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R.J. Henderson · E.N. Hegseth · M.T. Park

Seasonal variation in lipid and fatty acid composition of ice algae from the Barents Sea

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Abstract The fatty acid compositions of neutral lipid, glycolipid and phospholipid fractions from ice algae sampled from the Barents Sea in spring and autumn were examined for seasonal differences. The ice-algal assemblages were dominated by diatoms. In spring, *Nitzschia frigida* was the most common species whereas resting stages of *Thalassiosira bioculata* and *Actinocyclus* cf *curvatulus* predominated in autumn. With the exception of one spring sample, neutral lipids predominated over glycolipids and phospholipids in all algal samples. The lipid fractions displayed characteristic fatty acid compositions. In the spring samples the major fatty acids of the neutral lipid fraction were 16:0, 16:1(n-7) and 20:5(n-3) whilst the glycolipid fraction was characterised by higher levels of 20:5(n-3) and C16 polyunsaturated fatty acids, particularly 16:4(n-1). Phospholipids contained higher levels of 22:6(n-3) than the other two lipid fractions although 20:5(n-3) was still the major polyunsaturated fatty acid. In the autumn samples, the neutral lipid fraction contained higher proportions of saturated fatty acids and 16:1(n-7) than the two polar lipid fractions and 22:6(n-3) was most abundant in phospholipids. As with the spring samples, 20:5(n-3) was the major polyunsaturated fatty acid in all lipid fractions of the autumn algae. Overall, the fatty acid compositions of the lipid fractions from spring and autumn algal samples were similar and are consistent with diatoms being the predominant group in the ice algae studied. The high level of neutral lipids observed in both spring and autumn samples suggests that the production of neutral lipids is characteristic of ice algae regardless of season. Nevertheless, some species-specific differences in lipid production may exist since the neutral lipid content of autumn samples containing mainly *A. curvatulus* was substantially higher than those in which *T. bioculata* predominated.

R.J. Henderson (✉) · M.T. Park
N.E.R.C. Unit of Aquatic Biochemistry,
Department of Biological and Molecular Sciences,
University of Stirling, Stirling FK9 4LA, Scotland
e-mail: rjh4@stir.ac.uk; fax: (44) 1786 464994

E.N. Hegseth
Norwegian College of Fishery Science,
University of Tromsø, N-9037 Tromsø, Norway

Introduction

The annual blooms of ice algae associated with the sea ice of Arctic and Antarctic oceans are known to contribute significantly to marine primary production in these locations (Horner 1985). Most studies of sea-ice algae to date have been concerned with their growth parameters and photosynthetic efficiency (for review see Kirst and Wiencke 1995) and very few studies have examined their lipid composition. The limited amount of data that is available on the lipid composition of ice algae refers mainly to Antarctic sea-ice algae, either obtained directly from field samples (Nichols et al. 1989, 1993b; Skerratt et al. 1995) or cultured under laboratory conditions (Whitaker and Richardson 1980; Gillan et al. 1981). Only one study (Smith et al. 1993) has examined the lipid component of Arctic ice algae in any detail.

It is well established that each phylum of algae has its characteristic polyunsaturated fatty acid composition and direct correlation can often be made between the fatty acid composition of phytoplankton sampled in the field and the species present in the algal assemblage (Kattner et al. 1983; Pond et al. 1993). In aquatic poikilotherms, a basic adaptation to a change of environmental temperature is an increase in the proportion of polyunsaturated fatty acids (PUFA) in the polar lipids of biomembranes at low temperature (Cossins and Raynard 1988). This phenomenon has been demonstrated in both vertebrates and invertebrates, including photosynthetic algae (Henderson and Mackinlay 1989). Changes in light and nutrient conditions also influence the lipid composition of microalgae grown under laboratory conditions (Shifrin and Chisholm 1981; Thompson et al. 1990; Reitan et al. 1994) and the seasonal

changes observed in the lipid composition of phytoplankton sampled in the field can be related to changes in environmental parameters (Kattner et al. 1993). Sea-ice algae are subjected to large gradients in irradiance and salinity during the seasonal cycle of ice formation, growth of sheet ice and melting. Adaptation to such environmental changes may have specific effects on the lipid composition of the sea-ice algae.

