

Glen A. Smith¹**, John D. Davis¹, Ann M. Muscat², Richard L. Moe³ and David C. White¹

¹Institute for Applied Microbiology, University of Tennessee/Oak Ridge National Laboratory, 10515 Res. Dr. Suite 300, Knoxville, TN 37932-2567, USA

²California Museum of Science and Industry, 700 State Dr., Los Angeles, CA 90037, USA

³Department of Botany, University of California Berkeley, Berkeley, CA 94720, USA

Received 14 July 1988; accepted 3 March 1989

Summary. Benthic microbial communities of the Arthur Harbor area were described by analysis of their cell membrane phospholipid ester-linked fatty acids (PELFA) and metabolic rates. Analysis revealed a biomass averaging 6 nM (phospholipid) or 3.5×10^8 cells per gram dry weight (gdw) of sediment for the four sites. Only slight biomass differences were detected between the four peninsula sites. All Arthur Harbor sites were determined to have a biomass similar to the lowest amount reported for a previously described McMurdo Sound site at New Harbor. Community structure based on signature phospholipids indicated only slight differences between the four peninsula sites with greater relative amounts of diatom marker lipids at a deeper site. Bacterial biomarker lipids were also determined in relatively equal proportions for the four Arthur Harbor sites with only one site indicating a somewhat decreased proportion. Metabolic rates of sodium $[^{14}C]$ -acetate and methyl $[^{3}H]$ -thymidine incorporation into lipid and bacterial DNA respectively also indicated only slight relative differences in microbial communities of Arthur Harbor study sites. Lipid metabolism (¹⁴ C-acetate) ranged between 6 and 12 (\times 10⁴) DPM/g/h for the four sites with 8 being the average. Bacterial (excluding sulfate-reducing bacteria (SRB)) cell divisions per g per h indicated increased rates at a deeper site with 14×10^5 , compared to the average (5 × 10⁵) for the three remaining sites. Average estimated total bacterial (excluding SRB's) community turnover was on the order of 0.6%/h for the four sites. Metabolic rate comparisons of Arthur Harbor with those of previously determined McMurdo Sound indicated a somewhat increased lipid metabolism and an order of magnitude greater bacterial cell division rate at Arthur Harbor.

Introduction

Previous work by several investigators has demonstrated the utility of PELFA analysis in determining biomass and community structure of a variety of environments (Gillan 1981; Guckert et al. 1985; Nichols 1983; Smith et al. 1982a; White 1983; White and Findlay 1988). Phospholipid analysis enables one to determine the content in recent sediments of only those cells with potential viability, as cell death induces phospholipase activity with formation of neutral lipids (ie. free fatty acids and other products) from phospholipids. In addition such analysis avoids the bias encountered using methods which require quantitative culturing or microscopic analysis. Measurements of enzyme activity, muramic acid levels, total ATP, and respiratory activity have been found to correlate well with PELFA analysis (White et. al. 1979; White 1983; Balkwill et al. 1988).

Springer-Verlag 1989

With the recent advances in chromatographic instrumentation and small scale derivatization procedures, it has been possible to more accurately assign geometrical and stereochemical configuration to lipid components (Dunkelblum et al. 1985; Nichols et al. 1986b); Tunlid and Odum 1986). These and other technological improvements in instrumentation have resulted in a growing number of reported signature lipids identifying specific subgroups of microorganisms from various environments (Lechevalier 1977; Johns et al. 1979; Taylor and Parkes 1983; Sasser et al. 1984) further extending the usefulness of computer aided gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) analysis.

In this study of the near-shore benthic microbiota we have described the biomass, community structure and metabolic rates of four Arthur Harbor sites in their natural condition. However, this study deals only with the austral summer season and is believed to reflect the most productive season for this area (El-Sayed 1985; El-Sayed and Taguchi 1981). To our knowledge this represents the first quantitative study of the endemic microbiota and

^{*}This paper is part 4 in the series: Microbial Ecology in Antarctic Sea-Ice and Benthic Communities

^{**}Corresponding author

their metabolic activities for marine sediments from Arthur Harbor for any season. For this reason our report should be viewed as an initial determination of this vital region, and is intended to be a baseline study of an as yet unaltered ecosystem free of anthropogenic contamination.

Materials and Methods

Solvents were all of residue analysis quality or better (Burdick and Jackson Inc., Muskegon, MI). Standards and derivitization reagents were purchased from Supelco (Bellefonte, Pa); Applied Sciences Co. (State College, Pa) and Sigma Chemical Co. (St. Louis, Mo).

