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Fatty acid, sterol and hydrocarbon composition of Antarctic sea ice diatom communities during the spring bloom in McMurdo Sound

DAVID S. NICHOLS[1], PETER D. NICHOLS[1,2] and CORNELIUS W. SULLIVAN[3,4]

[1]*CSIRO Division of Oceanography, GPO Box 1583, Hobart, Tasmania,Australia, 7001*
[2]*CRC for the Antarctic and Southern Ocean Environment, University of Tasmania, GPO Box 252C, Hobart, Tasmania, Australia 7001*
[3]*Graduate Program in Ocean Sciences, Hancock Institute for Marine Studies, University of Southern California, Los Angeles, California 90089-0371, USA*
[4]*Present address: Office of Polar Programs, National Science Foundation, 1800 G Street, NW, Washington D.C. 20550, USA*

Abstract: The lipid composition of microalgal communities dominated by diatoms collected from the sea ice at three locations within McMurdo Sound during the austral spring bloom of 1989/90, was determined using gas chromatography (GC) and GC mass spectrometry. A range of C_{27}-C_{29} sterols were detected. The major sterols found at the three sites were 24-methylcholesta-5,22E-diene-3β-ol (Cape Armitage); *trans*-22-dehydrocholesterol, 24-ethylcholesterol and 24-methylenecholesterol (Erebus Ice Tongue); and 24-methylenecholesterol (Cape Royds). The difference in sterol profiles is believed to reflect the differing species composition at each site. The high relative levels (as % of total) of 24-ethylcholesterol at the Erebus Ice Tongue site (possibly related to *Amphiprora kufferathii*) supports the proposal that diatoms are a more probable source of C_{29} sterols in Antarctic lakes than are other algal groups or cyanobacteria. Changes in sterol composition over the course of the bloom were evident at the Cape Armitage site, particularly within the cellular free-lipid fraction. The major fatty acids identified were 14:0, 16:0, 16:1ω7c, 16:4ω1 and 20:5ω3 (Cape Armitage and Erebus Ice tongue sites); 16:0, 16:1ω7c and 20:5ω3 (Cape Royds site). All sites demonstrated high levels of PUFA (40–50% of total fatty acids), with an average 20:5ω3 level of 21% Erebus Ice Tongue, 20% Cape Royds, and 17% Cape Armitage. Variation was also observed in the percentage of 20:5ω3 for the Cape Armitage community over the sampling period. Levels of 22:6ω3 were between 0.4 and 1% of total fatty acids for the three sites. A $C_{25:2}$ isoprenoid hydrocarbon was present in samples from all sites, adding further evidence to the proposal that diatoms are probably a source of this and related isoprenoid alkenes in marine and coastal sediments.

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Key words: sea ice, diatoms, fatty acids, sterols, hydrocarbons

Introduction

Annually, vast areas of ice form and melt at the ocean surface surrounding Antarctica. The sea ice may grow from 1–5 m in thickness and at its maximum extent in August–October may form a continuous 400–1900 km wide barrier around the entire continent. Satellite observations of the Southern Ocean from 1973–75 show a minimum sea ice coverage of 4 million km^2 in February, peaking at an annual maximum of 20 million km^2 in September–October (Vincent 1988).

During the austral spring and summer, sea ice supports the growth of a wide array of microalgae, mostly diatoms found throughout the pack-ice and near the bottom of the hard congelation ice of near shore fast ice (Bunt 1963, Palmisano & Sullivan 1983, 1985, Vincent 1988, Kottmeier & Sullivan 1990). Consequently, the sea ice diatom population potentially represents a major component of the carbon / energy flux of the polar oceans, where the sea ice microalgae may contribute up to 50% of primary production in certain regions (Grossi *et al.* 1987). The diatoms also produce lipid components, such as 20:5ω3 and 22:6ω3 fatty acids, that are essential dietary components of many marine organisms which lack the ability to biosynthesise these themselves (Kanazawa *et al.* 1979). Several sea ice diatom studies have characterized the lipid components of natural sea ice diatom communities, or of individual diatom species isolated from these sources (Whitaker & Richardson 1980, Gillan *et al.* 1981, Nichols *et al.* 1986b, 1988,1990) but, only limited time series data has been reported concerning seasonal changes in lipid composition (Palmisano *et al.* 1988, Nichols *et al.* 1989). This is particularly true for the changes in fatty acid composition occurring during the annual summer bloom of microalgae under the land-fast sea ice.

This study was undertaken to determine the changes in fatty acid and sterol composition of several sea ice diatom communities during the 1989/90 summer sea ice algal bloom in McMurdo Sound. Such a study is important to those examining the organic geochemistry of sediments, chemotaxonomy and the transfer of essential lipids through marine food webs.

Materials and methods

Sea ice algal communities were sampled during the 1989/90 summer bloom from three locations within McMurdo Sound:

Cape Armitage, Cape Royds and the Erebus Ice Tongue. Congelation ice samples from each site were collected with a SIPRE ice auger (7 cm core diameter) from the annual sea ice. The bottom 20 cm of each ice sample, in which 99% of the chlorophyll *a* is found (Palmisano & Sullivan 1983) was then melted at less than 5°C in 1.2 l of filtered seawater. Selected samples were differentially separated at McMurdo station into whole cell (WC), membrane (MF), cell wall and free lipid (FL) enriched fractions by the lysis of dense diatom cell suspensions (1 g wet wt/ml) in a buffered medium contained in a loose fitting teflon tissue homogenizer by grinding at 0°C. Cell disruption was checked in a phase contrast microscope (Zeiss) and the lysate was separated into the following fractions using a three step differential centrifugation scheme: 1. 2000*g* (10 min), cell walls; 2. 10 000*g* (15 min), mitochondria; 3. 40 000*g* (60 min), membranes. The free lipid fraction remains at the top of the tube after the final centrifugation and was removed using a Pasteur pipette (Sullivan 1978). All samples were then frozen at -70°C for transport to CSIRO Marine Laboratories in Hobart for analysis and determination of subcellular localization (enrichment) of various lipid species.