The present study was undertaken to determine the lipid composition of sea-ice algae taken from the field in the Arctic in spring and autumn with a view to gaining information on the influence of environmental and species-specific factors on lipid composition.

Materials and methods

Ice algae

Sea-ice algae were sampled below 1-year and 2-year pack ice in the Barents Sea between 76–81.5°N and 23–35°E from May 1988, September 1988 and October 1987 (Table 1). Further descriptions of sampling stations are given in Hegseth (1992). Samples were collected by divers using an electric suction sampler as previously described (Hegseth 1992), and under-ice irradiance was measured using a hand-held irradiance meter (QSI-140, Biospherical Instruments, USA). Algal samples for counting and identification were preserved with neutralised 20% formaldehyde and counted in a counting chamber using a light microscope (Hegseth 1992).

Algae were harvested for lipid analysis by filtration of samples onto glass fibre filters (GF/C, precombusted to 300°C to remove organic material). Samples were stored frozen at −18°C in 10 ml chloroform : methanol (2:1, v/v), containing 0.05% butylated hydroxytoluene.

Lipid extraction and analysis

For lipid extraction, the volume of chloroform : methanol in each sample was increased to 16 ml. After vigorous shaking, 4 ml 0.88% KCl (w/v) was added and the sample shaken again. The sample was then centrifuged gently to aid the separation of organic and aqueous layers. After aspiration of the aqueous layer, the organic layer containing the extracted lipid was filtered through a filter paper that had been prewashed with chloroform:methanol (2:1, v/v). The organic solvent was then evaporated under a stream of nitrogen to leave the lipid extract which was then desiccated under vacuum overnight. The lipid was then redissolved at a known concentration in chloroform : methanol (2:1,v/v) and taken for analysis.

The lipid class composition of lipid extracts was determined by high performance thin-layer chromatography (HPTLC) using a double development system with methyl acetate:propan-2-ol: chloroform : methanol : 0.25% aqueous KCl (25:25:25:10:9, by volume) and hexane : diethyl ether : glacial acetic acid (80:20:2, by volume) as the solvent systems (Olsen and Henderson 1989). Separated lipid classes were visualised by spraying the developed chromatograms with 3% (w/v) copper acetate in 8% (v/v) phosphoric acid and charring at 160°C. The individual lipid classes were identified by comparison of Rf values with those of authentic standards and by chromatography of samples alongside a well-characterised lipid extract from a *Chroomonas salina*, a marine cryptomonad (Henderson and Mackinlay 1989). The identities of glycolipids, phosphatidylcholine and phosphatidylethanolamine were confirmed by spraying developed chromatograms with alpha-naphthol, Dragendorf and ninhydrin reagents, respectively (Henderson and Tocher 1992).

Table 1 Characteristics of sampling stations in the Barents Sea 1987–1988 (– not determined)

Year	Date	Station no.	Position	Ice type	Ice thickness (m)	Irradiance (μmol m^{-2} s^{-1})	Water temp (°C)	Salinity (ppt)	PO_4 (μmol l^{-1})	SiO_4 (μmol l^{-1})	NO_3 (μmol l^{-1})
1988	22.05[a]	7	76°22′N 31°03′E	One-year	1.0	30	−1.8	34.4	–	5.0	9.8
	26.05[a]	12	76°10′N 24°16′E	One-year/cave	1.5	25	−1.8	34.4	–	5.8	9.6
	27.05[a]	13	76°05′N 23°25′E	One-year	0.9–1.0	70	−1.8	34.5	–	5.8	10.5
1988	12.09[b]	Hk2	81°30′N 30°33′E	Two-year/cave	2.5–3.0	65	−1.8	–	–	–	–
1987	20.10[b]	12	78°12′N 31°40′E	Two-year	1.0–1.5	0.9	−1.8	34.1	0.5	0.7	3.3