Sample Collection

Sediment samples were collected from four sites within Arthur Harbor on Anvers Island, Palmer Peninsula, Antarctica (Fig. 1). Hand held cores were recovered by SCUBA divers at near-shore sites ranging in depth from 10 to 26 m. Physiochemical seawater parameters for the four sites averaged: dissolved oxygen 11.3 mg/l; light 17.3 μ E/m²/s; salinity 32.9% temperature -0.5° C; pH 6.8. Sediment CHN analysis indicated C/N ratios ranging from 5 to 8, and sediment size fractionation indicated

between 77% to 91% passed through a 500 μ m sieve (Table 1). Care was taken to prevent mixing of sediment layers within the 2.5 cm diameter core by stopping both ends and maintaining in an upright position upon removing the core. Samples were immediately returned to the Palmer Station laboratory in ambient seawater. Cores were then sectioned recovering the top 2 cm, and sieved through a 500 μ m screen to exclude meio- and macrofauna from the sediment and extracted with chloroform:methanol:buffer. Extraction efficiencies of sediments using the techniques of Federle and White (1982) have been used here to provide quantitative recovery of lipids from sediments. Lipid extraction and separation (including GC and GC/MS) analysis has been reported in detail elsewhere (Smith et al. 1986). The peak area quantification from GC analysis was carried out on a Nelson 2600 analytical chromatography system, with version 4.0 software. Fatty acid compositional data reported for these samples are expressed as average mol.% of the total mole quantity for each replicate sediment analysis (N = 3, 2 DF) and are therefore relative, except for Tukey's tests which provides significance at the $\alpha = 0.05$ level.

Fatty Acid 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*



Fig. 1. Arthur Harbor study sites. AH(10) = Arthur Harbor at 10m, AH(13) = Arthur Harbor at 13m, ER = Elephant Rock at 26m, HI = Hero Inlet at 10m

Table 1. Sediment size fractionation and particulate organic carbon to particulate organic nitrogen (C/N) ratio for four Arthur Harbor Sites

Sieve size	Percent of sediment ^a						
(mm)	AH(10) ^b	AH(13)	ER	HI			
4.000	10.5	4.0	7.0	4.5			
2,800	1.6	1.4	1.2	3.5			
1,400	3.2	1.7	1.3	4.7			
1,000	2.0	1.5	0.8	2.1			
0.500	6.3	5.2	5.5	7.3			
0.250	9.7	5.1	11.5	8.6			
0.125	16.0	12.9	20.0	14.2			
0.063	48.7	59.1	44.8	51.6			
pan	2.0	9.1	7.9	3.5			
Ċ/N°	5	5	6	8			

^a Percentage (by weight) of sediment retained by sieve

^b Study sites Fig. 1

° Ratio of particulate organic carbon to particulate organic nitrogen

geometry. The prefixes i and a refer to *iso* and *anteiso* branching, respectively. Other methyl-branching is indicated as position from the carboxylic acid (Δ) end, i.e., 10Me16:0.

Thymidine Incorporation into Bacterial DNA

Cores were subsampled and 1 g wet weight amounts of surface material was added to 15 ml disposable centrifuge tubes with 50 μ Ci Methyl [³H]-thymidine (50–80 Ci/mmol) and 40 μ l of a 10 nM thymidine solution in filter sterilized (0.22 μ m, Millipore) seawater kept at an ambient temperature of -0.5° C. Incubations were aerobic for 6 h, and kept under ambient light and seawater temperature conditions, which ranged from 5.9 to 31 μ E/m²/s and -0.8° to -0.5° C, respectively. Isotope dilution and time course experiments (Pollard and Moriarty 1984) were carried out prior to analysis to determine optimal conditions. Ethanol killed controls were run in parallel and were subtracted from analysis to correct for any unincorporated ³H carried through the procedure. Further purifications and analysis were carried out as previously reported (Smith et al. 1986) and recommended by Pollard (1987). The scintillation instrument used was an LKB 1217 LSC operated in the standard ratio mode.

Acetate Incorporation into Microbial Lipids

Freshly collected surface sediment samples (2 g wet weight) were added to 0.2 μ Ci sodium [¹⁴C]-acetate (SA 45–60 mCi/mmol) in 50 ml disposable centrifuge tubes. Incubations were aerobic at ambient seawater and light conditions as described above for 6 h. Formalin killed controls (10% in seawater) were run in parallel and were subtracted from analysis to correct for any unincorporated ¹⁴C carried through the procedure. Additional purification procedures are reported elsewhere (Smith et al. 1986) and the scintillation instrument was as described above.

Statistical Analysis

Analysis were performed on a laboratory IBM PC compatible system with a Statistical Analysis System (SAS Institute Inc., Cary, NC USA) program. The test for Tukey's Honestly Significant Difference (HSD) was performed on all fatty acid and metabolic activity data with the SAS program keeping the within-experiment, family-wise error rate set at α = 0.05.