Lipids were extracted from a known mass of sample using the modified one-phase $CHCl_3$-MeOH Bligh and Dyer extraction (Bligh & Dyer 1959, White *et al.* 1979). A portion of the total lipid extract was then saponified by reaction at 60°C for 3 h using 3 ml of a 5% KOH in 80% MeOH (w/v) solution. After the addition of water, the mixture was extracted with hexane/$CHCl_3$ (4:1, v/v) to yield a non-saponifiable neutral lipid fraction (predominantly hydrocarbons, alcohols, sterols) contained in the upper organic layer and free fatty acids (as potassium salts) in the lower aqueous layer. Sterols were converted to OTMSi ethers by reaction with bis(trimethylsilyl)trifluoroacetamide (BSTFA) reagent for 24 h. Subsequent fatty acid methylation was achieved by acidification of the aqueous layer and extraction with hexane/$CHCl_3$ (3 x 1.5 ml, 4:1 v/v). Solvent was evaporated under a stream of nitrogen before reaction for 1 h at 60°C with a MeOH/HCl/$CHCl_3$ (10:1:1 v/v/v) solution, followed by extraction of the resultant FAME with hexane/$CHCl_3$ (4:1 v/v).

Gas chromatographic (GC) analyses of the sterols (as OTMSi ethers) and fatty acids (as FAME) were performed with a Hewlett Packard 5890 GC equipped with a 50 m x 0.32 mm internal diameter cross-linked methyl silicone (HP-1) fused-silica capillary column and flame ionization detector. Samples were injected at 50°C in the splitless mode with a venting time of 2 min. After one minute the oven was temperature programmed from 50–150°C at a rate of 30°C min^{-1}, then at 2°C min^{-1} to 250°C and 5°C min^{-1} to a final temperature of 300°C which was maintained for 15 min. Hydrogen was used as carrier gas, and the injector and detector were maintained at 290 and 310°C respectively. Peak areas were quantified using chromatography software (DAPA Scientific Software, Kalamunda, Western Australia) operated using an IBM-XT personal computer.

Co-eluting fatty acids were separated using a Hewlett Packard 5890 GC equipped with a 60 m x 0.32 mm internal diameter Supelcowax column and a flame ionization detector. Samples were injected on-column at 45°C . After 1 min the oven was temperature-programmed from 45–120°C at a rate of 30°C min^{-1}, then at 3°C min^{-1} to 250°C. Hydrogen was used as the carrier gas, and the detector temperature was maintained at 280°C. Peak areas were quantified using a Shimadzu Chromatograph C-R2AX plotter/integrator.

Gas chromatography-mass spectrometry (GC-MS) analyses of samples were performed on a Hewlett Packard 5890 GC and 5970 Mass Selective Detector (MSD) fitted with a direct capillary inlet. The column, injector and chromatography conditions were similar to those described above for the non-polar column GC analysis, with helium used as the carrier gas. Mass spectra were acquired and processed using an HP 59970C Workstation operated in scan acquisition mode. Typical MSD operating conditions were: electron multiplier 2000–2200 volts; transfer line 310°C; autotune file DFTPP normalized; electron impact energy 70eV; scan threshold = 1500; 0.8 scans/sec; mass range 40–600 amu; solvent delay 7 min. Identification of compounds was confirmed by comparison of mass spectra with those previously reported and by comparison of relative retention time data.

Monounsaturated fatty acid double bond position and geometry was determined by GC-MS analysis of their dimethyl disulphide (DMDS) adducts, as described in Dunkelblum *et al.* (1985) and Nichols *et al.* (1986*a*).

Results and discussion

Species composition

Nichols *et al.* (1988) and Palmisano *et al.* (1988) found that the 1985 austral summer sea ice algal communities of McMurdo Sound were dominated by *Nitzschia stellata*, (Manguin), *Amphiprora* sp. and *Berkeleya* sp. at Cape Armitage, and *N. stellata, Amphiprora, Pleurosigma* and *N. kerguelensis* (O'Meara) at the Erebus Ice Tongue site.

Microscopic analysis of the samples collected in 1989 and analysed in this study showed the Cape Armitage site was dominated by *Nitzschia stellata* (>99%) and the Cape Royds community was virtually 100% the centric diatom *Porosira pseudodenticulata* (Hustedt). The Erebus Ice Tongue samples comprised a mixture of *N. stellata, Amphiprora kufferathii* (Manguin) and four small unidentified centric diatoms.

Hydrocarbons

GC-MS analysis of the hydrocarbons present from the Cape Royds whole cell (25 November) and Cape Armitage free lipid fraction (25 November) showed the presence of a C_{25} diunsaturated isoprenoid. This identification was reached by comparison of mass spectra and elution times with a positively identified $C_{25:2}$ isoprenoid ($brC_{25:2}$; retention index of 2088 on OV-1 stationary phase, Rowland & Robson (1990)). This compound was a dominant hydrocarbon in sea ice diatom communities collected during the 1985 austral spring bloom from McMurdo Sound (Nichols *et al.* 1988). Levels of the $C_{25:2}$