[a] Spring
[b] Autumn

For the analysis of fatty acid composition, total lipid extracts were applied as small streaks to 20 × 20 cm glass plates coated with silica gel G, which were subsequently developed using the same double development system as the HPTLC described above. The developed chromatograms were sprayed with 0.1% 2′,7′-dichlorofluorescein in methanol and viewed under UV light to visualise separated classes. Areas of adsorbent containing neutral lipid classes, glycolipids and phospholipids were marked and scraped separately from the plates into glass tubes. A known amount of 21:0 fatty acid was added as internal standard to each tube before each lipid fraction was subjected to acid-catalysed transesterification at 50°C to form the methyl ester derivatives of component fatty acids (Christie 1989). The resulting fatty acid methyl esters were purified by thin-layer chromatography using hexane:diethyl ether:glacial acetic acid (85:15:1, by volume) as developing solvent and eluted from the adsorbent with hexane. After concentration by reducing the volume of solvent, the fatty acid methyl esters were analysed by gas chromatography using a Carlo Erba 6000 gas chromatograph equipped with a flame ionisation detector and a fused silica capillary column (50 m × 0.32 mm i.d.) coated with CP Wax 51 (Chrompack). Sample application was by on-column injection and hydrogen was used as carrier gas. The oven temperature was programmed to increase from 50°C to 225°C during the course of a run. Component peaks were identified by reference to authentic standards and a well-characterised sardine oil (Fishing Industry Research Institute, Rosebank, South Africa). When necessary, the unsaturated nature of component methyl esters was confirmed by hydrogenation of the sample using PtO_2 as catalyst followed by re-analysis.The absolute amounts of fatty acids present in lipid fractions were calculated by reference to the added 21:0 internal standard.

Results

Abiotic factors

The temperature of the surrounding water was always low (−1.5°C/−1.8°C), and close to the freezing point of seawater. Nutrient levels in the surface layer, as indicated by SiO_4 and NO_3 concentrations, were high in spring, but were much lower in the autumn (Table 1). All samples were collected below ice-floes: in spring below 1-year ice, and in autumn, when the thinner ice was melted, below 2-year ice. The ice cover had a lot of ridging, creating caverns where ice algae could be found. Ice thickness in spring was in general 1–2 m, in autumn up to 3 m, but the caverns were hardly more than 0.9–1.5 m below the sea surface. Snow depth on the ice varied from up to 30 cm in spring down to 5 cm in autumn, and this had great impact on the light conditions below the ice. Irradiance in September was found to be as high as in late May.

Ice-algal assemblages

The ice-algal samples were all collected from the sub-ice assemblage loosely attached to the ice under-side, and hence none were covered in ice. Diatoms predominated, but the species composition was different between samples (Table 2). A few species are specialised cave dwellers, e.g. *Thalassiosira bioculata*. The most common species was *Nitzschia frigida* with its epiphytes, but *Fossula arctica* was also a common species. In the autumn the cells had entered a resting stage and the populations were almost monospecific, dominated either by *T. bioculata* or *Actinocyclus* cf *curvatulus*. Dinoflagellates were either not found or occurred in insignificant numbers in the samples counted.

Lipid composition

Analysis by HPTLC showed that the samples contained around 12–16 individual lipid classes. Neutral lipids corresponding to hydrocarbons, triacylglycerols, free fatty acids and sterols were present, along with trace amounts of diacyl- and monoacylglycerols. In the glycolipids, monogalactosyldiacylglycerols (MGDG) and digalactosyldiacylglycerols (DGDG) were always predominant, but slightly smaller amounts of two other glycolipids with Rf values between those of MGDG and DGDG were also evident in all samples. The occurrence of phospholipid classes was less consistent, but most samples contained phosphatidylcholine and phosphatidylethanolamine with smaller amounts of phosphatidylserine, phosphatidylinositol and phosphatidylglycerol. The quantification of separated lipid classes by scanning densitometry was not attempted since some samples showed streaking between lipid class components.