Results and Discussion

Measurements of total PELFA's for the four Arthur Harbor sites indicated sediment microbial biomasses were relatively similar. Only one site at AH(13) indicated a somewhat lower biomass of 2.7×10^8 cells/gdw (Table 2). The three remaining sites were determined to have relatively equal cell concentrations of: AH(10) 4.3, ER 3.6, HI 3.5 ($\times 10^8$) cells/gdw. Conversions from total PELFA mole amounts to cells were made using a liturature conversion factor of 100 μ M PELFA/gdw of average size bacteria, and 1 g (dry weight) of bacteria is equivalent to 5.9×10^{12} cells (White et al. 1979). These Arthur Harbor biomasses are lower than previously determined McMurdo Sound sites at Cape Evans, Cape Armitage, and New Harbor, which averaged 2.1×10^9 cells/gdw, with the lowest measurement at New Harbor containing 3.7×10^8 (Smith et al. 1986). Reasons for the biomass differences at the two Antarctic areas are not readily obvious. The Palmer Peninsula seawater temperature $(-0.5^{\circ}C)$ at Arthur Harbor does not reach the lower -1.9°C temperature at the higher latitude McMurdo Sound during the mid-summer months of December and January. Therefore one might assume this warmer water temperature to have a greater positive effect on microbial biomass at Arthur Harbor. However, this does not seem to be the case, and it appears that McMurdo Sound benefits greatly from the deep cold water current that provides nutrients (Littlepage 1965) and seasonal algal blooms that are swept under the fast ice during the spring (Palmisano et al. 1986) and summer seasons. In addition, inputs from the McMurdo Sound seasonal ice-algal bloom may provide carbon sources to the benthos upon senescence and sedimentation (Palmisano and Sullivan 1983). The effects of this rich current can be seen from the biomass (microbial, meiofaunal and macrofaunal) differences between the East and West side of McMurdo sound which reveal greater abundances on the East side (Smith et al. 1986; Dayton and Oliver 1977; Hodson et al. 1981). Relatively little sea-ice is present in Arthur Harbor, and that which does form produces only a relatively small algal community. There is however a water column bloom occurring in December and January which becomes quite extensive (personal observation). These factors may explain, at least in part, the observed difference in biomass for the two areas.

Community Structure

Descriptions of endemic microbial subgroup proportions are also provided by analysis of PELFA profiles (Lechevalier 1977; Bobbie and White 1980; Sasser et al. 1984; White 1983). The determination of PELFA positional isomers and geometry, as well as branching and mid-chain methyl group identification (Table 3) provides a greater degree of specificity in microbial taxonomic identification. In this study we relied heavily upon GC/MS analysis of PELFA's to provide confirmations of complex lipid components.

Table 2. Tukey's test of significant difference for biomass and metabolic rates for four Arthur Harbor sites

	Highest ^a		Lowest	
	AH(10) ^b	ER	HI	AH(13)
Total (×10 ⁸) microbial cells/gdw	4.3°	3.6	3.5	2.7
	ER	AH(10)	AH(13)	HI
Methyl [3 H]-thymidine (×10 4) (bacterial DNA)	4.5	4.0	2.0	1.9
	ER	AH(13)	HI	AH(10)
Sodium $[^{14}C]$ -acetate (×10 ⁴) (lipid metabolism)	12.0	9.0	6.0	6.0

^a Sites are ordered from highest to lowest for cells/gdw and DPM/gdw/h for biomass and incorporations respectively, and sites connected by a common line are not significantly different

^b Study sites as in Fig. 1

^c Total number of cells/gdw calculated from PELFA (100 μ M PELFA/g bact. the size of *E. coli* with 1 g bact. equivalent to 5.9×10^{12} cells (dry weight))

Bacteria

Signature PELFA from bacterial subgroups indicated similar overall proportions for three sites AH(10), ER, HI and a somewhat lower relative amount at the AH(13) site (Tables 4 and 5). Ratios of dominant bacterial acids (e.g. i + a $15:0 + 18:1\omega$ /c/16:0) indicated AH (13) contained 40% fewer bacteria when compared to the average of the other sites (Table 5). Tukey's HSD test of PELFA mol% signatures revealed greater amounts of bacteria at AH(10) and HI sites, as a whole. However; no significant differences were determined when the family error rate was set at $\alpha = 0.05$ and means were ordered from highest to lowest (Table 4). This trend was also apparent for the sediment subgroup of special eubacterial distinction (primarily SRB), with sites AH(10), ER, HI containing slightly greater amounts when compared by Tukey's HSD test and ratios (Tables 4 and 5).

 Table 3. Detailed Phospholipid ester-linked fatty acid profiles from four sites in Arthur Harbor, Antarctic Peninsula

Fatty acid methyl ester ^b	%composition ^a				
	AH(10)°	AH(13)	ER	HI	
i14:0	0.6	ndd	tr°	0.4	
14:1ω5c ^f	tr	tr	tr	tr	
14:0	2.6	1.5	1.4	2.0	
i15:1ω5 ^f	0.6	nd	0.3	0.5	
a15:1 ^f	0.5	nd	tr	tr	
i15:0	1.4	0.8	0.7	1.2	
a15:0	2.2	1.7	1.4	2.3	
15:1 ^f	0.7	0.3	tr	0.4	
15:0	1.1	0.7	0.6	0.9	
i16:1ω6 ^f	tr	tr	tr	tr	
16:3	0.4	0.4	0.4	0.3	
16:2	0.6	0.4	0.6	0.3	
i16:0	0.4	0.4	0.3	0.6	
16:1ω9c	1.8	2.0	2.4	2.3	
16:1ω7c	19.9	17.4	16.6	19.6	
16:1ω7t	1.1	1.2	0.5	1.2	
16:1ω5c	2.1	2.2	1.5	2.4	
16:1 <i>w</i> 13t	0.7	0.3	0.4	0.3	
			12.7	12.1	
i17:1ω7	0.6	0.5	0.4	0.5	

