the vernal bloom in various bottom ice communities. The nutrient chemistry of ice and sea water suggests that silicon is often more likely to become limiting than is nitrogen or phosphorus, in high Arctic and Antarctic locations (Cota et al. 1987, 1990, Dieckmann et al. 1991). It is interesting, in view of the high lipid contents reported for the diatom-dominated bottom ice algae in the high Arctic, that silicon deprivation is one of the most effective ways of stimulating high rates of lipid synthesis by diatoms (Taguchi et al. 1987).

Lipids comprise a functionally-diverse group of compounds (Gurr 1991). For example, triacylglycerols and some other neutral lipids are associated with energy and carbon storage, and in algal cultures they may be synthesized preferentially when nutrients are limiting (e.g. Parrish & Wangersky 1987, Lombardi & Wangersky 1991). By contrast, many polar lipids (especially phospholipids) are associated with various types of membranes and are likely to dominate the total lipid pool when light is limiting and/or nutrients are sufficient to support rapid growth (Parrish & Wangersky 1987). Relevant studies of lipid composition in natural microalgae are few, but there is evidence that coastal marine phytoplankton may indeed shift to a greater synthesis of neutral lipid when nutrients become limiting during the spring bloom (Morris et al. 1983, Parrish 1987a). Bottom ice algae in Antarctica have been shown to synthesize relatively more neutral lipid in the later stages of their vernal bloom, a change that was interpreted to indicate a response to improved light availability and/or limited nutrient supply (Palmisano et al. 1988, Nichols et al. 1989). No direct test of the relationship between light climate and/or nutrient status of an ice algal community and its lipid composition and abundance has yet been published.

One of the convenient features of bottom ice communities is that they afford a natural laboratory in which the effects of varying light climate can be investigated by comparing population development under differing depths of snow cover (Cota & Smith 1991a, b). Such an approach has provided much of the evidence for light limitation of the spring ice algal bloom. Our intention here was to use the same method to determine the effects of light climate on lipid content and composition of ice algae and, by implication, other microalgae living at low temperatures and irradiance levels. We hypothesized that the lipid composition of the algae would reflect a positive influence of light availability on the relative abundance of neutral lipids, as opposed to polar lipids. We furthermore hypothesized that neutral lipids would become relatively more important as the ice algal bloom progressed from its early, light-limited phase, to the later phase in which nutrient limitation may become a significant factor. A drawback to this approach is that the communities develop at different rates under different snow covers, and thus the effects of light may be influenced differentially by confounding factors such as nutrient limitation and species succession. We therefore also conducted a short-term manipulation of the snow cover to test the more immediate influence of light. The concentrations of 2 potentially important nutrients, nitrogen and silicon, in particulate matter were measured to test for evidence of shortage during the bloom and consequent effect on lipids.

MATERIALS AND METHODS

The study site was approximately 3 km offshore from Resolute, Northwest Territories, Canada (74° 41' N, 95° 50' W) in Resolute Passage. Water depth was about 100 m, and ice thickness varied from 1.5 to 2.0 m. Details of hydrographic conditions, water and ice characteristics, and abundance and nature of ice biota in the study area are provided elsewhere (Cota et al. 1987, 1990, Smith et al. 1988, Welch & Bergmann 1989).

We sampled in areas of 3 different snow covers, approximately 0, 5 and 20 cm, from March 31 to June 14, 1989. The 0 cm area was cleared of snow at the outset of the sampling season, and maintained by subsequent clearing as required. The 5 and 20 cm snow depths were in areas of relatively uniform, hard, old snow, estimated to be stable throughout the period of sampling. Samples for the determination of particulate organic matter in bottom ice were taken using a SIPRE corer. The lower 5 cm section of each core was melted without addition of sea water, and aliquots then collected on precombusted glass fibre filters (GF/F; approximately 0.8 µm particle retention) or 0.4 µm polycarbonate filters (silica measurements only) for later analysis. Organic material and inorganic nutrients can be lost during such sampling, due to the inefficient retrieval of material by the SIPRE corer (Welch et al. 1988) and leakage of low molecular weight material from the ice biota (Garrison & Buck 1986, Smith et al. 1990). Possible consequences for the present work will be addressed in the 'Discussion'. The methodology used here was consistent with relevant earlier studies (e.g. Nichols et al. 1989, Smith et al. 1989).

Samples collected for particulate organic carbon (POC) and nitrogen (PON) were frozen pending analysis on a CHN elemental analyzer. Samples for chl *a* were frozen, then extracted overnight with 90% acetone (within 3 wk of collection) and analyzed by a fluorometric method (Yentsch & Menzel 1963), with calibration against pure chl *a*. Samples for particulate biogenic silica were frozen pending analysis by the method of Paasche (1980).