With the exception of st. 12 in May, neutral lipid classes predominated in the lipid extracted from algae sampled in both spring and autumn (Table 3). This was particularly notable in the algae taken from st. 7 in May and from st. 12 in October 1987, in which neutral lipids

Table 2 Dominating algal species in samples from sea ice of the Barents Sea. Values in parentheses are % of total cell numbers

Year	Date	Station no.	Dominating species
1988	22.05[a]	7	*Nitzschia frigida* (51), *Synedropsis hyperborea* (16), *Acnanthes taeniatai* (23)
	26.05[a]	12	*Thalassiosira bioculata* (24), *N. frigida* (24), *Navicula vanhoeffenii* (20)
	27.05[a]	13	*Nitzschia frigida* (42), *Navicula vanhoeffenii* (34)
1988	12.09[b]	Hk2	*T. bioculata* (97)
1987	20.10[b]	12	*Actinocyclus cf curvatulus* (88)

[a] Spring
[b] Autumn

Table 3 Relative amount of fatty acids in lipid fractions in sea-ice algae from different sampling stations in spring and autumn. Values are % of total fatty acids

	Spring			Autumn	
	May 1988			Sept. 1988	Oct. 1987
	St. 7	St. 12	St. 13	St. Hk2	St. 12
Neutral lipids	94.2	37.4	72.5	54.6	83.3
Glycolipids	3.8	45.8	14.9	27.0	11.2
Phospholipids	2.0	16.8	12.6	18.4	5.5

accounted for 94% and 83% of the total lipids, respectively. In the lipids of all samples, the proportions of glycolipids were greater than those of phospholipids and in algae sampled from st. 12 in May glycolipids were the major lipid fraction. Phospholipids were always the smallest lipid fraction, accounting for 2–18% of the total lipid.

The fatty acid compositions of the neutral lipid, glycolipid and phospholipid fractions of the samples taken in spring are shown in Table 4. The fatty acid component shown as 16:1(n-13)t also contained small amounts of another unidentified saturated fatty acid which was not converted to 16:0 upon hydrogenation. Certain similarities were obvious among all the spring samples in terms of the fatty acid compositions of lipid fractions. Thus, 20:5(n-3) was always the principal polyunsaturated fatty acid, with the exception of the glycolipids of st. 7 which had a very low PUFA content in comparison with the other samples and fractions. The highest content of 20:5(n-3) was observed in the glycolipids of algae from st. 12, in which it accounted for 38.8% of the total glycolipid fatty acids. The glycolipids of all three spring samples contained substantial proportions of C16 polyunsaturated fatty acids, particularly 16:4(n-1) which composed nearly 16% of the glycolipid fatty acids in algae from st. 13. Phospholipids always contained the highest proportion of 22:6(n-3), although 20:5(n-3) was still the major PUFA of this lipid fraction. The fatty acid compositions of the neutral lipid fractions from the three spring samples were notably different. In particular, the neutral lipid of the st. 7 sample had a substantially higher content of PUFA than those of the

Table 4 Fatty acid composition (wt%) of lipid fractions from sea-ice algae sampled in spring. Values of less than 0.4% are not presented (*NL* neutral lipids; *GL* glycolipids; *PL* phospholipids; *sat* saturated fatty acids; *mono* monounsaturated; *PUFA* polyunsaturated fatty acids)