10Me16:0	0.7	0.8	0.8	0.8
i17:0	0.7	0.4	0.5	0.5
br17:0	nd	tr	tr	0.3
a17:0 ^g	1.7	1.7	1.5	2.2
17:1ω6	1.4	1.1	1.0	1.6
cy17:0	0.3	tr	tr	0.3
17:0	0.6	0.8	0.7	0.7
i18:1	tr	0.3	tr	0.3
18:5ω3 ^f	0.7	0.6	1.0	1.0
18:3ω6 ^f	tr	0.6	0.4	tr
18:4ω3 ^f	0.8	1.0	1.0	0.8
18:2ω6	0.7	0.9	1.1	1.0
18:3ω3 ^f	0.5	0.4	0.5	0.6
i18:0 ^f	0.5	nd	nd	nd
18:1ω9c	4.0	4.1	7.5	5.4
18:1ω7c	10.2	10.2	15.2	13.3
18:1ω7t	0.4	0.3	0.3	0.4
18:1ω5c	2.0	1.6	0.6	1.0
18:0	1.5	2.2	2.0	1.5
brl9:1 ^f	0.4	0.3	tr	0.3
19:1ω12c ^f	tr	0.3	tr	0.3
19:1ω8 ^f	0.6	0.3	0.3	0.3
19:1ω12t ^f	nd	tr	tr	tr
20:4ω6	5.2	2.6	3.5	4.9
20:5ω3	9.6	13.6	12.4	7.4
20:3ω6 ^f	tr	0.3	0.4	0.4
20:4ω3 ^f	0.3	0.3	tr	0.3
20:2\omega3f	tr	tr	tr	nd
i20:0	tr	0.4	0.5	tr
20:1ω9c	0.7	1.5	0.6	0.7
20:1 <i>w</i> 8c	tr	nd	tr	0.6
20:1ω9t	nd	1.2	1.0	tr
20:0	tr	tr	0.4	0.3
22:6w3	3.6	3.5	3.0	2.5
21:0	0.5	nd	nd	nd
22:4ω6	tr	0.4	0.3	0.3
22:5w3	0.3	nd	0.4	0.4
22:1ω9c	1.3	0.7	0.4	0.6
22:0	nd	tr	nd	tr
Total PELFA ^h	7.3	4.6	6.1	6.0

^a mol % composition of individual fatty acids

^b Phospholipid fatty acid methyl esters

^c Arthur Harbor study sites (Fig. 1)

^d nd = not detected

e tr = trace < 0.2%

^f Identified by GC retention time with authentic standards alone due to

insufficient detection of mass ions from GC/MS analysis

^g Contains 17:1ω8

^h Total nM/gdw of phospholipid ester linked fatty acids

Table 4. Tukey's Honestly Significant Difference test for phospholipid ester-linked fatty acids from four Arthur Harbor sediment sites with family error rate set at $\alpha = 0.05$

Fatty acid	Highest ^a		Lowest	
Bacterial ^b i14:0	AH(10)°	ні	ER	AH(13)
i15:0, 15:1, 15:0 ^d	AH(10)	HI	AH(13)	ER
a15:0	HI	AH(10)	AH(13)	ER
i16:1 <i>w</i> 6	HI	AH(13)	AH(10)	ER
16:1ω9c	ER	HI	AH(13)	AH (10)
i16:0, a17:0	HI	AH(10)	AH(13)	ER
i17:0	AH(10)	ER	HI	AH(13)
17:0	AH(13)	ER	HI	AH(10)
18:1 <i>w</i> 7c	ER	HI	AH(13)	AH(10)
Other bacteria i15:1ω5	AH(10)	HI	ER	AH(13)
a15:1ω5	AH(10)	ER	HI	AH(13)
i17:1ω7	AH(10)	HI	AH(13)	ER
10Me16:0	HI	AH(13)	ER	AH(10)
br17:0	ні	AH(13)	ER	
17:1 <i>w</i> 6	HI	AH(10)	AH(13)	ER
cy17:0	HI	AH(10)	ER	AH(13)
Algal/diatom 14:1ω5c	HI	AH(10)	AH(13)	ER
14:0, 16:1ω7c	AH(10)	HI	AH(13)	ER
16:3	AH(10)	AH(13)	ER	HI
16:2	ER	AH(10)	AH(13)	HI
16:1 <i>w</i> 13t	AH (10)	ER	AH(13)	HI
18:3 <i>w</i> 6	AH(13)	ER	HI	AH(10)
18:5 <i>w</i> 3, 18:3 <i>w</i> 3	HI	ER	AH(10)	AH(13)
18:4 <i>w</i> 3, 20:5 <i>w</i> 3	AH(13)	ER	AH (10)	HI
18:2 <i>w</i> 6	ER	HI	AH(13)	AH(10)
Microeucaryotes 18:1ω9c	ER	HI	AH(13)	AH(10)
20:4 <i>w</i> 6	AH(10)	HI	ER	AH(13)
20:3 <i>ω</i> 6	ER	HI	AH(13)	AH(10)
20:1ω9c	AH(13)	HI	ER	AH(10)
22:6 ω 3	AH(13)	ER	HI	AH(10)