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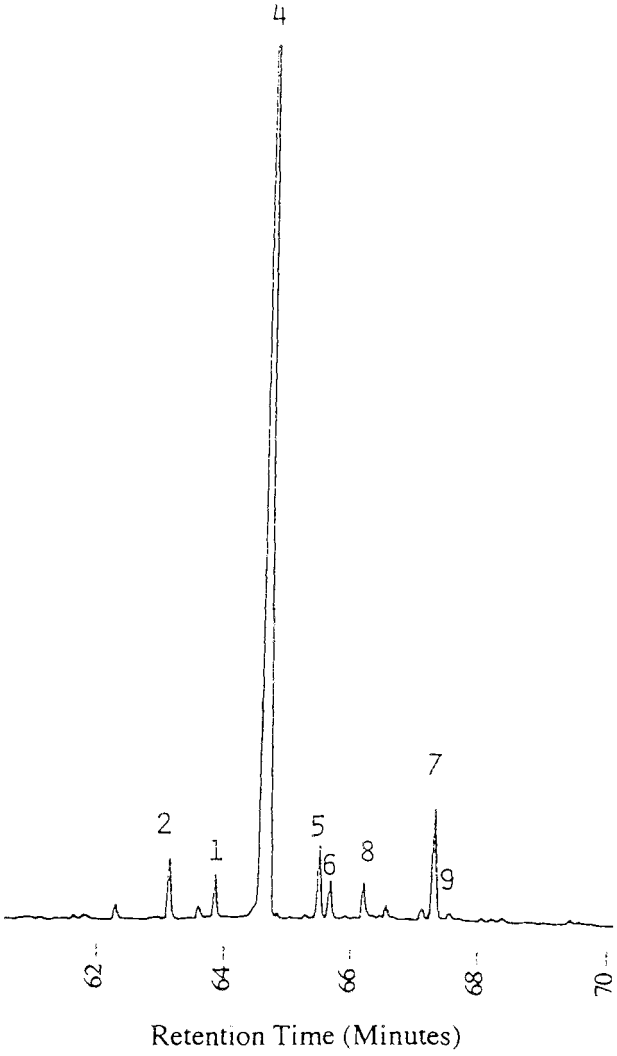

Fig. 1. Partial gas chromatogram (60–70 min) illustrating a representative sterol profile obtained for Cape Armitage sea ice diatom community dominated by *Nitzschia stellata*, December 1 1989. Peak numbers refer to Table II.

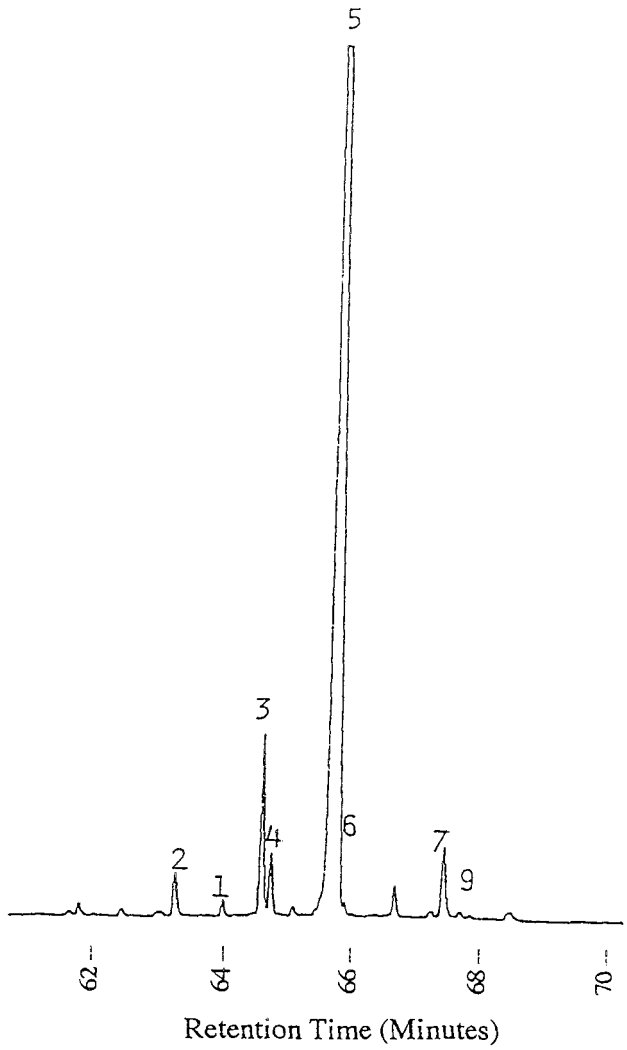

Fig. 2. Partial gas chromatogram (60–70 min) illustrating a representative sterol profile obtained for Cape Royds sea ice diatom community dominated by *Porosira pseudodenticulata*, November 22 1989. Peak numbers refer to Table II.

isoprenoid from the Cape Royds community were much higher than those from Cape Armitage (unpublished data). This may relate to the relative species distribution at each site. Trace amounts of a possible $C_{25:3}$ were also observed in nearly all samples but not in samples from the 1985 study (Nichols *et al.* 1988).

The presence of these unsaturated isoprenoids in sea ice diatom communities lends further support to the proposal by Nichols *et al.* (1988) that diatoms are a more probable source of C_{25} alkenes in marine sediment than are bacteria or other algae as suggested previously (Rowland & Robson 1990).

Sterols

A wide range of C_{27}–C_{29} sterols were identified in samples from all three sites (Tables I & II, Figs 1 & 2). However, the percentage composition of the major sterols at each site was found to differ markedly. 24-Methylcholesta-5,22E-diene-3β-ol was the major sterol at the Cape Armitage site dominated by *N. stellata*, while 24-methylenecholesterol was the major component from the Cape Royds community, dominated by *P. pseudodenticulata*. This difference in sterol profiles is believed to reflect the differing species composition at each site, suggesting that these lipids may serve as chemical "markers" of these species or groups of species.

The Erebus Ice Tongue sea ice diatom community contained 24-methylenecholesterol as the major sterol, but also possessed significant levels (>15% of total sterols) of both *trans*-22-dehydrocholesterol and 24-ethylcholesterol (Table II). High levels of 24-ethylcholesterol (29% of total sterols) were noted in the 1985 Erebus Ice Tongue and Cape Armitage sea ice diatom communities (Nichols *et al.* 1989) and are of relevance as this sterol has traditionally served as a marker for higher

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Table I. Sterol composition of sea ice algal samples collected furing the 1989 spring bloom at Cape Armitage in McMurdo Sound.