	St. 7			St. 12			St. 13		
	NL	GL	PL	NL	GL	PL	NL	GL	PL
14:0	6.5	–	1.3	4.9	3.0	6.6	6.2	3.9	7.7
i-15:0	1.1	–	–	6.7	6.7	5.4	0.8	2.0	0.8
15:0	0.7	–	–	1.2	0.7	0.6	0.6	0.7	0.6
16:0	13.5	16.1	16.0	16.4	4.1	10.5	18.0	9.2	10.5
16:1(n-7)	13.2	1.0	5.3	17.9	2.6	6.7	37.2	11.2	7.0
16:1(n-13)t	0.7	–	2.4	1.2	7.8	10.4	1.4	10.1	8.4
16:2(n-4)	2.1	1.9	1.3	0.6	1.1	0.6	1.8	2.7	2.1
16:3(n-4)	5.5	2.1	2.6	–	1.1	–	0.6	2.5	–
16:4(n-1)	4.8	4.0	2.8	1.5	12.3	1.8	3.4	15.8	2.0
18:0	3.3	25.7	12.0	7.3	7.1	6.1	2.4	6.6	4.2
18:1(n-9)	5.1	20.3	14.7	6.7	1.5	1.8	2.4	3.3	2.1
18:1(n-7)	1.0	6.9	9.3	–	–	1.2	0.2	1.3	0.7
18:2(n-6)	1.1	5.2	4.0	1.8	0.7	1.2	1.0	1.2	0.7
18:4(n-3)	5.7	–	–	1.2	2.6	5.5	2.0	1.4	5.6
20:1(n-9)	8.9	2.8	–	–	–	0.6	–	–	–
20:2	–	–	–	–	–	4.2	–	–	1.3
20:4(n-3)	0.9	2.0	2.6	–	–	–	0.4	–	0.4
20:5(n-3)	16.3	2.2	10.9	14.2	38.8	22.6	12.7	19.7	31.5
22:1(n-9)	0.7	–	–	1.5	–	–	–	–	–
22:5(n-3)	1.1	–	–	–	–	–	–	–	–
22:6(n-3)	5.7	–	6.9	0.9	1.9	4.4	0.6	1.3	10.5
24:0	–	–	–	0.9	2.2	3.0	0.1	2.6	2.8
24:1	0.6	2.8	1.3	–	–	–	–	–	–
Unidentified	0.7	7.0	6.6	13.3	5.8	6.8	7.2	4.5	1.1
Total sat	25.5	41.8	29.3	38.6	23.8	32.2	28.3	25.0	26.6
Total mono	30.2	33.8	33.0	27.6	11.9	20.7	41.4	25.9	18.2
Total PUFA	43.6	17.4	31.1	20.5	58.5	40.3	23.1	44.6	54.1

other two samples. The 16:1(n-7) content of the neutral lipid of all three samples was always higher than in the two polar lipid fractions, particularly in the case of the sample from st. 13 where it accounted for 37.2% of the total neutral lipid fatty acids.

As with the samples from spring, the lipid fractions of the algae sampled in autumn showed characteristic fatty acid compositions. Thus, the neutral lipid fractions contained a higher proportion of saturated fatty acids than either the glycolipids or phospholipids and had also a notably higher content of 16:1(n-7). In the glycolipid fraction of the algae sampled in autumn 1987, 18:1(n-9) and 20:5(n-3) were the principal fatty acids and 16:4(n-1) was absent. In contrast, 16:4(n-1) was a major fatty acid along with 20:5(n-3) in the glycolipid fraction of the ice algae sampled in autumn 1988 and 18:1(n-9) was a relatively minor component. The phospholipid fraction of the two autumn samples was characterised by a high content of polyunsaturated fatty acids, particularly 20:5(n-3) and 22:6(n-3), which constituted 31.7% and 17.8% respectively, in the October sample and 27.3% and 7.1% respectively, in the September sample. No major differences in fatty acid composition were obvious between the spring and autumn samples of sea-ice algae.

