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Triacylglycerol Fatty Acid and Sterol Composition of Sediment Microorganisms from McMurdo Sound, Antarctica*

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Summary. The triacylglycerol fatty acid and sterol profiles of microorganisms from three McMurdo Sound sediment sites, collected during the austral summer of 1984–1985, were determined using gas chromatography and gas chromatography-mass spectrometry. Comparison of the three sites indicated that Cape Evans contained the greatest concentration of triacylglycerol (TG) (220 nmoles/gram dry weight (gdw) of sediment), approximately six to seven times that determined for sediment microorganisms from the Cape Armitage and New Harbor sites. The relative proportion of triacylglycerol-derived polyunsaturated fatty acids (PUFA) revealed a somewhat different trend. New Harbor sediment contained the greatest relative proportion of PUFA (22% of triacylglycerol fatty acids), followed by Cape Evans (16%) and Cape Armitage (11%). The proportion of unsaturated fatty acids (poly- and monounsaturated) was relatively constant and ranged from 63% to 71% of the triacylglycerol fatty acids for the three sites. Sterol concentrations varied from 610 pmoles/gdw at Cape Evans, to 370 and 240 pmoles/gdw for Cape Armitage and New Harbor respectively, and was approximately 1% of the total determined lipid. Cholesterol was the major sterol component detected, occurring at similar relative levels (29%) for all three sites. Other sterols present in decreasing order of abundance were 22-dehydrocholesterol, brassicasterol, 24-ethylcholesterol and 24-methylcholesterol. 5α-stanols were only minor components of the three sediments, indicating that in situ biohydrogenation of stenols was not a major sterol transformation process in these recent surface oxic sediments.

Introduction

Several recent reports of microbial lipid profiles have focused on organisms from extreme environments. Microbial assemblages of deep sea trenches (Baird and White 1985) and subsurface aquifers (White et al. 1983a; Smith et al. 1986a) have been described by their fatty acid composition. Lipid compositions of thermophilic (Langworthy 1979; Brock 1978), methanotrophic (Nichols et al. 1985a), methanogenic (Mancuso et al. 1985), halophilic (Kushner et al. 1964; Kates 1978), sulfate-reducing (Taylor and Parkes 1983; Dowling et al. 1986), as well as psycrophilic organisms (Gillan et al. 1981; Nichols et al. 1985b; White et al. 1986; Smith et al. 1986b) have also been reported. Signature lipid data from such investigations have proven useful in identifying and quantifying the presence of these and other microbial assemblages of less extreme environments (Bobbie and White 1980; White et al. 1979; White 1983b; Perry et al. 1979).

Studies noted above have been accomplished following recent advances in gas chromatography (GC) and GC-mass spectrometry (GC/MS) (Boon et al. 1977; Dunkelblum et al. 1985; Tunlid and Odum 1986; Nichols et al. 1986). Such instrumentation when used with appropriate techniques and derivatization procedures can provide an accurate measurement of lipid components, and can readily be used as a taxonomic tool (White 1983b; Lechevalier 1977). We have previously reported phospholipid ester-linked fatty acid profiles and rates of ^{3}H-thymidine and ^{14}C-acetate incorporation by benthic marine microorganisms from McMurdo Sound, Antarctica (Smith et al. 1986b). Together these biochemical assays provided a quantitative and reproducible measure of the biomass, community structure and metabolic activity of representative McMurdo Sound sediments.

Previous studies of neutral lipid components from Antarctic marine environments have been limited for the most part to midwater marine fish and invertebrates (Reinhardt and Van Vleet 1984a, b). Recently Volkman et al. (1987) determined the lipid composition of algal and bacterial communities in a freshwater lake from the Vestfold Hills area of Antarctica. Sterol biomarkers have also been determined for several freshwater lake sedi-

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ments from three areas in the Antarctic, Syowa Oasis, Vestfold Oasis and the Dry Valley area (Matsumoto et al. 1983; Orcutt et al. 1986). In this study we report the detailed TG fatty acid and sterol profiles of three benthic sediments from McMurdo Sound, Antarctica. Evident from these analysis is the enhanced proportions of unsaturated fatty acids derived from TG. This is proposed as a microbial metabolic response providing increased fluidity of the neutral lipid storage components at low temperature.