		Percentage composition												
	Sample date:	8 Nov			12 Nov	15 Nov	17 Nov	21 Nov	25 Nov			28 Nov	30 Nov	1 Dec
	Sample code:	1MF	2FL	3WC	6WC	8WC	9WC	10WC	22MF	21FL	9WC	26WC	27WC	29WC
Sterol	Common name													
cholest-5-en-3β-ol	cholesterol	1.2	0.1	6.4	1.3	3.0	3.9	2.3	2.2	4.6	2.9	2.2	2.3	1.7
cholesta-5,22E-diene-3β-ol	*trans*-22-dehydrocholesterol	0.4	-	1.4	2.7	7.5	8.7	6.4	3.8	2.2	12.2	3.7	4.8	2.5
cholesta-5,24-diene-3β-ol	desmosterol	-	-	-	-	-	-	-	-	0.6	-	-	-	-
24-methylcholesta-5,22E-diene-3β-ol		87.6	99.7	84.3	89.0	78.3	69.5	76.5	73.5	75.4	71.5	82.8	79.2	83.0
24-methylcholesta-5,24(28)-diene-3β-ol	24-methylenecholesterol	2.2	0.1	0.5	3.5	4.2	7.0	4.9	3.8	3.2	5.1	3.5	4.3	3.3
24-methylcholest-5-en-3β-ol	24-methylcholesterol	3.0	0.1	4.6	1.8	2.4	2.9	1.3	2.6	3.6	tr	1.8	2.3	1.7
24-ethylcholest-5-en-3β-ol	24-ethylcholesterol	4.4	-	1.7	1.6	4.5	6.0	7.1	10.2	8.2	7.1	5.1	5.3	5.6
24-ethylcholesta-5,22E-diene-3βol		1.2	-	0.6	-	tr	1.9	1.4	3.7	2.0	1.2	0.8	1.8	1.7
24-ethyl-5α-cholestan-3β-ol	24-ethylcholestanol	tr	-	0.4	-	-	0.1	-	0.3	0.3	tr	0.2	tr	0.3
Total:		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Legend: WC = Whole cell; MF = Membrane fraction; FL = Free lipid fraction; tr = < 0.1%

Table II. Sterol composition of sea ice algal samples collected during the 1989 bloom from Erebus Ice Tongue and Cape Royds in McMurdo Sound.

			Percentage composition			
		Sample date:	22 Nov	22 Nov	24 Nov	25 Nov
		Sample code:	14WC	11WC	23FL	18WC
		Location:	EIT	CR	CR	CR
		Species composition:	Mixed	*P.pseud.*	*P.pseud.*	*P.pseud.*
Peak No	Sterol	Common name				
1	cholest-5-en-3β-ol	cholesterol	7.0	0.5	1.3	0.6
2	cholesta-5,22E-diene-3β-ol	*trans*-22-dehydrocholesterol	29.0	1.5	1.2	0.2
3	cholesta-5,24-diene-3β-ol	desmosterol	5.3	5.5	4.7	4.4
4	24-methylcholesta-5,22E-diene-3β-ol		3.5	1.6	9.6	2.3
5	24-methylcholesta-5,24(28)-diene-3β-ol*	24-methylenecholesterol	31.8	87.8	79.0	90.2
6	24-methylcholest-5-en-3β-ol*	24-methylcholesterol	1.2	0.3	1.0	0.3
7	24-ethylcholest-5-en-3β-ol	24-ethylcholesterol	17.3	2.6	2.9	2.0
8	24-ethylcholesta-5,22E-diene-3β-ol		3.6	-	0.1	tr
9	24-ethyl-5α-cholestan-3β-ol	24-ethylcholestanol	1.3	0.1	0.2	tr
		Total:	100.0	100.0	100.0	100.0

Legend: WC = Whole cell; FL = Free lipid fraction; EIT = Erebus Ice Tongue; CR = Cape Royds; *P.pseud.* = *Porosira pseudodenticulata;* tr = <0.1%.
* Percentages are approximate due to peak overlap

plants (Huang & Meinschein 1979). 24-Ethylcholesterol was a major sterol component of the Erebus Ice Tongue diatom community during two separate seasons (1985 & 1989), when the major species distribution remained similar. This result further strengthens the proposal by Nichols *et al.* (1989) that sea ice diatoms appear to be a more probable source of C_{29} sterols in the Antarctic environment than are cyanobacteria or other algal groups (Matsumoto *et al.* 1982, Orcutt *et al.* 1986).

If the production of 24-ethylcholesterol by the 1985 Erebus Ice Tongue and Cape Armitage communities was not the effect of coincidental environmental factors acting on the two communities, the presence of 24-ethylcholesterol at both sites could be due to the occurrence of one, or several, common diatom species. The only two major diatom species occurring at both the Cape Armitage and Erebus Ice Tongue sites in 1985 were *N. stellata and Amphiprora kufferathii* (Nichols *et al.* 1989), while both species were also important in the 1989 Erebus Ice Tongue samples. In contrast, the disappearance of *Amphiprora kufferathii* from the 1989 Cape Armitage community coincided with the reduction of 24-ethylcholesterol from a major (> 15% of total sterols) to a minor (< *c.* 5%) sterol component. It appears therefore that *Amphiprora kufferathii* is a possible major source of 24-ethylcholesterol in the sea ice algal communities.

As further supports the 1989 Cape Royds diatom community, dominated by *Porosira pseudodenticulata*, did not contain 24-ethylcholesterol as a major sterol. Examination of the minor sterols from the Cape Armitage and Cape Royds whole cell samples shows a general trend of higher cholesterol and *trans*-22-dehydrocholesterol in the Cape Armitage community,

whereas the Cape Royds diatoms possess desmosterol which was absent from the Cape Armitage sea ice algae.