Samples for lipid determination were placed immediately in 2:1 (v:v) chloroform:methanol. The overlying air was replaced with oxygen-free nitrogen gas, and the samples kept frozen (–20°C) pending analysis. After the samples were removed from the freezer, they were ground and filtered through a GF/F filter. The chloroform phase was separated and purified by adding a volume of 0.9% NaCl that was 20% of the solvent volume (Folch et al. 1957), mixing thoroughly, and centrifuging (with 2 repeats). The purified lipid extract was dried under oxygen-free nitrogen gas and stored frozen.

Lipid classes were determined by thin-layer chromatography with flame ionization detection (TLC-FID). A subsample of the lipid extract (6 to 40 µg) was spotted with a Hamilton syringe equipped with a TLC spotter onto silica-coated Chromarods-SII or SIII (RSS Inc.). The Chromarods were developed and analyzed sequentially in 4 increasingly polar solvent systems (Parrish 1987b). The first 2 solvent systems were hexane, diethyl ether, and formic acid in the ratios of 99:1.0:0.05 and 80:20:0.1, respectively. The third and fourth solvent systems were 100% acetone and a mixture of dichloromethanol, methanol, and water (5:4:1). Following solvent development, the Chromarods were scanned with an Iatroscan Mark IV (Iatron Labs, Tokyo) connected with a Hewlett-Packard 3392A integrator. A mixed lipid standard was prepared, containing one compound from each of the following lipid classes: hydrocarbon, sterol ester, triacylglycerol, free fatty acid, alcohol (aliphatic), sterol (alicyclic alcohol) and phospholipid (Parrish 1987b). The mixed standard was used for TLC-FID calibration and quantification. Concentration of acetone-mobile polar lipids (AMPL), a collection of lipids that may include photosynthetic pigments, glycolipids and monacylglycerols, was determined with the sterol standard curve. Calibration curves were determined over a range of 0.15 to 30 µg for each standard compound (Parrish et al. 1988). The mean recovery for the sum of the lipid classes, relative to the gravimetric determination of total lipid, was 67.5% ± 5.3 (SE, n = 39).

For alkane analysis, the chloroform extract was taken to dryness at 30°C under nitrogen and redissolved in pentane. It was then applied to an activated silica gel column (0.6 × 10 mm). The alkanes were eluted with 12 ml of pentane, evaporated to 1 ml under nitrogen, and analyzed on a gas chromatograph (HP 5890) equipped with a 30 m SE 54 capillary column (Supelco) and a flame ionization detector. An external standard with equal concentrations of *n*-C16 through *n*-C36 was used to establish retention times. Sample peaks were confirmed by analyzing portions of sample extracts spiked with standards.

As often as possible, the surface incident irradiance and the under-ice irradiance were measured using quantum sensors (cosine collectors) at or near local noon. The overlying snow and ice, and the algae themselves, attenuate the light so it is necessary to apply a model of light transmission to estimate the light actually available to algae living in the bottom 5 cm of the ice (Smith et al. 1988). The average irradiance within the 5 cm algal layer (µmol photons m^{-2} s^{-1}) is denoted I_a (Smith et al. 1989) and can be calculated as

$$I_a = \frac{I_u}{k \times chl} \times \frac{1 - e^{-k \times chl}}{e^{-k \times chl}} \quad (1)$$

where I_u is the irradiance measured just beneath the ice; *chl* is the integrated areal chl *a* concentration (mg m^{-2}); and k is the chlorophyll-specific diffuse attenuation coefficient (m^2 mg^{-1}). We used a relatively high value of 0.035 m^2 mg^{-1} for k, as determined in a previous study in the same area (Smith et al. 1988). If we used a smaller value, our estimates of I_a would be reduced.

A reciprocal light shift experiment was started on May 5. An apparently homogeneous area (3 m by 5 m) of 15 to 20 cm snow cover was cleared, and a similar depth of snow added to a comparably-sized area of the previously existing snow-free plot. An area adjoining each manipulated plot was left undisturbed as a control. The undisturbed areas were termed the high light control (HC, 0 cm snow) and low light control (LC, 15 to 20 cm snow), while the manipulated areas were termed the high-to-low (HL) and low-to-high (LH) light shift areas. Cores were taken from all areas on Days 0, 3, 7 and 14 for analysis of particulate organic material.

