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Biomarker and Stable Isotope Characterization of Coastal Pond Organic Matter, McMurdo Dry Valleys, Antarctica

Melissa Margaret Hage
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Melissa Margaret Hage entitled "Biomarker and Stable Isotope Characterization of Coastal Pond Organic Matter, McMurdo Dry Valleys, Antarctica." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Geology.

Chris Fedo, Major Professor

We have read this thesis and recommend its acceptance:

Linda Kah, Claudia Mora

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Claudia Mora
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Accepted for the Council:

Anne Mayhew
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Vice Chancellor and
Dean of Graduate Studies

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BIOMARKER AND STABLE ISOTOPE CHARACTERIZATION OF COASTAL POND ORGANIC MATTER, MCMURDO DRY VALLEYS, ANTARCTICA

A Thesis
Presented for the
Masters of Science
Degree
The University of Tennessee, Knoxville

Melissa Margaret Hage
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ABSTRACT

Small coastal ponds containing photosynthetic microbial mat communities represent a potentially significant source of labile organic carbon within the McMurdo Dry Valleys, Antarctica. To distinguish coastal pond derived organic matter (CPDOM) from other sources of organic matter in the dry valleys, I investigated bulk organic carbon and nitrogen isotopic signatures and phospholipid fatty acid (PLFA) profiles of benthic microbial mats located at two sites, Hjorth Hill Coast and Garwood Valley. The average $\delta^{13}C$ values at Hjorth Hill Coast and Garwood Valley are -10.91 ‰ and -10.19 ‰, respectively. The average $\delta^{15}N$ values are 3.73 ‰ and -1.25 ‰, respectively. Microbial mats from all ponds are dominated by monounsaturated PLFAs, which are indicative of gram negative bacteria, and polyunsaturated PLFAs, which are indicative of microeukaryotes. Specific biomarkers for aerobic prokaryotes, eukaryotes, photoautotrophic microeukaryotes, and sulfur-reducing bacteria are present in all samples. Benthic mats at Garwood Valley are thicker, more laminated, have a higher biomass, and greater % C and % N, suggesting greater productivity than mats at Hjorth Hill Coast. Greater productivity is supported, as well, by greater dissolved oxygen contents likely derived from greater photosynthetic productivity. Higher productivity at Garwood Valley likely results from greater influx of terrestrial surface water and concomitant nutrient loading.

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CHAPTER I

INTRODUCTION

Examination of biological communities that thrive in the Antarctic environment may aid in the identification of mechanisms that help sustain life in extreme environments, which is an important goal in the NASA Astrobiology Roadmap. Understanding the persistence and variability of life on Earth will provide an essential component for the search for life beyond Earth (Des Marais et al., 2003). Because liquid water is essential for all life on Earth, the study of inland waters, such as Antarctic coastal ponds, is a crucial element in the study of life in extreme environments.

Coastal ponds occur throughout Antarctica's margins, and are especially concentrated in the ice-free coastal areas of McMurdo Sound and the McMurdo Dry Valleys regions. Along the Victoria Land coast, over 300 ponds at the transition between marine and terrestrial ecosystem have been identified from topographic maps and aerial photographs and have been the focus of many biocomplexity and ecological studies (Berkman, 1997; Berkman et al., 1998; Peterson and Howard-Williams, 2000). Although the size of coastal ponds are relatively small compared to the large, perennially ice-covered lakes of the McMurdo Dry Valleys (i.e. Lake Hoare, Lake Bonney, Lake Fryxall), collectively they comprise a region ~500,000 m^2 suitable for primary production, and represent a prominent habitat for benthic microbial mats (Vincent and Howard-

Williams, 1986; Wynn-Williams, 1990; Hawes and Brazier, 1991; Schmidt et al., 1991; Hawes et al., 1993; Hawes and Schwarz, 1999).

One of the central themes of the McMurdo Long Term Ecological Research (LTER) site is understanding the structural and spatio-temporal linkages between ecosystem elements in terms of organic carbon transport and other processes affecting primary production (Moorehead and Priscu, 1998). Coastal ponds are a potentially significant, yet uninvestigated, component of the McMurdo Dry Valley organic carbon budget. The transient nature of these ponds results in highly labile coastal pond derived organic matter (CPDOM) being vulnerable to transport, and capable of supporting heterotrophic communities throughout the dry valley ecosystem.

This paper represents one of the first studies to characterize CPDOM. Identifying the role of CPDOM within the broader scope of the dry valleys ecosystem will provide a more detailed understanding of the linkages between modern ecosystem components, and will therefore further develop understanding of the organic carbon budget of the McMurdo Dry Valleys polar desert. To begin to distinguish CPDOM from other sources of organic matter in the dry valleys, I investigated bulk organic carbon and nitrogen isotopic signatures of coastal pond benthic microbial mats. Phospholipid fatty acid analyses provide a more detailed characterization of the CPDOM, including community structure and organism-specific biomarker profiles for the benthic microbial mats.

CHAPTER II

MCMURDO DRY VALLYES

Study Area

Less than 0.4% of the total area of Antarctica is ice-free. While this ice-free area is primarily mountain peaks emerging from the polar ice caps, a small percentage (~1-2%) of this ice-free environment consists of coastal oases. The largest oasis is the McMurdo Dry Valleys, which is situated near the coast of the Ross Sea (Hodgson et al., 2004). The McMurdo Dry Valleys (77°00'S, 163°00'E) represent one of the coldest, driest, windiest places on earth. Valley floor mean annual temperature ranges between -14.8 °C and -30.0 °C. This region receives less than 100 mm of annual precipitation (with as little as 7 mm recorded in a single year), which is usually limited to snow that quickly sublimates due to extremely low vapor pressures. Austral summer (~4 months) consists of 24 hours of sunlight; austral winter (~4 months) lacks sunlight completely. Only 4 months of the year have both days and nights (Doran et al., 2002).