To our knowledge this study presents the first determination of neutral lipid components from marine sediment microorganisms within the Ross Sea Antarctic region. These data are presented to provide a base for future food-web, microbial ecology and geochemical studies in this unique environment.

Materials and Methods

Solvents were all of residue analysis quality or better (J.T. Baker Chemical Co., Phillipsburg, NJ.). Standards and derivitization reagents were purchased from Supelco (Bellefonte, PA.); Applied Science Co. (State College, PA.) and Sigma Chemical Co. (St. Louis, MO.).

Sediment samples were collected from three sites in McMurdo Sound, Antarctica (Fig. 1). Two sites were on the more productive East Sound (Dayton and Oliver 1977), one at Cape Armitage (CA) and the other at Cape Evans (CE). The third site was located on the West side of the Sound at New Harbor (NH), and was a less productive area thought to resemble a deep sea sediment (Dayton and Oliver 1977) (Fig. 1). Triplicate sediment samples were collected for each site between 23 November to 22 December, 1984. A more detailed site description is provided in Smith et al. (1986b). Ice algal and *Phaeocystis* sp. blooms occur to varing degrees each austral summer in late December to early January, and are believed to be a major input of carbon to the benthos at certain Sound sites (Palmisano and Sullivan 1983; Palmisano et al. 1986). Sediments were collected before the algal blooms by SCUBA divers using plastic coring tubes at water depths of 14.5 m to 30.5 m. Care was taken to recover sediments intact without mixing of the layers. Transportation to the Eklund biological laboratory, sealed and in seawater at ambient temperature, was accomplished within 1 to 1.5 h from time of coring. The cores were then extruded and the top 2 cm was washed through a 500 µm mesh sieve into a 250 ml stainless steel centrifuge can. The sieving served to prevent any large meiofauna from being extracted and biasing the lipid analysis, as well as providing some consistency in sediment grain size.

Lipid Extraction and Fractionation

The collected sediment was centrifuged at 10000×g, for 10 min. The water phase was discarded and the sediment pellet extracted in a 250 ml centrifuge can using a single phase methanol:chloroform 2:1 (v/v) modification of the Bligh and Dyer (1959) technique (Guckert et al. 1985). The total lipid was roto-evaporated to dryness, transferred to teflon-lined screw capped test tubes and dried under nitrogen. At this point the dried lipid was stored at −70 °C until transported on dry ice to Florida State University for analysis. Details of the lipid extraction procedures are given in Smith et al. (1986b). The dried lipid was dissolved in chloroform and transferred with (3×) 200 µl washes, onto a 1 g column of dried and activated silicic acid (Unisil 100–200 mesh, Clarkson Chemical Co., Inc., Williamsport, Pa.). The neutral lipid fraction was collected by eluting the column with 10 mls of chloroform, dried under a steam of nitrogen, and then transferred in hexane with (3×) 200 µl washes onto a second 1 g column of silicic acid. Hydrocarbons, triacylglycerols, and free sterols were eluted with consecutive