RESULTS

Algal biomass and nutrients

The snow-free sampling area was cleared of its original 5 to 10 cm snow cover on March 28, 1989. The first ice cores, taken 4 d later, revealed 1 to 2 mg m^{-2} of chl *a* in the snow-free area, and 0.02 to 0.08 mg m^{-2} in areas of either 5 or 20 cm snow cover. Chl *a* concentrations increased rapidly with time in both the snow-free and 5 cm snow areas, but the initial increase was

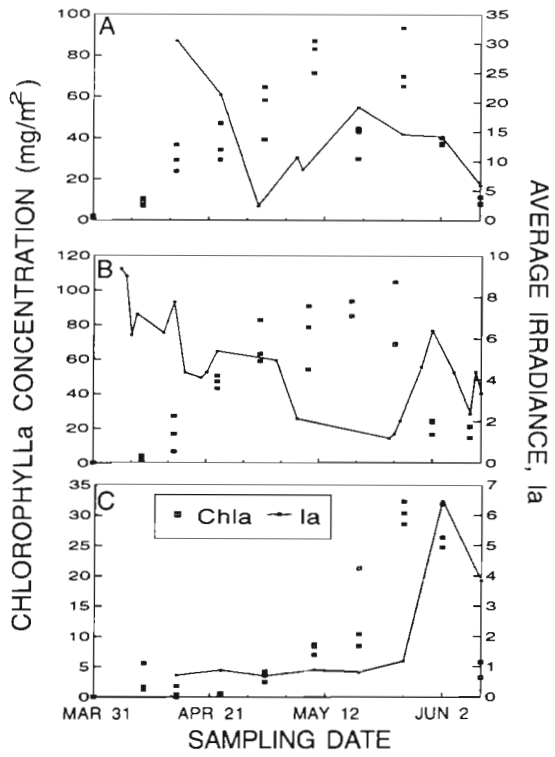

Fig. 1. Chlorophyll *a* concentration (Chla) and irradiance (Ia) in the bottom ice near Resolute, NWT, Canada, in 1989. (A), (B) and (C): results for 0, 5 and 20 cm snow cover respectively. Points are determinations for individual cores

slightly faster in the snow-free area (Fig. 1A, B). The observed maximum chl *a* concentrations were 80 to 100 mg m^{-2} in both snow-free and 5 cm snow areas, and were attained by early to mid May (Fig. 1A, B). Chl *a* concentrations were slower to increase under 20 cm snow cover, and attained an observed maximum of about 30 mg m^{-2} (Fig. 1C).

Systematic, quantitative determinations of algal species composition were not performed, but the bottom-ice community was dominated by diatoms throughout the bloom, with *Nitzschia frigida*, *N. grunowii*, *N. cylindrus*, *N. delicatissima*, *N. promare*, and *Chaetoceros* sp. the dominant taxa, in samples taken from various depths of snow cover in early and late parts of the season.

The average irradiance (I_a, Eq. 1) in the bottom ice algal layer was initially as high as 30 µmol photons m^{-2} s^{-1} at or near solar noon in the snow-free area, but decreased as algal biomass accumulated and self-shading became significant (Fig. 1A). Average noon-time irradiance beneath 5 cm of snow cover was initially 7 to 10 µmol photons m^{-2} s^{-1}, but declined to less than 2 µmol photons m^{-2} s^{-1} at the peak chl *a* concentration (Fig. 1B). In both areas, irradiance tended to increase again after the peak chl *a* concentration was past and self-shading was reduced, but other factors (such as weather) complicated the pattern. Algae growing in the area with 20 cm of snow cover received an extremely low noon-time irradiance of less than 1.0 µmol photons m^{-2} s^{-1} until early June, when values of 4 to 6 µmol photons m^{-2} s^{-1} were observed (Fig. 1C).

The mean concentrations of particulate organic carbon (POC) and nitrogen (PON) displayed their seasonal maxima of 1400 to 1500 mgC m^{-2} and 180 to 200 mgN m^{-2} respectively in the mid-May samples from both the snow-free and the 5 cm snow areas (Table 1). Given that the average coefficient of variation was 9.7% and 13.6% for POC and PON respectively, the difference in observed maxima between snow-free and 5 cm snow areas was not statistically significant ($p > 0.05$). Maximum observed POC and PON concentrations were lower in the area with 20 cm of snow cover (Table 1).

Table 1. Concentration and composition of particulate organic matter in bottom ice covered by different thicknesses of snow (cm) near Resolute, NWT, Canada, in 1989. POC and PON are particulate organic carbon and nitrogen respectively and LIPID is total particulate lipid (all in mg m^{-2}). C:CHL is the carbon:chl *a* ratio, C:N is the carbon:nitrogen ratio, and LIP:POC is the ratio of lipid-carbon to POC (all as mg mg^{-1}). Values are means for duplicate (usually) or triplicate (occasionally) cores; nm denotes no measurement

Date	Snow	POC	PON	LIPID	C:CHL	C:N	LIP:POC
Apr 9	0	170	16.7	137	22.0	11.3	0.60
May 18	0	1370	178	575	38.7	7.7	0.31
Jun 9	0	1016	105	754	108	9.7	0.56
Apr 9	5	85	8.8	93	34.2	9.7	0.82
May 18	5	1439	202	548	19.2	6.6	0.28
May 26	5	1345	203	542	19.5	6.6	0.30
Jun 9	5	1430	141	612	81.1	10.0	0.32
Apr 9	20	nm	nm	151	nm	nm	nm
Apr 15	20	121	7.2	nm	146	17.4	nm
May 18	20	256	39.5	178	17.2	6.5	0.52
May 26	20	542	85.9	386	17.8	6.3	0.53