Two field sites (Hjorth Hill Coast and Garwood Valley) were selected for detailed analysis and sample collection based on their high concentration of coastal ponds (Fig. 1). This allows us to see if there is a difference in the biogeochemistry of the coastal ponds within a single site and between sites. Ponds at both sites partially or completely freeze during winter, but most completely thaw by mid-summer.

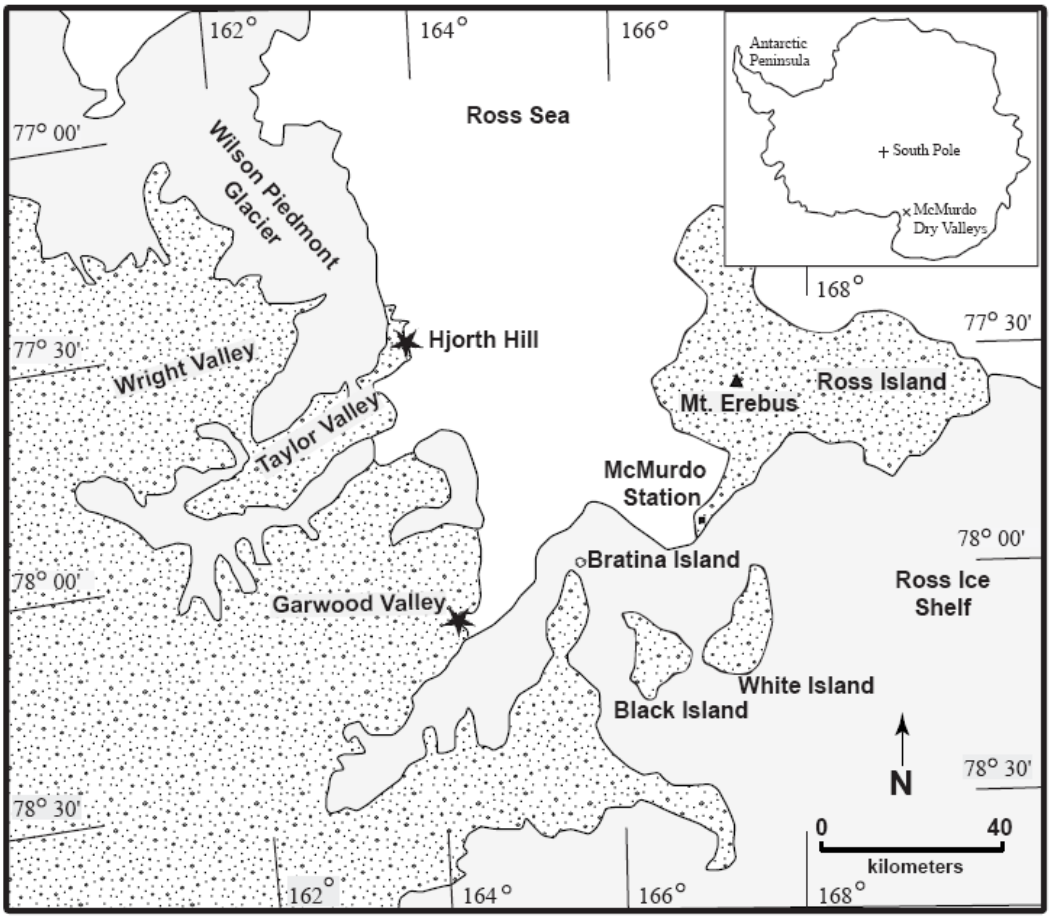

Figure 1. Map of field locations (Hjorth Hill Coast and Garwood Valley) located along the Victoria Land coast.

The northern study area (Fig. 1) is positioned near Hjorth Hill, with a coordinate of 77°30.628' S, 163°49.958' E at an altitude of 45 m. This site is ~ 2 km from the Ross Sea coast. An undetermined proportion of the Hjorth Hill coastal headland contains buried ground ice, ~0.5 m below the current surface. However, current geomorphic processes are dominated by periglacial activity. The winter freezing and summer thaws have produced an active layer ~0.5 m depth. Freeze thaw activity has resulted in the formation of distinctive small-scale landforms and sedimentary structures, with sorted, patterned ground. The inside of the polygons are characterized by deflation surfaces (P. Allen, unpublished data).

The southern site is located in Garwood Valley approximately 55 km south of Hjorth Hill Coast (Fig 1), with a coordinate of 78°02.040' S, 164°19.217' E. This site is ~2.5 km from the Ross Sea Coast. Garwood Valley is distinct within the Dry Valley's ecosystem due to the occurrence of the Garwood River, which starts at the Garwood Glacier and discharges into McMurdo Sound. Garwood Valley is a wide, steep valley, with evidence for recent glacial activity. The valley is open to maritime air masses, and deflation surfaces are widespread. Beneath the protective deflation surface is homogeneous fine silt. The sedimentary complex around the mouth of the Garwood Valley is an active periglacial zone, displaying patterned ground. An undetermined proportion of the Garwood Valley headland contains buried ground ice, ~0.5 m below the current surface (P. Allen, unpublished data). Differential melting of ground ice, combined with periglacial and aeolian processes has aided the development of

seasonal melt ponds at both locations. Dried, relict mat material is present along the edges of most ponds at both sites, but is more abundant at Garwood Valley.