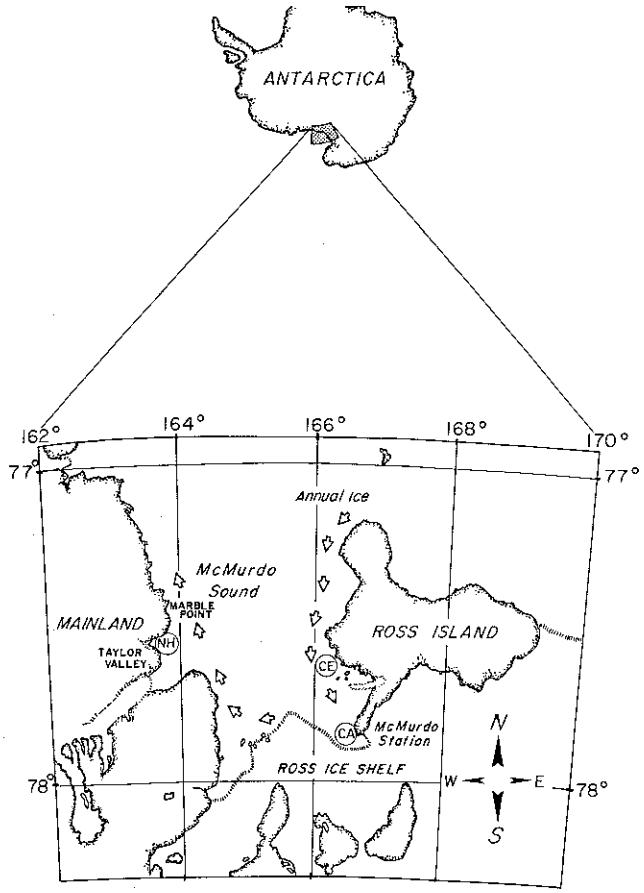

Fig. 1. McMurdo Sound, Antarctica, sampling sites. Cape Armitage (*CA*), Cape Evans (*CE*) and New Harbor (*NH*). *Arrows* indicate current direction

10:20:10 ml washes of hexane, hexane diethylether 9:1 (v/v), and hexane diethylether 8:2 (v/v) respectively (Kates 1986). Triacylglycerol fatty acids were transesterified to their corresponding methyl esters using a 0.2 M KOH in methanol solution (Gluckert et al. 1985).

Gas Chromatography

Fatty acid methyl esters (FAME) and sterols were taken up in hexane with methylnonadecanoate (19:0) as the internal injection standard. Initial identification of individual FAME and sterols was performed by high resolution gas chromatography using a Hewlett Packeard 5880A gas chromatograph equipped with a flame ionization detector. A detailed description of GC conditions and peak identification procedures has been previously reported (Smith et al. 1986b). Tentitave peak identification, prior to GC-MS analysis, was based on comparison of retention times with those obtained for commercial and previously identified laboratory standards. Elution order of detected sterols using these methods and instrumentation are shown in Fig. 2. Peak areas were quantified using a Hewlett Packard 3350 series programmable laboratory data system operated in an internal standard program. Fatty acid and sterol compositional data reported for these samples is the mean of three analysis.

Gas Chromatography-Mass Spectrometry

GC-MS analyses of FAMES (Table 1) and sterols (Table 2) were performed on a Hewlett Packard 5996A system fitted with a direct capillary inlet. Samples were injected in the splitless mode at 100 °C with an 0.5 min venting time after which the oven was programmed to 300 °C at either 3° or 4 °C/min. Helium was used as the carrier gas. MS

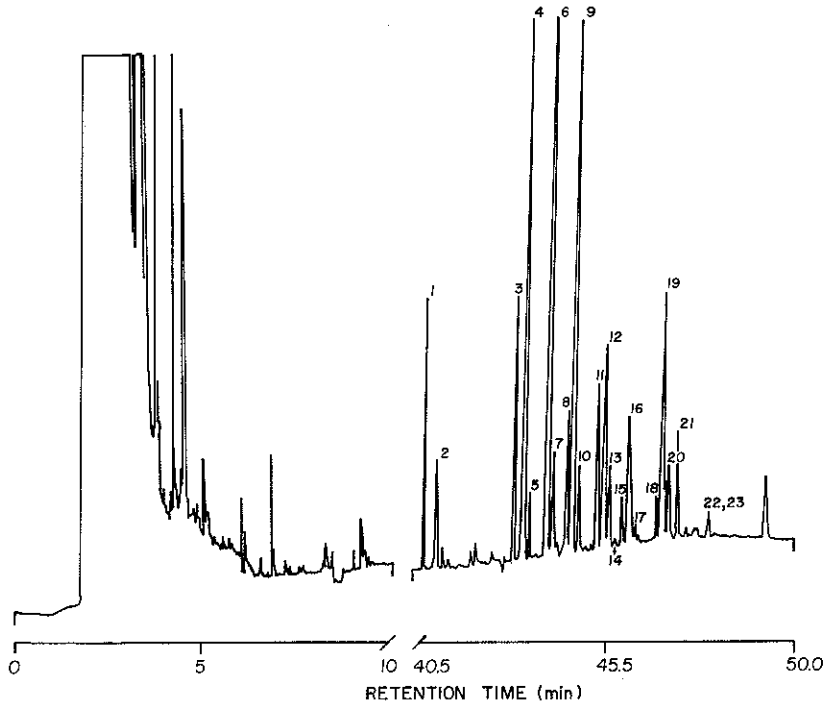

Fig. 2. Gas chromatographic trace of detected sterols indicating relative amounts. Numbers refer to sterols described in Table 5

operating parameters were: electron multiplier between 1300 to 1400 volts, transfer line 300 °C, source and analyzer 250 °C, autotune file DFTPP normalized, optics tuned at m/z 502, electron impact energy = 70 eV. Mass spectral data were aquired and processed using an HP-1000 series computer with version RTE-6/VM data system.