The carbon : chl *a* ratio (C : chl *a*) and carbon : nitrogen ratio (C : N) varied significantly ($p < 0.05$) with both snow cover and sampling date, according to 2-way analysis of variance (ANOVA). For the 5 and 20 cm depths of snow cover examined, C : chl *a* was minimal when chl *a* concentration in the ice was maximal (Table 1). Under snow-free ice, the minimum C : chl *a* was observed in the earliest sample, in early April (Table 1). C : chl *a* increased late in the season, in early June, under both 0 and 5 cm snow cover. A very high value of C : chl *a* was observed in mid-April under 20 cm snow cover (Table 1), a time of very low chl *a* concentration and irradiance (Fig. 1C). C : N displayed essentially the same patterns with time as did C : chl *a* (Table 1). Both C : chl *a* and C : N attained significantly lower values under 5 and 20 cm snow cover than under 0 cm snow cover in midseason ($p < 0.05$, ANOVA), when algal crops were well developed as judged from chl *a* concentrations.

The temporal variations of particulate biogenic silica concentration (PSi, Fig. 2) were broadly similar to those of chl *a*. The ratio of biogenic silica to POC (Si : C) ranged from 0.1 to 1.2, with the lowest values at the outset of the sampling season (Fig. 2). The maximum Si : C ratio observed during the sampling season varied directly and significantly with the depth of snow cover ($p < 0.05$, ANOVA). The ratio of PSi to chl *a* (Si : chl *a*) displayed a seasonal minimum during the middle of the sampling period, when algal abundance was increasing or at near maximum values (Fig. 2). The Si : chl *a* ratio did not vary significantly among snow covers during the seasonal minimum, but varied inversely and significantly ($p < 0.05$, ANOVA) with snow cover on the last 2 sampling dates, when ratios were higher under all snow covers (Fig. 2). The total range of Si : chl *a* ratios observed was approximately 9 to 130, with the highest values observed in the earliest samples from the natural snow sites.

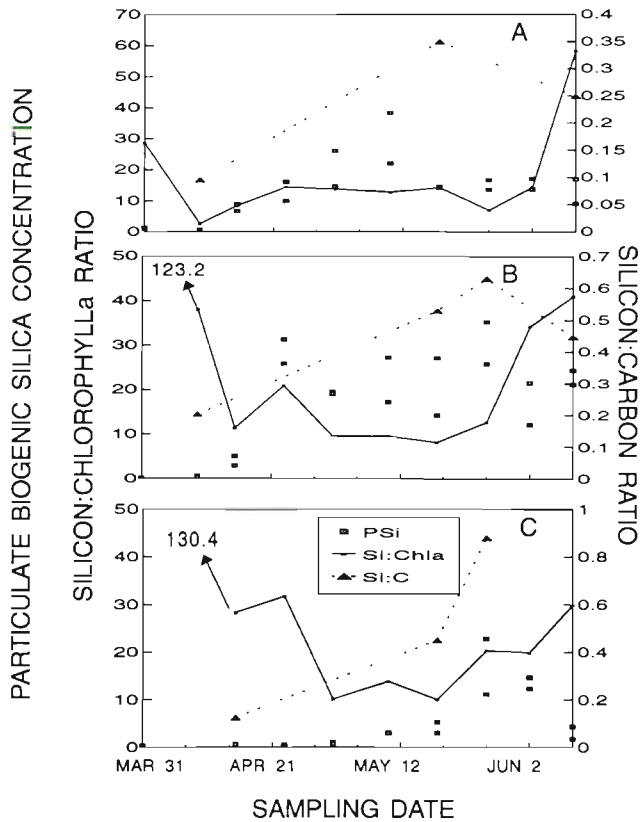

Fig. 2. Particulate biogenic silica (PSi, mmol m^{-2}) and its ratio to chlorophyll *a* (Si : Chla, g : g) and to POC (Si : C, g : g) in the bottom ice near Resolute in 1989. (A), (B) and (C): results for 0, 5 and 20 cm snow cover respectively. Points for PSi are determinations for individual cores; ratios are shown as means of duplicate or triplicate cores

Lipids

The particulate lipid samples always contained a suspiciously large hydrocarbon (HC) fraction (Table 2). Alkane hydrocarbons, analyzed by gas chromatogra-

Table 2. Lipid class composition of particulate organic matter from bottom ice covered by different thicknesses of snow (cm) near Resolute as percent of total lipid. HC: hydrocarbons; TG: triacylglycerols; FFA: free fatty acids; AMPL: acetone-mobile polar lipids; PL: phospholipids; POL: polar lipids (AMPL + PL); OT: other lipids (sterols, alcohols, wax esters); CHL: chl *a*