Discussion

In keeping with their general predominance in assemblages of sea-ice algae (Kirst and Wiencke 1995), diatoms composed the bulk of the algae from the Arctic ice of the Barents Sea examined in this study. At the same time, other microorganisms and detritus were also present. The finding that the lipid of most of the samples was dominated by neutral lipid substantiates the results of recent analyses of samples taken in a later year from the same location (Falk-Petersen et al. 1998) and agrees with the results of previous studies of communities of sea-ice diatoms from Antarctica in which neutral lipids were found to account for 16–63% of the total lipid (Nichols et al. 1988, 1989). The abundance of neutral lipid in sea-ice algae from Antarctica has been inferred by the existence of oil droplets within *Thalassiosira* cells isolated from the interstitial water between ice platelets (Fahl and Kattner 1993). Laboratory studies have shown that the lipid class composition of algae is greatly influenced by many factors, including temperature (Mortensen et al. 1988), light intensity and growth stage (Thompson et al. 1990), the latter being usually correlated

Table 5 Fatty acid composition (wt%) of lipid fractions from sea-ice algae sampled in autumn. Values of less than 0.4% are not presented. Abbreviations are as given in Table 4

	Sept. 1988			Oct. 1987		
	HK St. 2			St. 12		
	NL	GL	PL	NL	GL	PL
14:0	8.5	4.2	11.6	2.6	1.4	0.3
i-15:0	2.8	1.7	1.0	3.4	0.9	1.0
15:0	0.9	0.8	0.5	0.8	0.4	0.5
16:0	14.4	6.3	10.6	23.0	11.7	10.2
16:1(n-7)	10.8	3.4	7.6	29.6	6.3	1.3
16:1(n-13)t	0.5	14.0	7.6	–	–	1.0
16:2(n-4)	0.7	1.3	0.5	0.9	1.7	–
16:3(n-4)	–	–	–	0.6	0.9	–
16:4(n-1)	2.1	14.7	2.5	0.4	–	0.3
18:0	4.8	6.7	4.5	4.6	8.7	5.0
18:1(n-9)	5.3	6.3	1.5	6.5	27.8	5.1
18:1(n-7)	–	–	–	2.7	4.8	7.6
18:2(n-6)	3.4	2.1	2.0	2.5	4.3	3.0
18:4(n-3)	4.1	1.7	8.6	2.1	1.8	3.0
20:0	0.5	–	–	0.4	0.9	–
20:1(n-9)	–	–	–	1.5	1.7	2.6
20:2	–	–	4.0	–	–	–
20:4(n-6)	0.2	0.4	–	0.4	0.4	2.8
20:4(n-3)	0.7	–	–	1.1	0.5	2.9
20:5(n-3)	24.7	26.5	27.3	11.0	18.3	31.7
22:1(n-9)	–	–	–	1.7	1.6	–
22:5(n-3)	–	–	–	0.4	–	0.6
22:6(n-3)	1.4	1.3	7.1	2.1	1.9	17.8
24:0	0.5	–	2.0	0.6	1.7	0.5
24:1	–	–	0.5	–	–	2.3
Unidentified	12.8	8.6	0.6	–	1.9	0.5
Total sat	33.3	19.7	30.2	35.4	25.7	17.5
Total mono	16.6	23.7	17.2	42.9	42.3	19.9
Total PUFA	37.3	48.0	52.0	21.7	30.1	62.0

with nutrient availability (Reitan et al. 1994). Neutral lipids, usually in the form of triacylglycerols, account for high proportions of the total lipid in algae in the stationary phase, whereas high levels of polar lipids, particularly glycolipids, are associated with actively growing algae. Extrapolation of this to phytoplankton in the field predicts that algae that are actively growing in a bloom should contain less neutral lipid and more polar lipid than non-dividing algae of a late bloom when nutrients have been deleted (Kattner et al. 1983; Parrish 1987). The high proportion of neutral lipid observed here in the resting stage ice algae sampled in autumn is in keeping with this situation. However, in studies of photosynthetic carbon assimilation by Antarctic sea-ice diatoms it was found that, during the active growth of the bloom, most of the carbon fixed into lipid was incorporated into neutral lipids (Palmisano et al. 1988). Likewise, laboratory analysis of lipid composition has also shown that triacylglycerols predominate in the lipids of a log-phase pure culture of the sea-ice diatom *Nitzschia cylindrus* isolated from the Antarctic (Nichols et al. 1986).