The dimethyldisulfide (DMDS) adducts of monounsaturated FAME were formed, using the method of Dunkelblum et al. (1985) as modified by Nichols et al. (1986) to locate the double bond positions (Table 1). GC-MS analysis of the DMDS adducts showed major ions attributable to fragmentation between the two CH_3S groups located at the original position of unsaturation. Discrimination between cis and trans geometry of the double bond in the original monoenoic FAME was possible. The erythro isomer (originally the trans acid) eluted after the threo isomer (originally the cis acid). The different positional isomers of the same geometry were chromatographically separated under the GC conditions used in this study.

Nomenclature

Fatty acids are designated as total number of carbon atoms: number of double bonds followed by the position of the double bond from the ω (aliphatic) end of the molecule. The suffixes c and t indicate cis and trans geometry. Cyclopropyl fatty acids are designated with the prefix cy, and iso and anteiso branched fatty acids are indicated with the prefixes i and a. Sterols are designated by Chemical Abstracts Index substace name, and their diagnostic ions are presented in Table 2.

Table 1. Characteristic ion fragments of adducts formed following reaction of monounsaturated fatty acid methyl esters with dimethyldisulphide

Fatty acid	Diagnostic ions (m/z)		
	M^+	Δ^a	ω^b
16:1w7c	362	217	145
16:1w5c	362	245	117
i17:1w7c	376	217	159
17:1w6c	nd[c]	245	131
18:1w9c	390	217	173
18:1w7c	390	245	145
20:1w9c	418	245	173
20:1w7c	nd	273	145

[a] Δ: double bond position from carboxylic end of molecule
[b] ω: double bond position from aliphatic end of molecule
[c] nd: not detected

Results

Unsaturated fatty acids from the TG of Antarctic sediment microorganisms were found to be a major component of the determined lipids. Relative proportions of unsaturated fatty acids measured in the TG fraction were typically 60% or greater. Monounsaturated components averaged 50% and polyunsaturated 17% of the total TG

Table 2. Characteristic ions of major sterol components from three McMurdo Sound, sediments

Sterol[a]	Mass spectral data	
	MW[b]	Major ions detected (m/z)[c]
cholesta-5,22E-dien-3β-ol (*trans*-22-dehydrocholesterol)	456	69(100), 111(75), 129(70), 255(71), 327(86), 351(39), 366(64), 441(11), 456(18)
cholest-5-en-3β-ol (cholesterol)	458	129(54), 145(29), 225(26), 329(100), 353(44), 368(76)
24-methylcholesta-5,22E-dien-3β-ol (brassicasterol)	470	55(68), 69(92), 73(67), 81(43), 129(61), 159(35), 255(50), 341(23), 380(43)
24-methylcholest-5-en-3β-ol (24-methylcholesterol)	472	55(48), 73(55), 81(33), 105(27), 107(30), 129(51)
24-ethylcholest-5-en-3β-ol (24-ethylcholesterol)	486	43(45), 55(36), 73(59), 75(30), 129(56)

[a] Trivial name in parentheses
[b] Molecular weight of TMSi ether derivative
[c] m/z (relative intensity)

Table 3. Triacylglycerol fatty acid profiles of recent sediments from sites at Cape Armitage (CA), Cape Evans (CE) and New Harbor (NH), McMurdo Sound, Antarctica