Organic Carbon Dynamics

The McMurdo Dry Valleys presently contain both recalcitrant detrital carbon, derived from ancient glacial till, and modern labile carbon, derived from recent photoautotrophic organisms (Kellogg et al., 1978; Burkins et al., 2000; Howard 2006). Organic matter within glacial till is derived from westward-advancing Ross glaciations, which deposited marine sediments scoured from the bottom of McMurdo Sound within the valleys. Additionally, lower elevations in the valleys have repeatedly been sites of glacial melt-water pooling and lacustrine sedimentation during climatic warming (Glacial Lake Washburn) (Kellogg et al., 1978; Burkins et al., 2000). Long-term accumulation of glaciomarine and glaciolacustrine organic matter in the soil reservoir, facilitated by slow organic matter degradation rates in the polar desert environment, currently provides detrital carbon to modern Antarctic lakes, streams, and soils (Burkins et al., 2000). Modern organic matter produced by photoautotrophs is distributed via aeolian and hydrologic transport and sustains heterotrophic communities throughout the dry valley ecosystem. However, production of modern organic matter is limited by the presence of liquid water and radiant energy (Moorehead and Priscu, 1998). Thus, organic carbon in the dry valleys is divided between at least two pools: (1) a labile organic carbon pool replenished through photoautrophy and (2) a more recalcitrant organic carbon pool, which is less

bioavailable to heterotrophs, that records paleo-glacial and paleo-lacustrine environments (Burkins et al., 2001).

Unlike other components of the dry valleys (i.e. streams, large, permanently ice-covered lakes, soil), coastal ponds contain a significant amount of labile, organic carbon that can be readily distributed via aeolian and hydrologic transport throughout the ecosystem (Parker et al., 1982). The transient nature of coastal ponds results from dynamic interactions between the pond, surrounding ground ice, and evaporation/sublimation. Ponds persist when the volume of melt-water entering the pond is greater than the volume of water lost to evaporation and sublimation, and when ground ice persists adjacent to the pond margin (Hawes and Howard-Williams, 2003). When ground ice melts on one side of the pond, the pond drains, exposing active microbial mats. Subsequently, mat communities are exposed to prolonged periods of desiccation caused by freezing temperatures and the low vapor pressure typical of a polar desert ecosystem. Depending on the severity of desiccation, these mats may still contain viable microorganisms and, via aeolian and fluvial means, may be disseminated. Upon rewetting, new mat communities may be established and thereby continue to participate in the active carbon cycle of the dry valleys. Even if no viable organisms exist in the desiccated mat, aeolian and fluvial transport can still distribute labile organic carbon and nutrients that may be utilized by heterotrophic communities (Vincent and Howard-Williams, 1989; Hawes, 1993; Hawes et al., 1999).

Magnitude of the Coastal Pond Reservoir: Preliminary Estimates

The coastal pond component of the total organic carbon reservoir (ΣC_{org}) of the dry valleys region is a function of the concentration of organic carbon in each of the ponds, integrated over the total area of this environmental facies. Based on a preliminary survey of topographic maps and aerial photographs, there are approximately 300 modern ponds along the Victoria Land coast, ranging in size from 50-3000 m^2. This estimate is consistent with the description by Hall et al. (2000) and Torri et al. (1989) who document numerous small ponds at the mouth of Taylor Valley and in the Labyrinth area of Wright Valley. Although the exact organic carbon concentrations of the ponds are unknown, many of these ponds contain productive benthic mat communities that are similar to microbial mats found in the large, permanently ice-covered lakes (P. Doran, unpublished data), whose organic matter content has been studied (Parker et al., 1982; Parker et al., 1981). Since the ponds thaw during the summer, the coastal pond mats are assumed to have greater organic matter concentrations than the lakes, and thus, the coastal pond reservoir should contain, at a minimum, 25,600 g m^{-2} of carbon, which, integrated over the estimated total environmental facies (0.1 km^2), results in the coastal pond reservoir constituting at least 11% of the total organic carbon reservoir (Fig. 2) (M. Uhle and P. Doran, unpublished data). This is a conservative estimate because it does not take into account the carbon contribution from relict microbial mats, which are not discernable on topographic maps or aerial photographs but may contain a significant amount of labile organic carbon. Chemical and isotopic characterization of CPDOM is the first step in

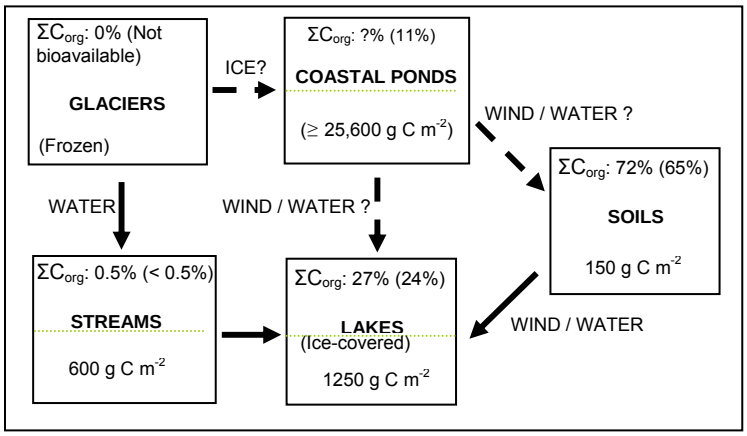

Figure 2. Model organic carbon budget in the McMurdo Dry Valleys. Total organic carbon reservoir index (ΣC_{org}) is for present day Taylor Valley (modified from Burkins et al., 2000). Values in parentheses are estimates which include carbon from modern coastal ponds (P. Doran and M. Uhle, unpublished data).

understanding the role this labile carbon plays in the organic carbon dynamics of the McMurdo Dry Valleys.