 Table 4 (continued)

Fatty acid	Highest ^a		Lowest	
22:4 <i>w</i> 6	AH(13)	ER	HI	AH(13)
22:5 <i>w</i> 3	ER	HI	AH(10)	AH(13)

^a Sites are ordered from highest to lowest according to their mol% values, and sites connected by a common line are not significantly different

^b Tukey's HSD test on signature fatty acids: bacterial, common eubacteria; other bacteria, mainly sulfate reducing bacteria: algal/diatom, includes diatoms as well as other microalgae; microeucaryotes, also includes some larvel forms of meiofauna < $500 \mu m$ in size

° Study sites as in Fig. 1

^d Grouped fatty acids show the same significance and abundance values using the Tukey's test

 Table 5. Ratios of selected phospholipid ester-linked fatty acids indicative of various microbial groups at four Arthur Harbor sites

Fatty acids	AH(10) ^a	AH(13)	ER	HI	
i+a15:0/16:0 ^b	0.29	0.15	0.16	0.28	
18:1ω7c/16:0	0.82	0.62	1.20	1.10	
i+a15:0, + 18:1ω7c/16:0	1.11	0.77	1.36	1.38	
10Me16:0/16:0	0.06	0.05	0.06	0.07	
16:1ω7c/16:0	1.60	1.06	1.31	1.61	
20:5ω3/16:0	0.77	0.83	0.98	0.61	
16:1ω7c+ 20:5ω3/16:0	2.37	1.89	2.29	2.22	
20:4 <i>w</i> 6/16:0	0.42	0.16	0.28	0.40	

* Arthur Harbor study sites as in Fig. 1

^b Saturated C—16 is found in all cell membranes in approximately equal proportions and is therefore used to normalize ratios

The acids 10Me16:0 and cy17:0 have been shown to be major components of Desulfobacter SRB cell membranes in marine systems (Taylor and Parkes 1983). Other branched and odd-chained PELFA's il5:1 ω 5 and il7:1 ω 7c are reported as being major components of the genus Desulfovibrio (Taylor and Parkes 1983; Boon et al. 1977; Dowling et al. 1986; Edlund et al. 1985), and have recently been reported in several Flexibacter (Nichols et al. 1986). Differences in the relative proportions of $i15:1\omega 5$ indicated an increased amount of Desulfovibrio at sites AH(10) and HI, with AH(13) again containing significantly less. The contribution to the sediment microbial community for all of the Arthur Harbor sites averaged 4% for the SRB biogroup which is significantly greater than the 2% average for the McMurdo Sound sediments. (Smith et al. 1986)

Diatoms

Proportions of major PELFA signatures for marine microalgae revealed little differences between the sites. Mid- and long-chain unsaturated fatty acids: $16:1\omega7c$,

16:2, 16:3, and 20:5 ω 3 are typically major components, along with lesser amounts of 14:0, 14:1 and 14:2 from Bacillariophyceae (Orcutt and Patterson 1975), and other marine algae (Ackman et al. 1968; Gillan et al. 1981; Volkmam et al. 1980a; Nichols et al. 1985). Several minor components (18:3 ω 3, 18:4 ω 3, and 18:5 ω 3) were also detected for all Arthur Harbor sites. Major algal PELFA indicated a higher proportion of $16:1\omega7c + 20:5\omega3/16:0$ (Table 5) within a deep trough site ER(26m), which was not expected due to low light levels (5.9 $\mu E/m^2/s$). There is however evidence that the algae within this area were senescent marine phytoplankters (microscopic investigation; R. Moe, personal communication) which had settled from the water column. Although the majority of algae at this site may have been senescent, it is believed that there was also a photosynthesising community, since algal photosynthesis has been determined for a benthic McMurdo Sound community at a much lower light level of 0.6 μ E/m²/s (Palmisano et al. 1985).

Metabolic Activities

Incorporation of precursor macromolecules into lipid (¹⁴C-acetate) and bacterial DNA (³H-thymidine) provided additional information about the activities of the sediment microorganisms. Analysis by Tukey's HSD test revealed no significant difference between the four Arthur Harbor study sites for either incorporation rate (Table 2). However, relative differences were evident from information on the ordered values for total DPM's, indicating the ER site was most active in both ¹⁴C-acetate and ³H-thymidine incorporations. Sites at Arthur Harbor were determined to be more active than those of previously determined McMurdo Sound sites, (except for Cape Evans which exhibited a greater ¹⁴C-acetate incorporation) with averages of 53% and 77% greater for ¹⁴C-acetate and ³Hthymidine, respectively, on a gdw per hour basis. This information is believed to reflect the ability of microorganisms to maintain enzymatic metabolic processes more easily under increased ambient seawater temperatures. Primarily the slight temperature difference (-1.9°) vs. -0.5° C), and greater light intensities to sediments at Arthur Harbor are thought to help facilitate greater metabolic rates. In addition to and as a consequence of the increased seawater temperature and light intensities at Arthur Harbor, there are increased numbers of photosynthetic organisms in both the sediments and overlying seawater. This increased photosynthesis and subsequent bacterial metabolism from utilization of algal exudates, as discussed by Kottmeier et. al. (1987) for sea-ice communities, is believed at least in part, to facilitate the overall increased sediment microbial metabolic rates at Arthur Harbor.