Fatty acid	Percent composition[b]		
	CA[a]	CE	NH
14:0	2.2	3.6	6.1
i 15:0	nd[c]	0.5	nd
a 15:0	0.7	0.7	nd
15:1	nd	0.5	nd
15:0	0.8	0.8	nd
i 16:0	2.1	0.2	1.2
16:1w9c	2.3	0.7	0.8
16:1w7c	25.8	36.9	32.0
16:1w7t	nd	0.8	nd
16:1w5c	1.4	0.7	1.0
16:1w13t	nd	0.3	0.7
16:0	19.6	21.0	17.2
i 17:0	2.5	0.1	0.8
17:0	0.9	0.9	1.2
17:1w6c	0.7	0.4	nd
cy 17:0	nd	0.3	nd
17:0	nd	0.4	nd
18:3w6	nd	nd	2.0
18:4w3	1.3	1.8	3.2
18:2w6	nd	1.0	2.9
18:3w3	1.6	0.4	0.8
18:2w3	nd	0.4	nd
18:1w9c	5.8	4.2	4.9
18:1w7c	9.5	4.0	6.9
18:1w7t	0.8	0.3	nd
18:1w5c	1.3	0.2	nd
18:0	6.4	2.0	2.6
20:4w6	0.9	0.4	2.6
20:5w3	7.8	9.2	10.7
i 20:0	nd	0.4	nd
20:1w9c	0.9	0.8	0.6
20:1w7c	2.0	0.5	1.8
20:0	0.7	0.3	nd
21:0	nd	0.3	nd
22:0	1.5	1.2	nd
22:6w3	nd	1.4	nd
22:4w6	nd	0.2	nd
22:5w3	nd	1.5	nd
24:0	nd	0.1	nd
Total unidentified[d]	0.5	0.6	nd
Total fatty[e] acids (nmoles/gdw)	20.1	222.8	32.1

[a] Mean of three replicates
[b] % mole composition
[c] nd: not detected
[d] Total of unidentified fatty acids (mostly C16 PUFA)
[e] Total of all fatty acids detected in nmoles per gram dry weight

for the three sites (Table 4). The absolute abundance of TG fatty acids from the three sediments sites indicated a similar pattern to that of reported phospholipid (Smith et al. 1986b). Cape Armitage was the exception to this phospholipid pattern having the lowest relative amount at 20 nmoles/gdw, followed by New Harbor at 32 and Cape Evans at 223 nmoles/gdw (Table 3). Conversely the membrane derived phospholipid fatty acids determined by a previous study indicated that both sites on the East Sound side (CA and CE) were present in greater abun-

Table 4. Composition of triacylglycerol fatty acids of recent sediments from Cape Armitage (CA), Cape Evans (CE) and New Harbor (NH), McMurdo Sound, Antarctica

	Percentage composition[a]		
	CA	CE	NH
Total sat.[b]	31.5	29.4	25.9
Total monounsat.	51.0	50.0	49.0
Total polyunsat.	11.6	16.3	22.2
Total unsat.[c]	62.6	66.3	71.2
Total other[d]	5.0	0.4	3.2
Total unsat. FA's[e] sum TG+PL	47.0	74.6	84.0

[a] Percent composition of triacylglycerol fatty acids
[b] Total of saturated fatty acids
[c] Total of all mono- and poly-unsaturated fatty acids
[d] Total of all cyclopropyl and branched fatty acids
[e] Ratio of triacylglycerol unsaturated FAME to sum of triacylglycerol and phospholipid fatty acids (phospholipid data from Smith et al. 1986b)

Table 5. Sterol profiles of recent sediments from Cape Armitage (CA), Cape Evans (CE) and New Harbor (NH), McMurdo Sound, Antarctica