Comparing the sterol composition of the whole cell, free lipid and membrane fractions (Tables I & II) provides information on the intracellular distribution of sterols. The 8 November Cape Armitage samples (Table I) show that 24-methylcholesta-5,22E-diene-3β-ol was the dominant sterol of the free lipid fraction (>99%). The membrane sterol fraction, while also consisting predominantly of 24-methylcholesta-5,22E-diene-3β-ol (87.6%), did contain at least trace levels of the eight minor sterols identified from the whole cell samples. By 25 November this distribution had changed markedly. Within the membrane fraction, the relative level of 24-methylcholesta-5,22E-diene-3β-ol had fallen to 73.5%, while the level of 24-methylcholesterol also dropped slightly. The remaining sterols all increased in relative abundance (from 1–6%). However, the greatest change in the sterol profiles occurred in the free lipid fraction (Fig. 3.). A decrease in 24-methylcholesta-5,22E-diene-3β-ol (from 99.7 to 75.4%) was accompanied by the appearance of the remaining cellular sterols that were previously absent, or only present in trace amounts, in the 8 November free lipid sterol fraction (Table I). In contrast, the free lipid sterol fraction extracted from the 24 November Cape Royds community (Table II) shows 24-methylenecholesterol as the major free lipid sterol and exhibits measurable levels of all other sterols identified from the corresponding whole cell samples. As no earlier free lipid samples from Cape Royds were available, it is not possible to determine whether these levels were the result of a similar change in free lipid sterol composition over the bloom, as at Cape Armitage, or whether they had remained relatively constant over this period.

The change in sterol composition during the sampling period of the Cape Armitage sea ice diatom community between 8 November and 1 December can be seen in Table I. Generally, there were no large changes in sterol composition of whole cells over the bloom period sampled. However, some trends in whole cell sterol content are evident. The proportion of *trans*-22-dehydrocholesterol rose to a maximum value of 12.2% on 25 November before falling to 3.7% by 28 November. Both 24-methylenecholesterol and *trans*-22-dehydrocholesterol also exhibited a smaller rise from 8 November to a secondary peak of 7.0 and 8.7% respectively on 17 November before declining toward 1 December. The proportion of cholesterol and 24-methylcholesterol generally decreased from maximal values of 4.6 and 6.4% respectively on 8 November while 24-ethylcholesterol rose from a minimum of 1.6% on 12 November to a broad maximum value of 7.1% over 21–25 November. The level of 24-methylcholesta-5,22E-diene-3β-ol, the major sterol present, remained relatively constant (*c.* 80%) over the bloom apart from a minimum of 69.5% on 17 November. Because of slight shifts in species composition during the bloom, we cannot unambiguously attribute all the observed changes in minor lipid composition to changes in nutrient status.

Nichols *et al.* (1989, 1990) reported the occurrence of

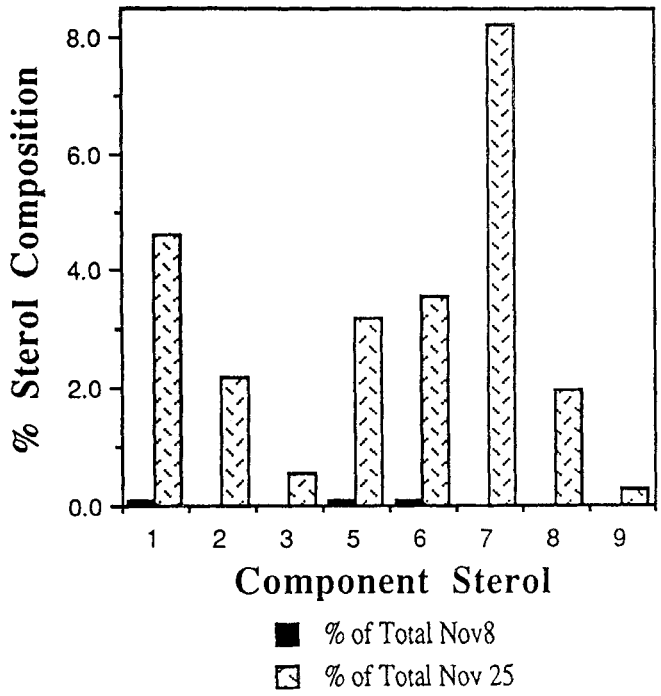

Fig. 3. Comparison of Cape Armitage diatom free lipid fractions on November 8 and 25. 1 = cholesterol; 2 = *trans*-22-dehydrocholesterol; 3 = desmosterol; 5 = 24-methylenecholesterol; 6 = 24-methylcholesterol; 7 = 24-ethylcholesterol; 8 = 24-ethylcholestanol; 9 = 24-ethyl-5α cholestan-3β-ol

significant levels of C_{30} sterols (10–14%) from the 1985 spring bloom at Cape Armitage and the Erebus Ice Tongue. In this study, C_{30} sterols appeared to be present as only trace amounts in several samples, with levels insufficient for positive identification by GC-MS. This difference may have arisen from changes in minor species composition at the two sites, or from differing environmental factors affecting sterol biosynthesis between the two seasons, or both.

Fatty acids

The fatty acid profiles for the Cape Armitage, Cape Royds and Erebus Ice Tongue sea ice diatom communities are shown in Tables III & IV. As was the case for sterols, the three communities contain a similar range of fatty acids, but in differing proportions. The Cape Armitage and Erebus Ice Tongue communities are dominated by 14:0, 16:0, 16:1ω7c, 16:4ω1 and 20:5ω3 fatty acids. The Cape Royds samples generally contained lower levels of 14:0 and 16:4ω1, but possessed higher levels of 16:0, 16:1ω7c and 20:5ω3. These major fatty acids are typical of most diatom species (Orcutt & Patterson 1975, Volkman *et al.* 1989, Gillan *et al.* 1981).

Tables III & IV show that all three communities contained high levels of polyunsaturated fatty acids (PUFA), Cape Armitage and Cape Royds averaging 40% and Erebus Ice Tongue 50% of total fatty acids. These values are similar to those for pure cultures of the sea ice diatoms *S. amphioxys* and *Navicula*

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Table III. Fatty acid composition of sea ice algal samples collected during the 1989 spring bloom at Cape Armitage in McMurdo Sound.