CHAPTER III

BIOCHEMICAL FRAMEWORK OF COASTAL POND ORGANIC MATTER

Isotopic Characterization

Potential sources of organic matter in the dry valleys include marine-derived organic matter (MDOM), lacustrine-derived organic matter (LDOM), soil-derived organic matter (SDOM), ornithogenic organic matter (OOM), and endolith-derived organic matter (EDOM) (Burkins et al., 2000). Geochemically and isotopically fingerprinting the CPDOM is critical in isolating this organic matter from other sources of organic matter in the dry valleys.

Bulk $\delta^{15}N$ compositions distinguish between potential sources of organic matter in the Antarctic dry valleys (Fig. 3) (Burkins et al., 2000). Using $\delta^{15}N$ as a primary tracer for organic matter sources is unconventional as a result of the complexities of the nitrogen cycle, for instance, the numerous and concurrent abiotic and biotic isotopic fractionations that occur in most ecosystems. The absence of higher plants and animals, however, and extremely slow rates of organic matter decomposition in the Antarctic polar desert ecosystem simplifies the nitrogen cycle (Burkins et al., 2000). In fact, $\delta^{15}N$ values for potential sources of organic matter determined by Burkins et al. (2002) are in agreement with straightforward $\delta^{15}N$ fractionations in the Antarctic environment: MDOM has $\delta^{15}N$ values similar in range to marine nitrate ($\delta^{15}N$ = +5.8‰), the dominant source of nitrogen for marine phytoplankton; OOM has a $\delta^{15}N$ signature

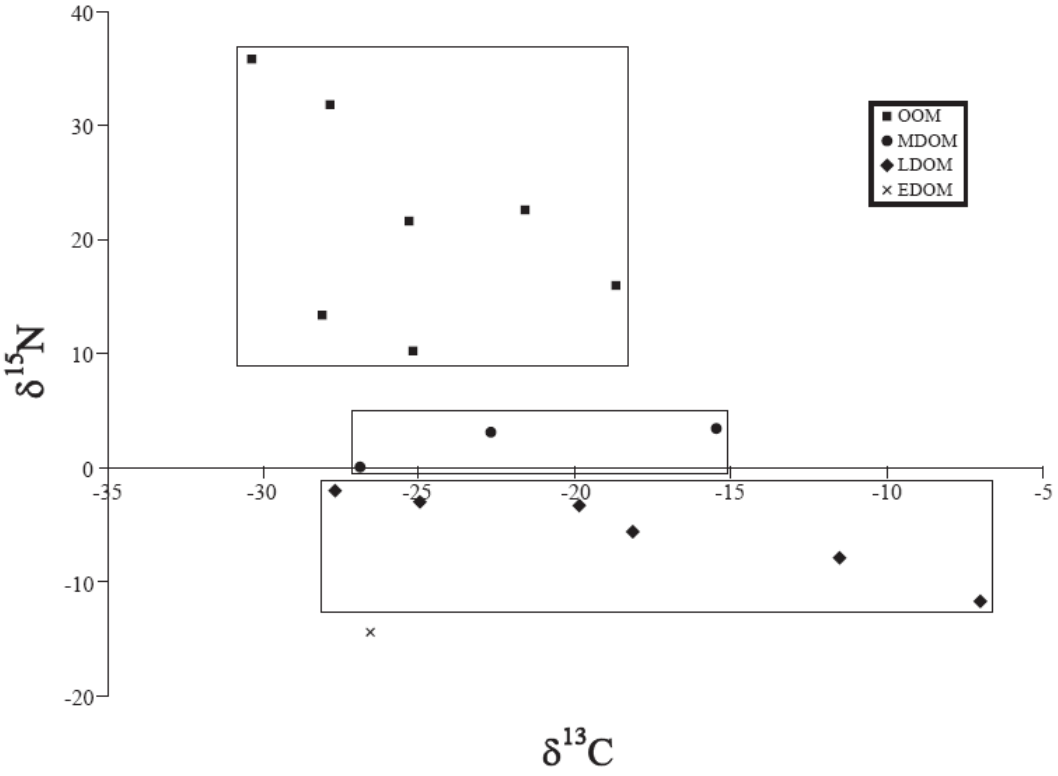

Figure 3. Potential sources of organic matter in the McMurdo Dry Valleys. There is overlap in $\delta^{13}C$ values, but not $\delta^{15}N$ values. OOM = ornithogenic organic matter; MDOM = marine-derived organic matter; LDOM = lacustrine-derived organic matter; EDOM = endolithic-derived organic matter (Burkins et al., 2000).

extremely enriched in ^{15}N relative to other organic matter sources, which can be explained by ^{15}N enrichment through marine trophic levels, as well as through secondary volatilization of ammonia in ornithogenic excrement; and $\delta^{15}N$ of LDOM is consistent with the release and recycling of ^{15}N-depleted NH_4^+ from the decomposition of organic matter in the lake water column, as well as the addition of ^{15}N-depleted nitrate from the surrounding soil ecosystem (Burkins et al., 2000).

In contrast to nitrogen isotopes, carbon isotopes show considerable overlap, and therefore do not universally discriminate sources of organic matter. However, carbon isotopes can provide information as to which component of dissolved inorganic carbon (DIC) is being utilized by the microbial mats (CO_2 vs. HCO_3^-) and the relative degree of $[CO_2]_{(aq)}$ saturation.