Date	Snow	HC	TG	FFA	AMPL	PL	OT	LIP : CHL	TG : POL	AMPL : PL
Apr 9	0	17	0	0	70	10	2	14.5	0	7.0
May 18	0	13	32	12	28	13	2	12.8	0.77	2.11
Jun 9	0	15	37	8	27	9	3	63.8	1.00	2.95
Apr 9	5	17	9	2	56	12	3	30.8	0.12	4.50
Apr 15	5	32	2	4	50	10	2	35.5	0.04	5.00
May 18	5	10	14	6	43	23	3	5.9	0.21	1.83
May 26	5	17	20	10	36	12	5	6.4	0.42	3.00
Jun 9	5	12	44	7	23	11	2	28.4	1.28	2.03
Apr 9	20	14	0	3	76	7	0	246.0	0	11.0
May 18	20	31	0	9	50	9	0	9.5	0	5.60
May 26	20	20	14	3	44	16	3	10.3	0.22	2.78

phy in 6 samples of widely varying total hydrocarbon content, were dominated by C-20 to C-30 molecules, with peak abundance in the C-24 to C-28 range. Although such alkanes have also been reported from Antarctic ice algal communities (Nichols et al. 1989), they accounted for only a small fraction of the total hydrocarbon in our samples. We were unable to characterize the remaining hydrocarbon, but contamination from various sources is hard to avoid when field camps and sampling equipment are powered by diesel and kerosene fuels, and seems a likely explanation of the remarkably high values. Most of the nonhydrocarbon lipid was found in the triacylglycerol (TG), free fatty acid (FFA), acetone-mobile polar lipid (AMPL) and phospholipid (PL) classes (Table 2). Sterols were usually undetectable and always less than 3.4% of total particulate lipid. Acetone-mobile polar lipid, that includes pigments and photosynthetic membrane components, was the dominant lipid class in the early samples from all 3 snow covers. It remained important, albeit markedly decreased in its share of total lipid, to the end of the observation period (Table 2). Triacylglycerols and FFA increased markedly through the observation period under all 3 snow covers, and together were the dominant forms of lipid by the end of the season under 0 and 5 cm snow cover.

The ratio of TG to total polar lipids (AMPL + PL) showed that triacylglycerols increased relative to polar lipids during the season, increasing earliest under 0 cm snow cover, then under 5 cm snow, and latest under 20 snow cover (Table 2). Within our observation period, TG never attained as great a relative importance under 20 cm snow cover as it did under the 2 thinner snow covers. The ratio AMPL:PL was maximal in the earliest samples, and attained its minimum coincident with the maximum chl *a* standing crop under each snow cover (Table 2).

Total lipid was estimated conservatively by summing the acyl lipid classes (TG, FFA, AMPL and PL) determined by thin-layer chromatography and flame ionization detection (Table 1). The HC class, believed to be largely the result of contamination, was thus excluded from the total. Nonetheless, we observed some large total lipid concentrations in the ice. Assuming that the carbon content of the lipid was on average 77, 71, and 65% of the lipid weight for neutral, acetone-mobile and phospholipids respectively, this calculation indicates that 28 to 82% of the POC was in the form of lipid (Table 1). Exact percentages should be interpreted with caution, however, because thin-layer chromatography with flame ionization detection is only a semiquantitative technique. The highest lipid-C:POC ratios were observed in the earliest samples from the 0 and 5 cm snow areas; minimal values were observed under 5 cm snow at the seasonal peak of chl *a* concentration. The ratio of total lipid to chl *a* varied in roughly similar fashion with season and among snow depths, but increased more in the late season than did the lipid-C:POC ratio (Table 2).

Light shift experiment

Because of large differences in self-shading between high and low light control areas, the shifted areas experienced extreme changes in irradiance as a result of manipulation (Table 3). Significant changes in chl *a* concentration resulted within 7 d (ANOVA, $p < 0.05$). Chl *a* concentrations decreased more slowly with time in the high-to-low light shift area than in the corresponding high light control area (Fig. 3). However, there was no significant difference in POC concentration between the 2 treatments, even after 14 d (Table 3). Significant ($p < 0.05$, ANOVA) compositional differences developed between the 2 treatments by Day 14: C:chl a, TG:POL and C:N were all higher in the high light control than in the high-to-low shift area (Table 3).

In the complementary half of the shift experiment, significant differences in chl *a* concentration did not emerge until Day 14, when the low light control area had a higher concentration than did the low-to-high shift area (Fig. 3). The control area was also moderately, but significantly, higher in POC after 14 d than was the shift area. The C:chl *a* of the low-to-high shift samples was much higher than that of the low light control (or even the high light control) after 14 d, and C:N was also higher in the shift area. However, the lipid composition ratios of the shift area were not significantly different from control values (Table 3).