Peak no.[b]	Sterol	Percentage composition[a]		
		CA	CE	NH
1	22-trans-24-norcholesta-5,22-dien-3β-ol	3.6	3.7	6.7
2	5-α-22-trans-24-norcholest-22-en-3β-ol	2.5	0.3	1.1
3	cholesta-5,22Z-dien-3β-ol	5.0	2.0	2.2
4	cholesta-5,22E-dien-3β-ol[c]	12.2	17.1	20.2
5	5α-cholest-22E-en-3β-ol	1.1	0.9	3.5
6	cholest-5-en-3β-ol[c]	25.9	34.8	26.7
7	5α-cholestan-3β-ol	2.4	2.1	3.4
8	cholesta-5,24-dien-3β-ol	3.5	1.8	1.3
9	24-methylcholesta-5,22E-dien-3β-ol[c]	14.2	12.0	7.6
10	5α-24-methylcholest-22E-en-3β-ol	2.1	1.0	1.4
11	unknown[d]	3.1	1.5	0.8
12	24-methylcholesta-5,24(28)E-dien-3β-ol	4.3	6.4	5.6
13	24-methylcholest-5-en-3β-ol[c]	2.1	3.0	2.2
14	5α-24-methylcholestan-3β-ol	nd	1.0	1.2
15	23,24-dimethylcholesta-5,22E-dien-3β-ol	1.1	0.9	nd
16	24-ethylcholesta-5,22E-dien-3β-ol	4.2	1.8	3.2
17	5α-24-ethylcholest-22E-en-3β-ol	0.5	0.6	nd
18	23,24-dimethylcholest-5-en-3β-ol	1.0	0.5	nd
19	24-ethylcholest-5-en-3β-ol[c]	5.7	5.6	9.0
20	5α-24-ethylcholestan-3β-ol	1.9	1.2	1.7
21	unknown[d]	2.2	0.9	0.9
22	unknown[d]	{1.4	0.6	1.3
23	unknown[d]			
24	unknown[d]	nd	0.4	nd
Total sterols[e]		370	610	240
Stanol/stenol[f]		0.13	0.08	0.14

[a] Percent sterol component from the total
[b] Numbers refer to peaks in Fig. 2
[c] Identified by GC-MS ions; all other peaks identified by GC retention times with known standards
[d] Unknown sterol
[e] Total of sterols in pmoles/gdw
[f] Ratio of stanols to stenols

dances compared to (NH) on the West (Smith et al. 1986b). Proportions of unsaturated TG fatty acids were greater at New Harbor with high abundances of several mono- and polyenoic C_{18} and C_{20} acids. Noted were the greater proportions of: 18:3w6, 18:4w3, 18:2w6, 20:4w6 and 20:5w3 (Table 3). New Harbor contained 22% of these acids while Cape Armitage and Cape Evans contained only 10 and 12%, respectively. Polyenoic acids of C_{22} (22:6w3, 22:4w6, and 22:5w3) were of greater abundance at Cape Evans (3%), while they were below detection at the other two sites.

Sterol abundances more closely resembled the previously determined phospholipid pattern, with the East Sound sites containing higher relative amounts (Table 5). Cholesterol was clearly the dominant sterol component averaging 29% of all sterols detected. The remaining sterols were found to be in average proportions of: tran 22-dehydrocholesterol 17%, brassicasterol 11%, and 24-ethylcholesterol 7% for the three sites (Table 5). Sterols accounted for approximately 1% of the detected lipid components (phospholipid, triacylglycerol and sterol). Compounds eluting immediately after identified sterols were in too low abundance (approximately 4% of sterols) to be positively identified by GC/MS. These compounds are tentatively identified as sterols by comparisons of retention times with standards and diagnostic ions detected in sufficient abundance (Table 5).

Discussion

Recent investigations have reported relatively high proportions of TG for organisms in polar regions indicating neutral lipid components are important energy reserves maintained within these organisms. Values of 25% to 75% of the total lipid during a 12 day North Atlantic phytoplankton bloom were determined to be contributed by fatty acids from TG (Morris 1984). Neutral lipids accounted for as much as 54% – 77% (during a summer bloom) of the total ^{14}C-labelled lipid when $NaH^{14}CO_3$ was incorporated by a sea-ice diatom community from McMurdo Sound (Palmisano et al. 1988). In addition, analysis of a monoalgal culture of an Antarctic ice diatom *Nitzschia cylindrus* from McMurdo Sound indicated abundances of neutral lipid fatty acids at 34% of the total fatty acids (Nichols et al. 1985b). Volkman et al. (1986a) likewise determined high TG content in the calinoid copepod *Paralabidocera antarctica* from an Antarctic saline lake. Reinhardt and Van Vleet (1984a, b) determined TG from mid-water fish and invertebrates from Croker Passage on the Antarctic peninsula to be approximately 42% and 51%, respectively, of the total lipid. Neutral lipid components (i.e. triacylglycerol and sterol) from McMurdo Sound in this study accounted for an average 65% of the determined lipids (phospholipid, triacylglycerol and sterol) for the three sites. This investigation aid in substantiating the fact that organisms inhabiting low temperature environments contain relatively large amounts of neutral lipids. Within this large neutral lipid fraction are high proportions of unsaturated fatty acids from the TG, indicating adaptive strategies by organisms in these environments to provide fluidity for metabolic processes (Cossins and Prosser 1978). Observations by Marr and Ingrahm (1962) have agreed with this and even submitted that temperature and it's effect upon lipids may set the limits for microbial growth.