Biomarkers

Molecular biomarkers are organic compounds specific to a limited group of organisms (Boschker and Middleburg, 2002; Zhang, 2002). It is commonly preferred to target a class of compounds in which biomarkers for various groups of organisms can be found, such as phospholipid fatty acids (PLFAs) (Table 1; Boschker and Middelburg, 2002). PLFAs are strong indicators of extant organisms, as they exist within bacterial and eukaryotic cell membranes, which break down within days of a cell's death (Green and Scow, 2000; Boschker and Middelburg, 2002). PLFAs are made from glycerol, 2 fatty acids and a phosphate

TABLE 1. RECOGNIZED PLFA BIOMARKERS AND ORGANISIMS FROM WHICH THEY DERIVE

Organism	**PLFA Biomarkers**
Eukaryotes (poly unsat.)	16:4ω1[a], 16:3[a], 18:3ω6[a], 18:2ω6[a], 18:3ω3[a], 20:4ω6[a], 20:5ω3[a], 22:5ω6[a], 22:6ω3[a]
ω6 "animal" series	18:3ω6[a], 18:2ω6[a], 20:4ω6[a], 22:5ω6[a]
ω3 "plant" series	18:3ω3[a], 20:5ω3[a], 22:6ω3[a]
Photoautotrophs	16:1ω13t[a], 18:3ω3[a], 18:1ω9[a]
Microeukaryotes	16:4ω1[a], 16:3[a], 18:3ω6[a], 18:3ω3[a], 20:3ω6[a], 20:4ω6[a], 20:5ω3[a], 22:5ω6[a], 22:6ω3[a]
Bacteria (saturated)	i14:0[e], i15:0[e], 18:1ω7c[e] (mono unsat), cy19:0[e]
Algae (poly unsat)	20:5ω3[e], 18:3ω3[e]
Fungi (poly unsat)	18:2ω6[e]
Actinomycetes	10Me17:0[e], 10Me18:0[e]
Prokaryotes (saturated)	i15:0[a], a15:0[a], 15:0[a], i17:0[a], a17:0[a], 17:0[a], 18:1ω7c[a] (mono unsat), cy19:0(ω7,8)[a], 10Me16:0[a], cy17:0(ω7,8)[a]
Desulfobacter	10Me16:0[a], cy17:0(ω7,8)[a]
Bacteria, anaerobic desaturase pathway	18:1ω7c[a]
Bacillus-type Gram positive bacteria	i15:0[a], a15:0[a], i17:0[a], a17:0[a]
Gram-positive prokaryotes and other anaerobic bacteria	14:0[a], a15:0[a], i15:0[a], 15:0[a], i16:0[a], 16:1ω13t[a] (mono unsat; not anearobic)
Sulfate-reducing bacteria and other anaerobic prokaryotes	16:0[a], 10Me16:0[a, e], a15:0[b,c, d], i15:0[b.c, d], a17:0[a,b.c], i17:0[a,b,c], cy17:0[a], 17:0[a], i17:1[e] (mono unsat), 18:0[a], cy19:0[a]
Aerobic prokaryotes and eukaryotes (mono unsat)	16:1ω5[a], 16:1ω7c[a], 17:1ω6[a], 17:1ω9[a], 18:1ω7c[a], 18:1ω9[a], 18:2ω6[a]
Diatoms (poly unsat)	16:4ω1[a], 16:0[a] (saturated), 16:3[a], 20:5ω3[a]

group. The hydrocarbon tail of the fatty acids is hydrophobic and the phosphate group end is hydrophilic, making PLFAs soluble in both water and oil and relatively easy to extract. In addition, total microbial biomass can be estimated from the sum of the masses of all detectable PLFAs (White et al., 1979; Bossio et al., 1998; Green and Scow, 2000), and PLFAs can provide a rough estimate of levels of stress experienced by the microbial community (Guckert et al., 1986; Navarrete et al., 2000).

Environmental stresses induce predictable changes in the structural development of PLFAs. If metabolic stress-induced lipid loss occurs while the cell is alive, the cis-monenoic fatty acids are preferentially utilized and the trans to cis ratio (trans/cis 16:1ω7c and trans/cis 18:1ω7c) increases to greater than 0.1. Also, the cis-monenoic fatty acids are modified to their cyclopropyl derivatives, and the cyclopropyl to cis ratio (cy 17/16:1ω7c) increases to greater than 0.1 (Guckert et al., 1986; Navarrete et al., 2000).

Metabolically different groups exist together, each in distinct layers, in a mat approximately 5-10 mm thick. Benthic microbial mats are typically dominated by only a few functional groups of microbes, and the majority of autotrophic production results from algal and cyanobacterial photosynthesis (van den Ende and van Gemerden, 1994). Microbial mats developed in shallow water, such as the coastal ponds investigated in this study, are composed primarily of oxygenic cyanobacteria in the upper layers, purple and green sulfur bacteria in the middle layers, and sulfate-reducing bacteria at the base (Navarrete et al., 2000).

CHAPTER IV

METHODS

Sample Collection and *In-situ* Measurements

Samples were collected from five ponds at Hjorth Hill Coast and four ponds at Garwood Valley during two consecutive austral summers, Dec. 03 - Jan 04, and Dec. 04 - Jan. 05. At Hjorth Hill Coast the ponds are informally named Obelisk, Tom, Skua, Big Sister, and Little Sister (Fig. 4). At Garwood Valley, the ponds are informally named Marina, Susan, Cody, and Garwood MET (Fig. 5). Benthic microbial mat samples and *in situ* measurements of pond water properties (temperature, pH, salinity, and dissolved oxygen) were collected at three different locations within each pond. Pond water was also collected from one location in each pond. All samples were kept frozen until analyzed.