	Percentage composition												
Sample data:	8 Nov			12 Nov	15 Nov	17 Nov	21 Nov	25 Nov			28 Nov	30 Nov	1 Dec
Sample code:	1MF	2FL	3WC	6WC	8WC	9WC	10WC	22MF	21FL	19WC	26WC	27WC	29WC
Fatty acid													
14:0	12.4	11.9	11.4	9.7	11.0	12.9	12.4	12.7	9.9	11.8	9.8	12.6	10.0
15:0	0.3	0.3	0.8	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
16:0	17.7	24.5	16.0	13.5	17.2	18.1	18.4	15.1	21.4	14.4	19.3	19.1	18.3
18:0	tr	tr	1.6	0.1	0.2	0.1	0.2	0.1	0.2	tr	0.1	tr	tr
Σ Saturates:	30.5	36.6	29.8	23.7	28.7	31.5	31.5	28.4	32.0	26.6	29.6	32.2	28.7
16:1ω7c*	22.3	34.1	23.1	21.8	26.1	28.3	28.3	19.7	33.9	26.7	31.4	29.4	30.2
16:1ω5c	0.3	0.2	0.4	tr	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.4	0.3
16:1ω13t	1.8	0.3	2.3	1.4	1.2	1.4	1.4	1.5	0.4	1.1	1.0	1.7	1.0
18:1ω9c	1.6	3.1	1.6	1.3	1.9	1.7	1.7	1.3	2.7	1.2	2.2	1.6	2.0
18:1ω7c	0.5	tr	0.8	0.3	0.3	0.3	0.5	0.7	0.1	0.2	0.4	0.7	0.3
20:1	tr	-	0.1	-	-	-	-	tr	-	0.2	tr	-	-
Σ Monounsat.:	26.5	37.8	28.2	24.9	29.9	32.0	32.3	23.5	37.4	29.7	35.3	33.8	33.8
16:2ω4**								5.5	3.2				
16:2ω7**								0.5	-				
16:3ω4#	5.0	3.1	7.1	6.1	4.8	5.4	5.1	5.9	3.2	5.4	3.9	5.7	4.1
16:4ω1	9.8	7.9	12.9	12.7	10.3	11.1	10.9	11.4	7.3	10.2	9.0	10.0	9.1
18:2ω6	1.5	1.2	1.7	1.1	1.2	1.3	1.2	1.6	1.6	1.0	1.1	1.0	0.9
18:3ω3	0.4	0.4	0.6	0.5	0.5	0.4	0.4	0.7	0.7	0.4	0.5	0.3	0.4
18:3ω6	0.2	0.1	0.4	0.3	0.7	0.6	0.8	0.9	1.0	0.8	0.6	0.5	0.6
18:4ω3	2.1	1.5	2.3	2.6	2.7	2.3	2.3	2.6	1.8	2.5	2.2	2.2	2.3
20:2ω6	-	-	-	-	tr	-	-	tr	-	0.1	tr	-	-
20:3ω6	-	-	-	-	tr	tr	tr	0.1	tr	tr	0.1	tr	tr
20:4ω3	tr	-	0.1	0.3	0.3	0.2	0.2	0.3	0.2	0.2	0.3	tr	0.3
20:5ω3##	23.6	11.3	16.4	26.4	19.8	14.7	14.7	18.7	14.5	22.1	16.4	13.9	19.2
22:6ω3	0.5	-	0.7	1.4	1.0	0.6	0.6	tr	0.3	0.9	1.0	0.4	0.7
Σ PUFA	43.0	25.6	42.0	51.5	41.3	36.5	36.2	48.1	33.8	43.7	35.0	34.1	37.5
Total:	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Legend: WC = Whole cell; MF = Membrane fraction; FL = Free lipid fraction; tr = < 0.1%; * Also includes small amounts of co-eluting 16:2ω4; # Also includes small amounts of co-eluting 16:2ω7; ## = Also includes small amounts of co-eluting 20:4ω6; ** Levels only determined for samples 21 & 22.

glaciei, (40–50% PUFA) (Gillan *et al.* 1981, Whitaker & Richardson 1980), but are not significantly higher than known temperate diatom species, which average between 12 and 53% PUFA (Orcutt & Patterson 1975, Volkman *et al.* 1989). The Cape Armitage diatoms appear to contain a greater proportion of PUFA within the membrane fraction than the free lipid, implying that PUFA are incorporated preferentially within cell membrane polar lipids rather than triacylglycerol storage lipids.

The average levels of 20:5ω3 present in the whole cell samples from the three communities were similar (Erebus Ice Tongue 20.8%; Cape Royds 17.1%; Cape Armitage 20.4%). These levels are comparable to those found for pure cultures of other Antarctic diatoms (24–26%) (Gillan *et al.* 1981) and similar to levels reported for related temperate species such as *Nitzschia angularis* (21% 20:5ω3) (Kates & Volcani 1966).

It can be seen from Fig. 4 that the level of 20:5ω3 (which includes 20:4ω6 present as a trace constituent, < 0.5%) within the Cape Armitage diatoms fluctuates significantly over the course of the bloom. Two maxima are present, on 12 November (26.4%) and 25 November (22.1%). Both of these are immediately followed by minima of 14.7 and 14.2% on 17–21 November and 30 November respectively. This suggests that since the majority of Antarctic diatom species appear to contain comparable levels of 20:5ω3, the observed fluctuations in the percentage of 20:5ω3 within the Cape Armitage community is more likely to be due to the effect of changing environmental factors during the bloom than to a shift in the species composition of the community. Thermal variation, however, is unlikely to have caused the observed fluctuation in 20:5ω3 levels, as the temperature within the sea ice habitat remains at -1.86 ± 0.2°C

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Table IV. Fatty acid composition of sea ice algal samples collected during the 1989 spring bloom from Erebus Ice Tongue and Cape Royds in McMurdo Sound