Triacylglycerols were the main neutral lipid present in the Barents Sea ice algae studied here in both spring and autumn and were also abundant in algae from the same region sampled in June when they accounted for up to 46% of the total lipid (Falk-Petersen et al. 1998). It is notable that the proportion of triacylglycerols in the lipid increased throughout the growth period of Canadian Arctic sea-ice algae from less than 10% of total lipid in April to 44% in June (Smith et al. 1993). The increase in neutral lipid content of actively growing sea-ice algae is characteristic of sea-ice algae and may result from changes in one or more of the environmental parameters. However, a common feature of ice algae is the low growth rates for natural populations (for review, see Cota and Smith 1991a). This is due to a combination of factors, with low light and low temperature being the most important. Thus, slow growth occurs in combination with a high content of neutral lipids, but no single factor has been identified as the sole explanation for this observation. For instance, no significant relationship was found for allocation of photosynthates to total lipid with varying irradiance for a large number of algal samples (Cota and Smith 1991b). In the present study, the ice algae sampled in spring in the Barents Sea had comparable low growth rates, with a calculated max. value of 0.2 divisions day^{-1} (Johnsen and Hegseth 1991). The proportion of neutral lipid was, however, substantially lower in the lipid of the st. 12 May sample than in that of the other two spring samples. Although the inorganic nutrient levels were similar for all three spring samples, the ice algae from st. 12 had been subjected to a different light climate than the ice algae in the other two samples. According to the diver, the colour of light inside the cavern was much more orange-red than that under flat ice. This indicates that under conditions of non-limiting inorganic nutrients, the light spectrum and perhaps also the light intensity can influence the amount of neutral lipid produced. Studies with Canadian sea-ice algae in which light intensity was manipulated have suggested that triacylglycerol synthesis is not enhanced by high light intensity alone, but rather requires a combination of high light intensity and high biomass concentration (Smith et al. 1993).

Growth of the algae in the autumn samples had almost ceased, and the cells had reached a resting stage. It is notable that the proportion of neutral lipid in the lipid of algae sampled in October 1987 was considerably higher than that observed for algae obtained in September of the following year. Although this difference may be related to different conditions, it may also be a consequence of the fact that the species compositions of the two autumn samples were entirely different. The lowest proportion of neutral lipid in autumn ice algae was observed with a sample composed almost entirely of *Thalassiosira bioculata*. This species was also a major component of the spring sample which contained the lowest proportion of neutral lipid. Thus, *T. bioculata* may produce less neutral lipid than *N. frigida*, the principal species present in the two other spring samples. A high level of neutral lipid may also be a feature of *Actinocyclus* cf *curvatulus*, at least under the environmental conditions that prevailed at the time of the sampling in October 1987. Species-specific variations have been noted both in natural populations from Antarctica (Priscu et al. 1990) and in cultured species (Thompson et al. 1990), but on the basis of the present results no definite conclusions can be drawn as to the ability of different species of sea-ice algae to produce neutral lipids since the samples were not monospecific or grown under controlled environmental conditions.

The present study showed that the lipids of the algae associated with Arctic sea ice contained 20:5(n-3) as the predominant polyunsaturated fatty acid, and that significant amounts of C16 polyunsaturates were also present. It is well established, mainly from studies with laboratory-grown algae, that diatoms, including those isolated from sea-ice, contain lipid that is characterised by high levels of 20:5(n-3) and C16 PUFA (McConville 1985; Nichols et al. 1993b; Viso and Marty 1993; Dunstan et al. 1994; Skerratt et al. 1995). Thus, the observed fatty acid profile is consistent with diatoms being the predominant class of algae present in the samples. Previous studies of the lipid composition of algae from sea ice have examined mainly the fatty acid composition of total lipid (Fahl and Kattner 1993; Nichols et al. 1993b). In agreement with the general situation in microalgae, the PUFA content of the neutral lipid fraction from most of the algal samples was lower than that of the glycolipid and phospholipid fractions (Henderson and Mackinlay 1989). Nevertheless, 20:5(n-3) was a major component of the neutral lipid fraction, which also contained significant amounts of C16 PUFA, particularly 16:4(n-1). This may be due to the free fatty acids present in the neutral lipid being enriched in PUFA liberated from algal polar lipids during sampling and lipid extraction, or already present in detritus. However, 20:5(n-3) has recently been shown to be the major PUFA of triacylglycerols in sea-ice algae from the Arctic