Pond Water Analysis

Water from each pond was analyzed for dissolved cations (Na^{+}, K^{+}, Mg^{+2}, Ca^{+2}) and anions (Cl^{-}, SO_4^{-2}) on a Dionex DX-120 Ion Chromatograph at the McMurdo Research Center by Kathy Welch (Byrd Polar Research Center, Ohio State University). For the cations, a Dionex IonPac CS12A analytical column (4x250 mm) and a CG12A guard column (4x50 mm) were used. The eluent was a 0.13% methanesulfonic acid solution, and a CSRS Ultra Cation Self-Regenerating Suppressor was used. For the anions, a Dionex IonPac AS14 analytical column (4x250 mm) and an AG14 guard column (4x50 mm) were used. The eluent was a

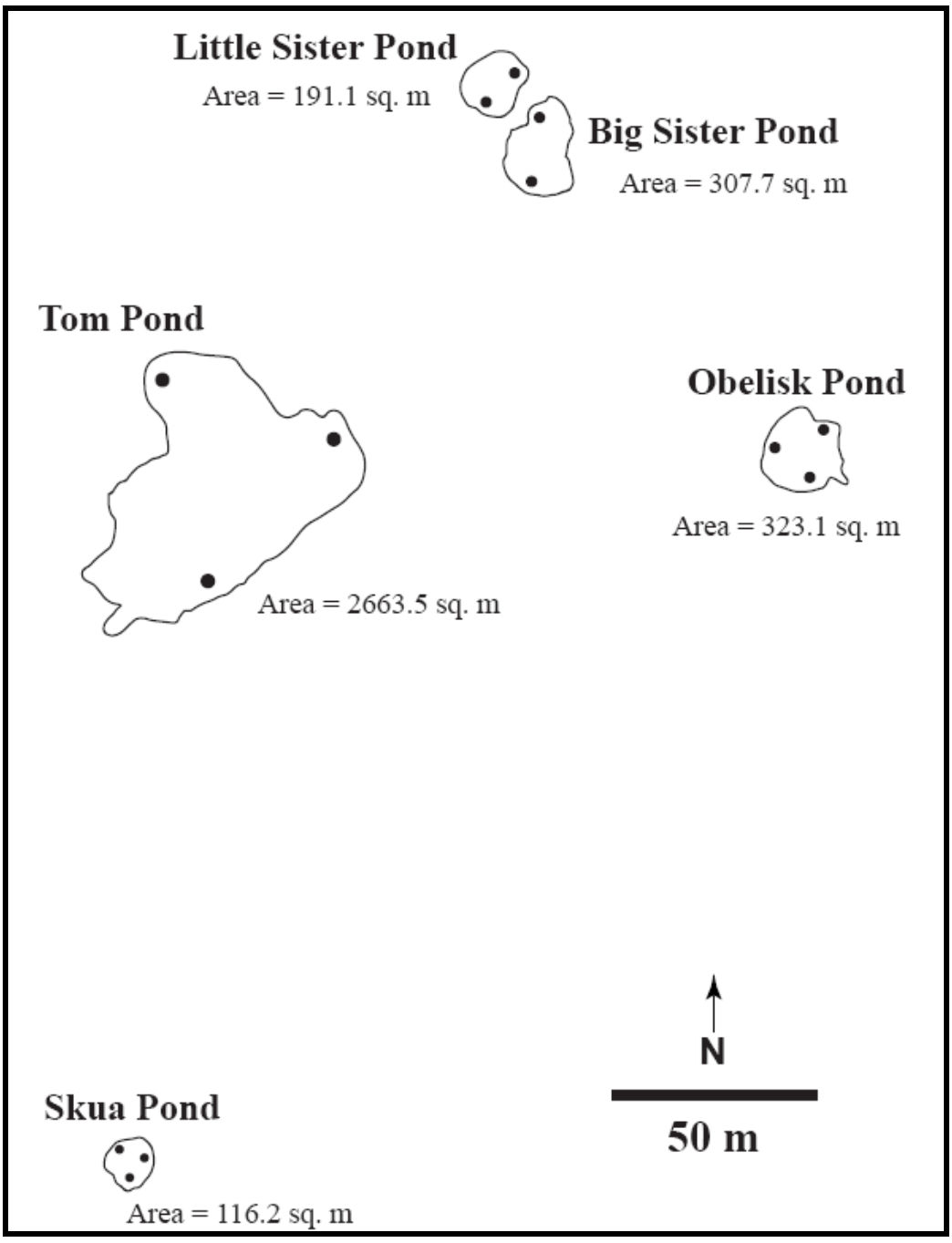

Figure 4. Map of Hjorth Hill Coast field site, depicting the relative location of the 5 ponds sampled for this study. Dots represent locations within the pond where samples were collected. The Ross Sea coast is located ~2 km to the east of Obelisk Pond.