	Percentage composition			
Sample data:	22 Nov	22 Nov	24 Nov	25 Nov
Sample code:	14WC	11WC	23FL	18WC
Location:	EIT	CR	CR	CR
Species composition:	Mixed	*P.pseud.*	*P.pseud.*	*P.pseud.*
Fatty acid				
14:0	14.8	7.6	6.3	1.3
15:0	0.2	0.3	0.2	0.3
16:0	12.9	17.9	13.8	17.8
18:0	0.1	0.3	0.5	0.2
Σ Saturates:	28.0	26.1	20.8	19.7
16:1ω7c*	19.5	28.8	34.5	31.0
16:1ω5c	tr	0.1	0.1	0.1
16:1ω13t	1.6	1.0	0.2	1.2
18:1ω9c	0.5	0.9	2.5	0.6
18:1ω7c	0.1	0.1	0.3	0.1
20:1	-	tr	tr	-
Σ Monounsat.:	21.7	30.8	37.6	33.2
16:2ω4	4.3	2.9	2.2	3.2
16:2ω7	0.5	0.2	-	0.4
16:3ω4#	6.9	3.2	2.4	3.2
16:4ω1	10.4	7.0	7.2	7.2
18:2ω6	1.3	1.3	0.9	1.1
18:3ω3	0.1	0.3	0.4	0.3
18:3ω6	0.6	0.5	0.5	0.2
18:4ω3	5.1	8.7	6.2	8.5
20:2ω6	-	-	tr	-
20:3ω6	-	-	tr	-
20:4ω3	0.1	0.7	0.8	0.6
20:5ω3##	20.8	17.2	20.0	18.9
22:6ω3	0.1	1.1	1.1	0.6
Σ PUFA:	50.2	43.1	41.6	47.2
Total:	100.0	100.0	100.0	100.0

Legend: WC = Whole cell; MF = Membrane fraction; FL = Free lipid fraction; EIT = Erebus Ice Tongue; CR = Cape Royds; tr = < 0.1%; *P.pseud.* = *Porosira pseudodenticulata;* * = also includes small amounts of co-eluting 16:2ω4; # = Also includes small amounts of co-eluting 16:2ω7; ## also includes small amounts of co-eluting 20:4ω6

during the sample period.

The levels of 22:6ω3 were low (between 0.4 and 1%) for all three sites, but within the range found for pure cultures of other diatom species (Volkman *et al.* 1989, Nichols *et al.* 1986, Gillan *et al.* 1981, Kates & Volcani 1966).

A trend was also noted between the levels of 16:0 and 16:1ω7c from the Cape Armitage samples, where the ratio of 16:0/16:1ω7c remained relatively constant over the bloom period sampled.

Table V. Lipid composition of sea ice algal samples collected during the 1989 spring bloom from Cape Armitage in McMurdo Sound.

	mg g^{-1} dry wt					
Sample data:	8 Nov			25 Nov		
Sample code:	1MF	1FL	3WC	22MF	21FL	19WC
Phytol	0.66	tr	0.01	0.55	0.05	0.24
$C_{25:2}$ Hydrocarbon (x1000)	-	2.85	4.28	1.49	0.40	0.01
Total fatty acids	8.55	6.56	0.12	5.93	21.7	3.01
Total sterols	0.58	0.01	tr	0.35	0.36	0.05
Total lipid (mg g^{-1} dry wt)	98	66	1.34	68.3	221	32.9
TFA/phytol	13.0	6565	20.5	10.9	463	12.7
$C_{25:2}$HC/phytol	-	4.00	0.71	tr	0.01	tr
Total sterols/phytol	0.89	16.0	0.57	0.65	7.75	0.19
TFA/sterols	14.7	574	36.0	16.7	59.7	66.3

N.B. Lipid abundance data is expressed as mg g^{-1} dry wt of each cell fraction. Data represents the sum of component areas from gas chromatograms. Legend: WC = Whole cell; MF = Membrane fraction; FL = Free lipid fraction; TFA = Total fatty acids; $C_{25:2}$HC = $C_{25:2}$ Isoprenoid hydrocarbon; tr = < 0.01 mg g^{-1}.

Lipid abundance

The relative concentration of phytol (the isoprenoid side chain of chlorophyll) from the Cape Armitage community increased in whole cell samples from 8–25 November implying that there was an increase in biomass between these two dates. Phytol, and therefore chlorophyll, was concentrated in the membrane fraction (Table V). The concentration of total sterols also followed this general trend, implying there was no marked increase in sterol biosynthesis by the diatoms in the sea ice communities between 8–25 November. There was, however, a change in the intracellular sterol distribution. Early in the bloom (8 November) sterols were concentrated in the membrane fraction of cells. By 25 November the proportion of sterols in the free lipid fraction had increased significantly to an almost equal proportion to that of the membrane fraction. The proportion of total fatty acids in whole cell samples increased sharply from 8–25 November. This is consistent with a rise in the assimilation of carbon into storage lipid (triacylglycerols) and is supported by the marked increase in fatty acid concentration within the free lipid fraction (containing triacylglycerols) of the diatom community on 25 November when compared to 8 November (Table V). A similar pattern was observed by Nichols *et al.* (1989) when studying the 1985 Cape Armitage diatom community, although the increase in storage lipid occurred somewhat later in the season, from 7–13 December.

In conclusion, significant changes in the lipid composition of sea ice algal communities can be found that may relate directly to changes in environmental conditions and thus physiological status. Analysis of individual lipid classes (sterols, fatty acids and hydrocarbons) has yielded information which is important for studies of the chemotaxonomy, microbial ecology and organic geochemistry of diatom communities. Sea ice diatoms

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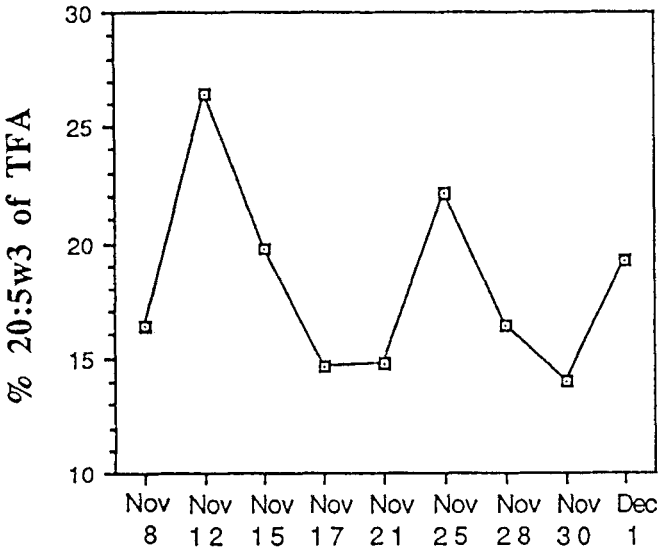

Fig. 4. Variation in the percentage of 20:5ω3* of total fatty acids in the Cape Armitage diatom community from 8 November to 1 December (* also included small amounts of co-eluting 20:4ω6).

have been demonstrated to contain high levels of essential PUFA, together with other lipid components of organic geochemical and taxonomic significance. The results of this study, therefore, lend further support to the hypothesis that sea ice diatoms play a much wider role in marine ecosystems than was previously thought.