Table 3. Concentration and composition of particulate organic matter in bottom ice during snow shift experiment. Treatments are HC, high light control; HL, high-to-low light shift; LC, low light control; LH, low-to-high light control. I_a is the average irradiance (near local noon) in the bottom ice algal layer. Other abbreviations as in Tables 1 & 2 and Fig. 1

Date	Treatment	POC	C:CHL	C:N	TG:POL	I_a
May 5	HC, HL	1635	13.7	5.7	0.23	nm
May 5	LC, LH	341	36.5	7.4	0.26	nm
May 8	HC	1782	13.1	6.3	0.22	19.2
May 8	HL	1971	15.8	5.6	0.16	3.0
May 8	LC	382	31.8	9.4	0.06	9.4
May 8	LH	376	39.1	7.4	0.07	60.2
May 19	HC	1145	29.2	7.5	0.84	35.1
May 19	HL	1290	17.1	6.2	0.16	3.9
May 19	LC	384	10.7	6.3	0.14	6.3
May 19	LH	212	97.3	7.5	0.14	68.8

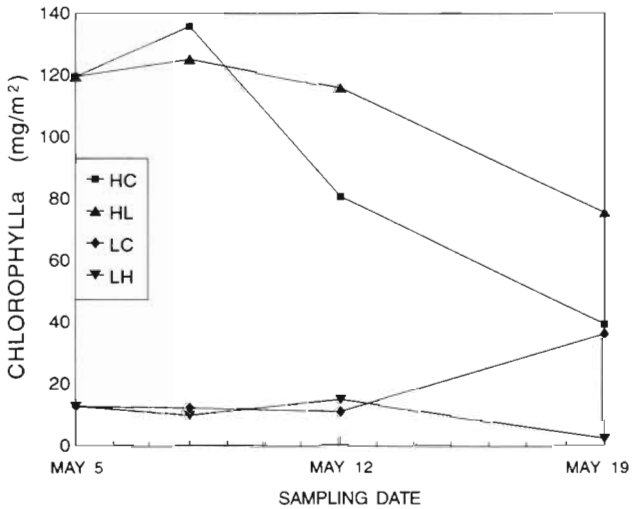

Fig. 3. Chlorophyll *a* concentration in bottom ice consequent to experimental manipulation of the overlying snow cover. HC: high light control; HL: high-to-low light shift; LC: low light control; LH: low-to-high light shift. Points are means for triplicate cores

DISCUSSION

Bloom development and limiting factors

The seasonal abundance patterns of bottom ice algae at Arctic and Antarctic sites (Cota & Smith 1991b), including the present study area (Cota et al. 1987, Smith et al. 1988, 1989), clearly demonstrate the importance of light in limiting the onset and early progress of many bottom ice algal blooms. The more rapid increase of chl *a*, POC and PON concentrations under the thinner snow covers (Fig. 1, Table 1) confirmed that light was limiting to algal biomass accumulation, or net production, for at least the earlier part of the bloom in the present study as well. Composition ratios, however, were not entirely typical of light-limited algae in the earliest samples. Carbon:chl *a* and C:N ratios were relatively high in some of the early samples, especially from 5 and 20 cm snow covers (Table 1). This seeming paradox may partly reflect a significant influence of nonliving material in the analyses when algal populations are still small, although previous study suggested that such interference was not likely to be important (Smith et al. 1989). A better idea of how actively growing algae respond to limiting irradiance may be gained by considering the samples taken near the peak of the bloom. Such samples represent material accumulated during the preceding period of light-limited growth, and the contribution of nonliving material becomes much less important (Smith et al. 1989).

Samples taken near the time of peak chl *a* concentrations (May 18 and 26, Table 1) revealed relatively low C:chl *a* and C:N ratios, generally consistent with previous reports for ice algae (Cota & Smith 1991a and references therein) and more consistent with the expected composition of shade-adapted algae (McConville 1985). Both ratios increased systematically from the deepest to the shallowest snow cover, paralleling the increase in I_a (Table 1, Fig. 1). Thus, these 2 relatively well-known indices of algal condition were both consistent with a graded response to variable degrees of light limitation under the different snow covers during the early phase of the bloom studied here.

From mid-May onward, light limitation was no longer the only factor influencing the measured concentration of organic matter in the ice under the thinner snow covers. Chl *a*, POC and PON ceased to increase under 0 and 5 cm snow cover, even though I_a remained much greater than the 0.5 to 1.0 µmol photons m^{-2} s^{-1} that supported algal increase earlier in the bloom under 20 cm snow cover (Fig. 1C). Furthermore, the concentrations under 0 cm declined relative to those under 5 cm snow cover (Table 1), indicating that irradiance had become supra-optimal. Previous studies in the same area (Cota et al. 1987, Smith et al. 1988, 1989) similarly found that algal biomass, measured as chl *a* or POC, attained higher concentrations under 5 to 10 cm of snow than under 0 cm.