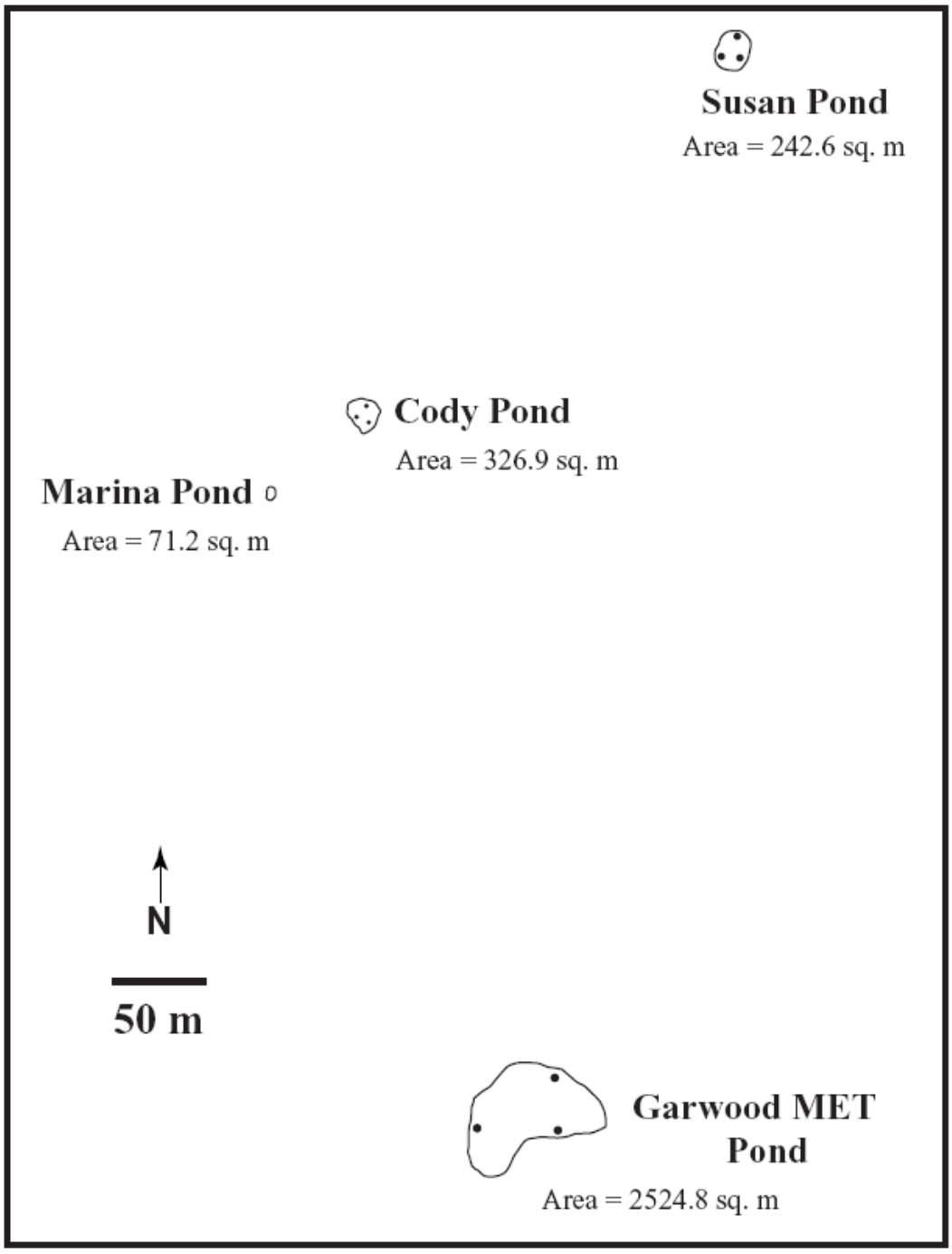

Figure 5. Map of Garwood Valley field site, depicting the relative location of the 4 ponds sampled for this study. Dots represent locations within the pond where samples were collected. Ross Sea located ~2.5 km to the north of Garwood MET pond.

1.0 mM $NaHCO_3$ and 3.5 mM Na_2CO_3 solution, and an ASRS Ultra Anion Self-Regenerating Suppressor was used.

Bulk Carbon, Nitrogen, and Sulfur Isotope Measurements

Bulk organic carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$) and sulfur ($^{34}S/^{32}S$) compositions were determined for modern microbial mat samples by Steve Macko at the University of Virginia with a Fisons NA 1500-R Series 2 elemental analyzer equipped with an autosampler and Waters-Isochrom diluter coupled to a Waters Optima isotope ratio mass spectrometer (EA/IRMS). All values are reported in δ notation, where $\delta = [(R_{spl}/R_{st}) - 1] \times 10^3$, where R_{spl} = isotope ratio of sample and R_{st} = isotope ratio of the standard, in permil using the standards PeeDee Belemnite for carbon, atmospheric air for nitrogen, and Canyon Diablo Troilite for sulfur.

All mat samples were freeze-dried and ground to a powder. Prior to $\delta^{13}C$ analysis, carbonate carbon was removed from the mat samples by dropwise addition of HCl. Samples were loaded into a combustion reactor where flash combustion occurred at 1020 ^oC for $\delta^{13}C$ and $\delta^{15}N$ analyses and 1090 ^oC for S analysis. A flow of ultra pure helium carried combustion products to an oxidation furnace, maintained at 1020 ^oC, and then to a reduction furnace, maintained at 650 ^oC. The sample combustion products were then passed through a perchlorate water trap and introduced to a packed column (Porapak QS 50-80 mesh) for separation of N_2 and CO_2. Following column separation, a thermal conductivity detector (TCD) measured concentrations and a mass spectrometer measured isotopic compositions.

Lipid Extraction, Identification, and Analysis

The single-phase chloroform:methanol:water extraction system of Bligh and Dyer (1959), as modified by White et al. (1979), was used to quantitatively extract the lipid-soluble components from viable cells. Lyophilized mats were extracted with 50 mM phosphate buffer (pH 7.4): methanol:chloroform (4:10:5 v.v). Once this first-phase extraction was complete (2-18 hours), chloroform and water were added to provide a final solvent volume ratio of 1:1:0.9 (v.v) for chloroform:methanol:water/buffer. After the sample had gravitationally separated (approximately 18 hours), the denser organic phase was collected via the stopcock, and the solvents were removed.