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References

Bunt, J.S. 1963. Diatoms of Antarctic sea ice as agents of primary production. *Nature*, **14**, 197-200.

Bligh, E.G. & Dyer, W.J. 1959. A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911–917.

Dunkelblum, E., Tan, S.H. & Silk, P.J. 1985. Double-bond location in monounsaturated fatty acids by dimethyl disulfide derivatization and mass spectrometry. *Journal of Chemical Ecology*, **11**, 265-277.

Gillan, F.T., McFadden, G.I., Wetherbee, R. & Johns, R.B. 1981. Sterols and fatty acids of an Antarctic sea ice diatom *Stauroneis amphioxys*. *Phytochemistry*, **20**, 1935-1937.

Grossi, S.M., Kottmeier, R.L., Moe, G.T., Taylor, G.T. & Sullivan, C.W. 1987. Sea ice microbial communities VI. Growth and production in bottom ice under graded snow cover. *Marine Ecology-Progress Series*, **35**, 153-64.

Huang, W.Y. & Meischein, W.G. 1979. Sterols as ecological indicators. *Geochimica et Cosmochimica Acta*, **43**, 739-745.

Kanazawa, A., Teshima, S.I. & Kazuo, O. 1979. Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. *Comparative Biochemistry and Physiology*, **63B**, 295-298.

Kates, M. & Volcani, B.E. 1966. Lipid components of diatoms. *Biochimica et Biophysica Acta*, **116**, 264-298.

Kottmeier, S.T. & Sullivan, C.W. 1990. Bacterial biomass and production in pack ice of Antarctic marginal ice edge zones. *Deep-Sea Research*, **37**, 1311–30.

Matsumo, G., Torii, T. & Hanya, T. 1982. High abundance of algal 24-ethylcholesterol in Antarctic lake sediment. *Nature*, **299**, 52-54.

Nichols, P.D., Guckert, J.B. & White, D.C. 1986a. Determination of monounsaturated fatty acid double-bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyl disulphide adducts. *Journal of Microbiological Methods*, **5**, 49-55.

Nichols, P.D., Palmisano, A.C., Smith, G.A. & White, D.C. 1986b. Lipids of the Antarctic sea ice diatom *Nitzschia cylindrus*. *Phytochemistry*, **25**, 1649-1653.

Nichols, P.D., Palmisano, A.C., Rayner, M.S., Smith, G.A. & White, D.C. 1989. Changes in the lipid composition of Antarctic sea ice diatom communities during a spring bloom: an indication of community physiological status. *Antarctic Science*, **1**, 133-140.

Nichols, P.D., Palmisano, A.C., Rayner, M.S., Smith, G.A. & White, D.C. 1990. Occurrence of novel C_{30} sterols in Antarctic sea ice diatom communities during a spring bloom. *Organic Geochemistry*, **15**, 503-508.

Nichols, P.D., Volkman, J.K., Palmisano, A.C., Smith, G.A. & White, D.C. 1988. Occurrence of an isopreniod C_{25} diunsaturated alkene and high neutral lipid content in Antarctic sea ice diatom communities. *Journal of Phycology*, **24**, 90-96.

Orcutt, D.M. & Patterson, G.W. 1975. Sterol, fatty acid and elemental composition of diatoms grown in chemically defined media. *Comparative Biochemistry and Physiology*, **50B**, 579-583.

Orcutt, D.M., Parker, B.C. & Lusby, W.R. 1986. Lipids in blue-green algal mats (modern stromatolites) from Antarctic sea ice oasis lakes. *Journal of Phycology*, **22**, 523-530.

Palmisano, A.C., Lizotte, M.P., Smith, G.A., Nichols, P.D., White, D.C. & Sullivan, C.W. 1988. Changes in photosynthetic carbon assimilation in Antarctic sea ice diatoms during spring bloom: variation in synthesis of lipid classes. *Journal of Experimental Marine Biology and Ecology*, **116**, 1-13.

Palmisano, A.C. & Sullivan, C.W. 1983. Sea ice microbial communities (SIMCOs) 1. Distribution, abundance, and primary production of ice microalgae in McMurdo Sound 1980. *Polar Biology*, **2**, 171-177.

Palmisano, A.C. & Sullivan, C.W. 1985. Pathways of photosynthetic carbon assimilation in sea ice microalgae from McMurdo Sound, Antarctica. *Limnology and Oceanography*, **30**, 674-678.

Rowland, S.J. & Robson, J.N. 1990. The widespread occurrence of highly branched acyclic C_{20}, C_{25} and C_{30} hydrocarbons in recent sediments and biota - a review. *Marine Environmental Research*, **30**, 191-216.

Sullivan, C.W. 1978. Isolation and characterisation of diatom membranes. *In* Hellebust, J.A. & Craigie, J.S. *eds. Handbook of Phycological Methods Vol. III. Physiological and Biochemical Methods*. New York: Cambridge University Press, 39-55.

Vincent, W.F. 1988. *Microbial ecosystems of Antarctica*. Cambridge: Cambridge University Press, 304 pp.

Volkman, J.K., Jeffery, S.W., Nichols, P.D., Rogers, G.I. & Garland, C.D. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology*, **128**, 219-240.

Whitaker, T.M. & Richardson, M.G. 1980. Morphology and chemical composition of a natural population of an ice-associated Antarctic diatom *Navicula glaciei*. *Journal of Phycology*, **16**, 250-257.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D. & Bobbie, R.J. 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. *Oecologia*, **40**, 51-62.