The occurrence of 22-dehydrocholesterol and brassicasterol (Table 5) are believed to be contributed by diatoms. These two sterols have been identified as major components of sea-ice diatoms from the Antarctic area. Nichols et al. (1985b) determined 22-dehydrocholesterol to be 66% of the sterol composition in the sea-ice diatom *Nitzschia cylindrus* and Gillan et al. (1981) determined brassicasterol composition of 79% in a similar sea-ice diatom *Stauroneis amphioxyes*. Although these are sea-ice species, they are believed to contribute to the benthic near shore flora upon seasonal ice ablation (Palmisano and Sullivan 1983). We therefore propose that the amount and species of sterols determined in this study of sediments from McMurdo Sound are indicative of a contribution by sea-ice algae. This however seems to be a seasonal occurrence coinciding with the ice-algal bloom and ice ablation during the late Austral summer season. In addition to the sea-ice algal input to the benthos, a seasonal water column algal bloom of *Phaeocystis* sp. (Palmisano et al. 1986), with a brassicasterol content of 67% (unpublished data), occurs in varying amounts on a seasonal basis at McMurdo Sound near-shore sites. Evidence of the Sound's current regime and the contribution of the *Phaeocystis* sp. to the benthos is indicated by the proportions of brassicasterol at East Sound sites of Cape Evans (12%) and Cape Armitage (14%), compared to New Harbor (8%) on the West (Table 5). Coincidently, indications of a greater overall microeucaryotic sediment community at Cape Evans is evidenced by the higher relative abundance of TG (81%) and sterols (50%) of the means at this site.

The sterol 22-dehydrocholesterol has also been found by Gillan et al. (1981) to be a major component of other diatom species. Matsumoto et al. (1982) determined high proportions of 24-ethylcholesterol in dry valley, Antarctic lake sediments and contributed its presence to epibenthic algae, mostly blue-greens. This sterol is also found in high proportions in terrigenous organic matter (Volkman 1986b), but this source can be excluded in the interpretation of data obtained from recent marine sediments from Antarctica. Studies by Orcutt et al. (1986) have recently reported both brassicasterol and 24-ethylcholesterol in relatively high proportions from several Dry Valley, Antarctic lakes. They proposed that blue-green algae were the probable major source of 24-ethylcholesterol to the lake sediments. In this study microscopic examination of fresh unpreserved sediment samples failed to reveal the presence of blue-green algal cells. Similarly blue-green algae were not present in Antarctic sea-ice diatom communities (Nichols et al. 1988) where 24-ethylcholesterol was a major component (unpublished data). A growing

number of reports have documented the presence of 24-ethylcholesterol in unicellular eucaryotic algae (Volkman 1986b; Volkman et al. 1988). The data presented here again support the view that care should be taken in the use of 24-ethylcholesterol as a marker for terrigenous input, and caution is needed in the utilization of this sterol as an indication of blue-green algae.

The percentage of stanols present (Table 5) may be contributed by viable phytoplankton and zooplankton in these recent sediments (Nishimura and Koyama 1977). Our analysis determined similar stanol percentages among the three sites in McMurdo Sound (Table 5). Hence, these data provide information on the presence of small amounts of stanols in the natural population of Antarctic water column and benthic organisms. In addition, high amounts of stenols in surface sediments are indicative of viable organisms, since stenol hydrogenation is known to occur rapidly in young microbially active sediments (Nishimura and Koyama 1977). There is at this time no clear evidence to indicate reduced hydrogenation activity in low temperature environments. At the present time, no literature is available on the lipid, particularly sterol, composition of Antarctic benthic marine algae. Until such data is reported, it will not be possible to determine completely the relative contributions of the potential TG and sterol sources to recent near-shore sediments.

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