When DPM's for the determinations of incorporated ³H-thymidine are converted into moles of thymidine, cell divisions per gdw per hour can be calculated (Table 6). Factors for conversion were determined by Pollard and Moriarty (1984) and have been estimated to be 2.2×10^{18}

Table 6. Calculated bacterial metabolic rates (cell divisions) for four Arthur Harbor sites, using membrane phospholipids and incorporation of ³H-thymidine

	AH(10) ^a	AH(13)	ER	HI
Total bact. PELFA ^b	30.9	30.8	32.4	34.8
Bact. cells $(\times 10^8)^c$	1.4	0.8	1.2	1.2
³ H-thymidine $(\times 10^5)^d$	5.8	3.7	14.0	5.8
Percent bact. divisions ^e	0.4	0.5	1.2	0.4

^a Arthur Harbor study sites (Fig. 1)

^b Total mol % of major bacterial phospholipid signature fatty acids, excluding sulfate-reducing bacteria. (i15:0, a15:0, 15:1, 15:0, i16:0, 16:1 ω 9c, 16:1 ω 5c, i17:0, a17:0, 17:1 ω 6c, 17:0, 18:1 ω 7c, 18:1 ω 7t, and 50% of 16:0)

^c Calculated number of bacterial cells per gram dry weight (calculated from total phospholipid fatty acids, and converted by a factor of $100\mu M$ phospholipid/gm bact. the size of *E. coli*, with 1gm bact. equivalent to 5.9 $\times 10^{12}$ cells (dry weight))

^d Estimation of bacterial divisions/gdw/hr from incorporation of ³H-thymidine $(2.2 \times 10^{18} \text{ cell divisions/mole }^{3}\text{H-thy}; \text{ Pollard and Moriarty } 1984)$

^e Calculated percent of dividing bacterial cells (ratio of total bact. cells to estimated cell divisions from ³H-thymidine incorporation)

cell divisions per mole of ³H-thymidine incorporated into non-SRB bacteria (SRB's are not capable of direct incorporation of precursor thymidine). Recent studies by Carman et al. (1988) have indicated possible problems with macromolecule catabolism and recyling within the microbial community. If catabolism is assumed to be minimal in such low temperature environments, and as in this study, dilution pools and time course work is carried out for each specific sediment analyzed, then site to site estimates can be made. The average for all Arthur Harbor sites when converted to their cell divisions per gdw per hour was 7.3 $\times 10^5$, with ER again containing a significantly greater (14.0×10^5) rate. The increased metabolic rate for ER is believed to be the result of released metabolic byproducts from the microalgae during later life stages. Similar observations (as stated above) have been determined by recent investigations of bacterial production during a two and a half month period for Antarctic sea ice communities dominated by microalgae.

Utilizing PELFA signature biomarkers for bacteria (excluding SRB's), one can calculate total bacterial cells and determine along with the ³H-thymidine incorporation rate the number of bacterial cells dividing within the total population (Table 6). Such analysis showed an average percentage of bacterial cell divisions in Arthur Harbor of 0.6%/h, with ER (1.2%/h) having the greatest percentage (Table 6). It should be noted that the calculated number of bacterial cells from PELFA's is believed to be a slightly conservative estimate, since only those components with a solely (or nearly so) bacterial origin were summed to provide this estimate.

Analysis of sediment microorganisms by their cellular PELFA provided a useful and convenient technique in profiling this extreme environment. The biomass and taxonomic information obtained from this technique made it possible to compare Arthur Harbor sites with those of McMurdo Sound, as well as more temperate and deep sea environments (White et al. 1984; Smith et al. 1988). Likewise, the usefulness of this baseline profiling of biomass, community structure and metabolic activities will prove beneficial for future monitoring of these vulnerable benthic microbial communities. With the apparent future increase in human encroachment upon this region, a greater amount of anthropogenic contaminants are inevitable. These baseline profiles, of the as yet uncontaminated system will greatly enhance the monitoring of future adverse effects. Changes in the above mentioned parameters for benthic microbial communities have been shown to be excellent indicators of various impacted systems (White 1983; Parker et al. 1984; Smith et al. 1985; Smith et al. 1982b; Schropp et al. 1988).

Continued research by our laboratory and others is intended to extend our knowledge into deeper sediment areas, and to investigate the obviously important input of phytoplankton (upon senescense) to the benthos during this productive season. Important also is the development of sensitive methods for following stable isotopes through food chains to provide insight into grazing and algal bacterial coupling questions.