The mechanism by which the higher irradiance under 0 cm (and, ultimately, 5 cm) of snow appears to inhibit algal biomass accumulation is unknown. In Hudson Bay, the seasonal change in under-ice irradiance and algal adaptation results in photosynthetic photoinhibition during the late bloom (Gosselin et al. 1985). The same situation may prevail here, but numerous measurements of photosynthesis in light-gradient incubators have failed to show significant photoinhibition of ice algae from the Resolute area at environmentally relevant irradiance levels (Smith et al. 1988, Bergmann et al. 1991, Smith & Herman 1991). Most such measurements have been short (approximately 1 h), so perhaps the photoinhibition is slow to develop. However, incubations up to 24 h in length under 33 µmol photons m^{-2} s^{-1} have also failed to demonstrate photoinhibition (Smith et al. 1987).

An alternative explanation might be that thinner snow covers permit an earlier and more extensive ablation of the extreme lower layers of the ice due to light absorption and consequent heating (Welch et al. 1988, Welch & Bergmann 1989). The soft ice immediately adjacent to the water-ice interface (skeletal layer), where the algae are frequently most concentrated (Smith et al. 1990), would be the most vulnerable to such ablation. Even if not previously ablated, the skeletal layer may easily be disrupted in the process of coring, particularly if it has also been warmed by light

absorption (Welch et al. 1988). Using a novel sampling method that should avoid such losses (Smith & Herman 1991), we found that the SIPRE method indeed underestimated the chl *a* concentration in the bottom ice during the present study. The SIPRE method recovered, on average, 83, 74 and 72% of the chl *a* measured by the more efficient technique in April, May and June respectively (Smith unpubl. data). Most previous work, like ours, has depended mainly on surface coring, and future work should evidently seek to employ more efficient methods where possible. While significant, however, the sampling problem did not appear likely to explain most of the variation observed among snow depths, or through the season, in the present case.

Regardless of the precise mechanism involved, the composition ratios C:chl *a* and C:N increased during the late bloom under the 2 thinner snow covers in a manner consistent with increasing limitation by some factor other than light, such as inorganic nutrients (Sakshaug et al. 1989, Smith et al. 1989). The changes may have been a physiological response of the bottom ice community to developing nutrient shortage as the large biomass of algae depleted the readily available stocks of inorganic nutrients (Maestrini et al. 1986, Cota et al. 1987, Gosselin et al. 1990). Alternatively, ablation of the extreme lower layers of the ice (or their loss during sampling) may have left the remaining material relatively enriched in algae deriving from older ice, farther removed from the ice-water interface. C:N and C:chl *a* ratios of such algae are higher than those of algae living closer to the interface, and may reflect their restricted access to inorganic nutrients (Smith et al. 1990). Both mechanisms (ablation or physiological change) therefore imply that the later samples contained algae that had experienced an environment limited not so much by light as by some other resource, most likely an inorganic nutrient.

Nutrients

Direct evidence for nutrient limitation in the study area is lacking. The existing indirect evidence is from indices of algal condition (Smith et al. 1987, 1989) and models of nutrient supply-to-demand balance (Cota et al. 1987). The C:N ratios reported here were typical of previous observations in being systematically higher than the Redfield value, and the ratios increased yet further in the late bloom under thin snow covers (Table 1). The values were not high enough to point unambiguously to nitrogen limitation, however, especially considering the scarcity of information concerning nutrient requirements of ice algae at the low ambient temperatures. Limited data from antarctic phytoplankton (Sommer 1991) and mesophilic freshwater phytoplankton (Rhee & Gotham 1981, VanDonk & Kilham 1990) indicate that higher nutrient quotas are needed to support optimal growth rates at low temperatures, but the magnitude of the effect is highly variable among species and nutrients. Comparable data for ice algae appear to be entirely lacking.

Measurements of dissolved nutrients in previous years have shown that silicon in bottom ice is in shorter supply than nitrogen or phosphorus (Cota et al. 1990, Smith et al. 1990). Nonetheless, the Si:C ratios reported here were high compared to the typical ratios of silicon-sufficient mesophilic diatoms in culture (Conley et al. 1989) or even the more silicon-rich Antarctic phytoplankton studied by Sommer (1991). Measurements of dissolved nutrients (unfortunately not done) might have shown 1989 to differ from previous years in terms of nutrient chemistry. Alternatively, the relatively heavily silicified species that dominate the ice algae may have unusually high silicon requirements compared to planktonic diatoms. The seasonal variation of both Si:C and Si:chl *a* composition ratios provided little or no evidence of developing silicon shortage in the late bloom. Additional experiments, including culture studies of representative species, will be needed to properly resolve the role of nutrients in the productivity of bottom ice algae.