sampled in summer (Falk-Petersen et al. 1998), and a significant amount has also been found in the triacylglycerols of algae taken from the interstitial water of sea ice in the Antarctic (Fahl and Kattner 1993).

The occurrence of 20:5(n-3) as the major PUFA in the glycolipid fraction agrees with previous analyses of laboratory-grown cultures of the marine diatom *Phaeodactylum tricornutum* (Arao et al. 1987), but is at odds with the apparent lack of this PUFA in the glycolipids of *Nitzschia cylindrus* isolated from sea ice and grown under laboratory conditions (Nichols et al. 1986). The phospholipid fraction of the latter species was also devoid of 20:5(n-3) and other PUFA in comparison with the samples of Arctic sea-ice algae analysed here. In all of the samples examined in this study, 22:6(n-3) was most abundant in the phospholipid fraction. Marine diatoms are characterised by a low content of 22:6(n-3), but the present data suggest that the 22:6(n-3) is concentrated in the phospholipid fraction as is found in other classes of microalgae (Henderson and Mackinlay 1989; Bell et al. 1997), although some of the phospholipids in the samples analysed here may have been associated with detritus rather than living diatoms. Since only very low levels of flagellates were observed in all samples it was assumed that the contribution of non-diatom species to the overall lipid composition of samples was also very low. However, the observed differences in lipid and fatty acid composition of the st. 7 sample in comparison with the other samples may nevertheless still be due, at least in part, to the presence of other groups of microorganisms. In particular, the ratio of 16:0 to 16:1(n-7) in polar lipids was higher in the st. 7 sample than in the samples from the other sites. An increase in this ratio has been shown to be associated with an increase in the level of prymnesiophytes in the water column particulates in Antarctic coastal waters (Skerratt et al. 1995). At the same time, the higher levels of 18:1(n-7) observed in the st. 7 polar lipids may reflect the presence of bacteria since 18:1(n-7) predominates over 18:1(n-9) in bacteria, including those from polar waters that are capable of producing 20:5(n-3) and 22:6(n-3) (Nichols et al. 1993a).

Although the fatty acid composition of total lipid from diatoms has been frequently studied, that of the component glycolipids and the phospholipids has received much less attention. The levels of polyunsaturated fatty acids present in the neutral lipid, glycolipid and phospholipid fractions in this study (up to 62%) are similar to those observed previously in the total lipid of diatoms from ice cores of Antarctic sea ice (Nichols et al. 1993b). They are, however, substantially higher than those reported previously for the same fractions in *N. cylindrus* isolated from Antarctic sea ice and grown at 4°C (Nichols et al. 1986) and those reported for total lipid of marine diatoms grown in laboratory cultures at higher temperature (Viso and Marty 1993; Reitan et al. 1994). An increase in the proportions of polyunsaturated fatty acids in the polar lipids of biomembranes is a known response of algae grown in laboratory cultures to lowered growth temperatures (Henderson and Mackinlay 1989) and the low environmental temperatures experienced by the sea-ice diatoms studied here may have influenced the degree of unsaturation of the fatty acid composition of the algae.

The complex nature of the samples analysed in the present study obscures any relationships between lipid composition, species-specific factors and environmental parameters. The continuous monitoring of specific sites is difficult, but would aid the elucidation of these relationships.

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