Acknowledgements. The authors wish to thank the excellent Palmer station crew and especially the help of station manager Rob Robbins and biology lab manager Sandra Ackley. Christopher Low is thanked for his help with the statistical analysis. Hewlett Packard is thanked for their generous contribution of the HP-1000 computer with RTE/6-VM data system for the HP-5996A GC/MS. This work was funded by a NSF/DPP contract 86–13248 to Drs. Anna Palmisano/David White.

References

- Ackman RG, Tocher CS, McLachlan J (1968) Marine phytoplankter fatty acids. J Fish Res Board Can 25:1603-1620
- Balkwill DL, Leach FR, Wilson JT, McNabb JF, White DC (1988) Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface aquifer sediments. Microbiol Ecol 16:74–84
- Bobbie RJ, White DC (1980) Characterization of benthic microbial community structure by high resolution gas chromatography of fatty acid methyl esters. Appl Environ Microbiol 39:1212-1222
- Boon JJ, Leeuw JW de, Hoek GJ van der, Vosjan JH (1977) Significance and taxonomic values of iso and anteiso monoenoic fatty acids and branched-hydroxy acids in *Desulfovibrio desulfuricans*. J Bacteriol 129:1183-1191
- Carman KR, Dobbs FC, Guckert JB (1988) Consequences of thymidine catabolism to estimates of bacterial production: An example from a costal marine sediment. Limnol Ocenogr 33:1595-1606
- Dayton PK, Oliver JS (1977) Antarctic soft-bottom benthos in oligotrophic and eutrophic environments. Science 197:55-58
- Dowling NJE, Widdel F, White DC (1986) Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulphate reducers and other sulfide forming bacteria. J Gen Microbiol 132:1815-1825
- Dunkelblum E, Tan SH, Silk PJ (1985) Double bond location in monounsaturated fatty acids by dimethyl-disulfide derivatization and mass-spectrometry: application to analysis of fatty acids in pheromone glands of four *Lepidoptera*. J Chem Ecol 11:265–277
- Edlund A, Nichols PD, Roffey R, White DC (1985) Extractable and lipopolysaccharide fatty acids and hydroxy acid profiles from *De*sulfovibrio sp. J Lipid Res 26:982-988
- El-Sayed SZ (1985) Plankton of the Antarctic seas. In: Bonner W,

Walton D (eds) Key environments Antarctica. Pergamon Press, New York, pp 135-153

- El-Sayed S, Taguchi S (1981) Primary production and standing crop of phytoplankton along the ice-edge in the Weddell Sea. Deep-Sea Res 28A:1017-1032
- Federle TW, White DC (1982) Preservation of estuarine sediments for lipid analysis of biomass and community structure of microbiota. Appl Environ Microbiol 44:1166-1169
- Gillan FT (1981) Lipids of aquatic ecosystems. PhD Thesis, University of Melbourne, Australia
- Gillan FT, McFadden GI, Wetherbee R, Johns RB (1981) Sterols and fatty acids of an Antarctic sea ice diatom *Stauroneis amphioxys*. Phytochemistry 20:1935–1937
- Guckert JB, Antworth CP, Nichols PD, White DC (1985) Phospholipid ester-linked fatty acids profiles as reproducible assays for changes in procaryotic community structure of estuarine sediments. FEMS Microbiol Ecol 31:147-158
- Hodson RE, Azam F, Carlucci AF, Fuhrman JD, Karl DM, Holm-Hansen O (1981) Microbial uptake of dissolved organic matter in McMurdo Sound, Antarctica. Mar Biol 61:89-94
- Johns RB, Nichols PD, Perry GJ (1979) Fatty acid composition of ten marine algae from Australian waters. Phytochemistry 18:799-802
- Kottmeier ST, McGrath Grossi S, Sullivan CW (1987) Sea ice microbial communities. VIII. Bacterial production in annual sea ice of McMurdo Sound, Antarctica. Mar Ecol Prog Ser 35:175-186
- Lechevalier MP (1977) Lipids in bacterial taxonomy—a taxonomist's view. Crit Rev Microbiol 5:109-210
- Littlepage JL (1965) Oceanographic investigations in McMurdo Sound, Antarctica. Antarct Res Ser 5:1-37
- Nichols PD (1983) Biological markers in the marine environment. PhD Thesis, University of Melbourne, Australia
- Nichols PD, Palmisano AC, Smith GA, White DC (1985) Lipids of the Antarctic sea diatom *Nitzschia cylindrus*. Phytochemistry 25:1649-1653
- Nichols PD, Stulp BK, Jones JG, White DC (1986a) Comparison of fatty acid content and DNA homology of filamentous gliding bacteria Vitreoscilla, Flexibacter and Filibacter. Arch Microbiol 146:1-6
- Nichols PD, Guckert JB, White DC (1986b) 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. J Microbiol Methods 5:49-55
- Orcutt DM, Patterson GW (1975) Sterol, fatty acid and elemental composition of diatoms grown in chemically defined media. Comp Biochem Physiol B50:579-583
- Palmisano AC, Sullivan CW (1983) Sea ice microbial communities (SIMCO). I. Distribution, abundance and primary productions of ice microalgae in McMurdo Sound, Antarctica. Polar Biol 2:171-177
- Palmisano AC, Soo Hoo JB, White DC, Smith GA, Stanton GR, Burckle L (1985) Shade-adapted benthic diatoms beneath Antarctic sea ice. J Phycol 21:664-667
- Palmisano AC, Soo Hoo JB, Soo Hoo SL, Kottmeier ST, Craft LL, Sullivan CW (1986) Photoadaptation in *Phaeocystis pouchetii* advected beneath annual sea ice in McMurdo Sound, Antarctica. J Plankton Res 8:891-906
- Parker JH, Nickels JS, Martz RF, Gehron MJ, Richards NL, White DC (1984) Effects of well-drilling fluids on the physiological status and microbial infection of the reef building coral *Montastera annularis*. Arch Environ Contam Toxicol 13:113–118
- Pollard PC, Moriarty DJW (1984) Validity of the tritiated thymidine method for estimating bacterial growth rates: Measurements of isotope dilution during DNA synthesis. Appl Environ Microbiol 48:1076-1083
- Pollard PC, (1987) Dialysis: a simple method of separating labelled bacterial DNA and tritiated thymidine from aquatic sediments. J Microbiol Methods 7:91-101
- Sasser MJ, Fieldhouse D, Carter C (1984) Computer assisted identification of bacteria based on fatty acid analysis. Abstr Annu Meet Am Phytopathol Soc A739. Phytopathology 74:882
- Schropp S, Lewis FG, Eubanks W, Carman K, White DC (1988) Biochemical characterization of estuarine benthic microbial communities for use in accessing pollution impacts. Am Soc Testing Materials, Philidelphia, PA (in press) (000)