Lipid concentration and composition

Our results are in good agreement with those for bottom ice algae in the Antarctic reported by Nichols et al. (1989) and agreed with our hypothesis that the lipid content and composition would change in response to changes in the factors limiting algal growth. Only the percentage of lipid in hydrocarbon was greatly different in our study (10 to 32%) compared to Nichols et al. (1 to 7%) and we believe much of the difference is due to contamination of our samples. Both studies revealed a comparable range of total lipid:chl *a*, and a tendency for the ratio to increase late in the bloom, as biomass began to stagnate or decline (Table 1). The range of TG:POL was similar in both cases, and showed a strong increase as the bloom progressed into its later stages. Like Nichols et al. (1989), we found sterols to be a very minor component of the total lipid. It is interesting that 2 algal communities so far apart can produce such similar results, and we might guess that the underlying factors were similar in both cases. By measuring and manipulating the light climate of the algae *in situ*, we were able to better resolve the role of light, and show that some other limiting factor(s) was an important determinant of the prominent seasonal increase of TG relative to polar lipids at our study site.

Diatoms in culture may contain up to approximately 75 % lipid carbon (Shifrin & Chisholm 1981, Taguchi et al. 1987), with the highest values occurring when light is nonlimiting and inorganic nutrients (especially silicon) are limiting. Under such conditions, neutral lipids (usually thought to be principally triacylglycerol) are the predominant lipid class synthesized (Parrish & Wangersky 1987, Lombardi & Wangersky 1991). Previous study of ice algae in the present study area indicated total lipid of up to 64 % of cell carbon (Smith et al. 1989), with highest values observed both early in the bloom, and again in the late bloom under minimal snow cover. The present study confirmed, using an independent method, that very high lipid contents may occur in samples of particulate organic matter early in the bloom, and again in the late bloom under 0 cm snow cover (Table 1). However, TG did not necessarily dominate when total lipid contents were high; acetone-mobile polar lipids (AMPL) were predominant in the samples with the 2 highest total lipid contents, which occurred in early April (Table 1), and in all the samples from the 20 cm snow area. There is some indication that total lipid content may have been high and dominated by polar lipid in the early stage of an Antarctic ice algal bloom as well (Nichols et al. 1989), but the relevant data were sparse and the AMPL were not resolved from other polar lipids.

The AMPL are thought to comprise mainly chloroplast membrane lipids and pigments (Parrish 1987a, b, Gurr 1991), so their predominance was consistent with the expected condition of extreme shade-adaptation in early samples and in those from 20 cm snow cover. However, the combination of such high lipid contents with a predominance of polar lipids is anomalous relative to the known behaviour of microalgae in culture (Shifrin & Chisholm 1981, Parrish & Wangersky 1987). Possibly the flame ionization detector is unusually sensitive to one or more of the compounds that may comprise the AMPL in samples of natural algal communities, causing us to overestimate the lipid content of the algae when AMPL were predominant. It seems unlikely that any such inconsistency in detector response could be large enough to alter substantially the basic observed pattern of AMPL predominance in early samples and those from 20 cm snow cover, although it could significantly alter our estimates of lipid-C:POC in some samples. Studies of ice algae in culture, under controlled conditions, would help to elucidate the basis of this seemingly distinctive aspect of ice algal lipid composition.

The abundance of lipid in early samples may help to explain why the light-limited bottom ice algae can have relatively high C:N ratios (Table 1). The C:N ratio of chlorophyll, for example, is approximately 11.8 by weight. Most other constituents of the AMPL class would have even higher C:N ratios. The C:chl *a* ratios of AMPL could also be relatively high if membrane lipids, rather than pigments, were predominant. The condition of the algae in the very early stages of the spring bloom may therefore be more analogous to the condition of greening tissue in higher plants than to light-limited, but actively growing, phytoplankton in culture.

Light clearly had an influence on lipid composition, as evidenced by the systematic increase in the relative importance of TG from deepest to thinnest snow cover in mid-May, at the conclusion of the light-limited phase of the bloom (Table 2). Light alone was not a simple explanation of the compositional differences among snow covers, however, because the experimental shift from low to high light failed to elicit a similar response (Table 3). The 14 d duration of the experiment corresponded to approximately 1 to 2 ice algal generation times under typical conditions (Cota et al. 1987, Cota & Smith 1991b), so there should have been sufficient time for a compositional response to become evident, especially if it were a relatively short-term energy storage response. It appeared to require a combination of high light and high biomass concentration (lacking in the low-to-high shift but present in the high light control) to elicit enhanced TG content, again suggesting that algal nutrient demand might be important. The high-to-low shift did show that TG accumulation could at least be delayed by a drastic reduction in light, however, confirming that adequate energy supply is important in the accumulation of neutral lipids by the ice algae.

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