- Smith GA, Nickels JS, Davis WM, Martz RF, Findlay RH, White DC (1982a) Perturbations in the biomass, metabolic activity, and community structure of the estuarine detrital microbiota: resource partitioning in amphipod grazing. J Exp Mar Biol Ecol 64:125-143
- Smith GA, Nickels JS, Bobbie RJ, Richards NL, White DC (1982b) Effects of oil and gas well drilling fluids on the biomass and community structure of microbiota that colonize sands in running seawater. Arch Environm Contam Toxicol 11:17-23
- Smith GA, Nickels JS, Davis JD, Findlay RH, Vashio P, Wilson JT, White DC (1985) Indices identifying subsurface microbial communities that are adapted to organic pollution. In: Durham NN, Redelfs AE (eds) Conference on Ground Water Quality Research. Oklahoma State University Printing Services, Stillwater, Okla, pp 210-213
- Smith GA, Nichols PD, White DC (1986) Fatty acid composition and microbial activity of benthic marine sediments from McMurdo Sound, Antarctica. FEMS Microbiol Ecol 38:219–231
- Smith GA, Nichols PD, White DC (1988) Benthic near-shore microbial communities of McMurdo Sound and Arthur Harbor, Antarctica: Microbial biomass, community structure and metabolic activity. 5th SCAR Symp Proceedings (submitted, Aug 1988)
- Taylor J, Parkes JR (1983) The cellular fatty acids of the sulfate-reducing bacteria, *Desulfobacter* Sp., *Desulfobulbus* Sp., and *Desulfovibrio desulfuricans*. J Gen Microbiol 129:3303-3309
- Tunlid A, Odum G (1986) Ultra-sensitive analysis of bacterial signatures

by gas chromatography/mass spectrometry. In: Mequsar E, Gantar M (eds) 4th Intl Symp Microb Ecol. Ljubljana, Yugoslavia. Slovene Society for Microbiology, pp 447–454

- Volkman JK, Eglinton G, Corner EDS (1980a) Sterols and fatty acids of the marine diatom *Biddulphia sinesis*. Phytochemistry 19:1809-1813
- Volkman JK, Johns RJ, Gillan FT, Perry GJ, Bavor Jr HJ (1980b) Microbial lipids of an intertidal sediment I. Fatty acids and hydrocarbons. Geochim Cosmochim Acta 44:1133-1143
- White DC (1983) Analysis of microorganisms in terms of quantity and activity in natural environments. In: Slater JH, Whitenbury R, Wimpenny JWT (Eds) Microbes in their natural environments. Soc Gen Microbiol Symp 34:37-66
- White DC, Findlay RH (1988) Biochemical markers for measurement of predation effects on the biomass community structure, nutritional status, and metabolic activity of microbial biofilms. Hydrobiologia 159:119-132
- White DC, Bobbie RJ, Herron JS, King JD, Morrison SJ (1979) Biochemical measurements of microbial mass and activity from environmental samples. In: Costerton JW, Colwell RR (eds) Native Aquatic bacteria: enumeration, activity and ecology. ASTM STP 695, Am Soc Testing Materials, pp 69-81
- White DC, Smith GA, Stanton GR (1984) Biomass, community structure and metibolic activity of the microbiota in Antarctic benthic marine sediments and sponge spicule mats. Antarct J US 29:125-126