Silicic-acid column chromatography was used to separate total lipid extracts into general lipid classes (neutral lipids, glycolipids, and polar lipids) using solvents of increasing polarity to selectively elute the lipid classes from the silicic-acid stationary phase. Neutral lipids were eluted with chloroform, glycolipids with acetone, and polar lipids with methanol (Guckert et al., 1985).

Esterified lipids in the polar lipid fraction underwent mild alkaline methanolic transesterification, as reported by Guckert et al. (1985) and modified by Mayberry et al. (1993), to cleave the fatty acids from the phospholipid glycerol backbone, replacing the glycerol bonds with methyl groups to form fatty acid methyl esters (FAMEs). The dried polar lipid fraction was redissolved in chloroform and methanol, vortexed, and incubated at 60 °C for 30 minutes. The sample was neutralized with acetic acid, hexane was added, and the phases were separated by centrifugation. The less dense phase containing the FAMEs was

collected at this point, and the denser aqueous phase was re-extracted with hexane twice more.

FAMEs were quantified by capillary gas chromatography using a 50 m nonpolar column (RTX-1ms, 200 μm I.D., 0.25 μm film thickness). Compound identification was made by gas chromatography-mass spectrometry (GC-MS) using an Agilent 6890 series gas chromatograph interfaced with an Agilent 5973 mass spectrometer. Spectra were compared to standards and NIST 98 spectral library for identification and quantification. The GC ran for 27.4 minutes with the following temperature program: initially 60 °C, isothermal 2.00 min; ramp 10 °C/min to 150 °C; ramp 30 °C/min to 312 °C.

Fatty acid nomenclature is in the form of "A:BωC" where "A" designates the total number of carbons, "B" the number of double bonds, and "C" the distance of the closest unsaturation from the aliphatic (ω) end of the molecule. The suffixes "c" for *cis* and "t" for *trans* refer to geometric isomers. The prefixes "i", "a", and "me" refer to iso and anteiso methyl branching, and midchain methyl branching, respectively. Cyclopropyl rings are indicated by "cy" (Navarrete et al., 2000).

Compound-Specific Isotope Analysis

The $\delta^{13}C$ of the FAMEs were quantified by William Holmes at the University of Michigan using a Delta Plus isotope ratio mass spectrometer with a GC/C III interface (Thermo Electron, San Jose, CA) and a 6890 gas chromatograph (Agilent, Palo Alto, CA; Boschker et al., 1998). Samples were run on a 50 m nonpolar column (HP-1ms, 200 μm I.D., 0.25 μm film thickness)

and the GC ran for 27.4 minutes with the following temperature program: initially 60 °C, isothermal 2.00 min; ramp 10 °C/min to 150 °C; ramp 30 °C/min to 312 °C.

CHAPTER V

RESULTS

In-Situ Measurements and Pond Water Analysis

Antarctic coastal ponds in close proximity to each other are known to be highly variable with regard to aqueous chemistry (de Mora at al., 1994; Fernandez-Valiente et al., 2001; Jungblut et al., 2005). Pond water temperatures ranged from 0.2 °C to 6.5 °C at Hjorth Hill Coast and 0.87 °C to 7.03 °C at Garwood Valley (Table 2). Pond water temperatures from both seasons at both sites are in the range of similar ponds found on the McMurdo Ice Shelf (~0.8 °C to ~8.4 °C) (Hawes et al., 1993; de Mora et al., 1994). All pond water from both seasons is slightly basic, with pHranging from 8.53 to 10 at Hjorth Hill Coast and from 8.57 to 11.7 at Garwood Valley (Table 2). Values from this study coincide with pH measured from ponds on the McMurdo Ice Shelf (6.3 to 10.8) (de Mora et al., 1994).

Ponds at both Hjorth Hill and Garwood Valley are typically supersaturated with regard to dissolved oxygen. Dissolved oxygen concentrations range from 69.2% to 122.07% at Hjorth Hill Coast and 97% to 191.8% at Garwood Valley (Table 2). Dissolved oxygen supersaturation is also observed for analogous ponds near Bratina Island (Hawes et al., 1993). With the exception of Garwood MET Pond, all ponds at Garwood Valley have higher concentrations of SO_4^{2-}, Cl^-, Na^+, K^+, Mg^{2+}, and Ca^{2+} than ponds at Hjorth Hill Coast (Table 2).

TABLE 2. *IN SITU* MEASUREMENTS OF POND WATER PROPERTIES AND CONCENTRATIONS OF MAJOR ANIONS AND CATIONS, HJORTH HILL COAST AND GARWOOD VALLEY

	HJORTH HILL COAST										GARWOOD VALLEY							
Pond	Tom		Obelisk		Big Sister		Little Sister		Skua		Garwood MET		Cody		Susan		Marina	
Area	2663.5		323.1		307.7		191.1		116.2		2524.8		326.9		242.6		71.2	
Season	04-05	03-04	04-05	03-04	04-05	03-04	04-05	03-04	04-05	03-04	04-05	03-04	04-05	03-04	04-05	03-04	04-05	03-04
Temp (°C)	3.67	0.6	6.57	3.07	6.5	0.3	6.9	0.2	2.57	1.03	0.87	4.07	6.83	7.1	7.03	5.1	6.67	4.41
pH	10	8.87	9.26	9.73	8.53	8.24	8.83	8.79	8.83	8.97	8.97	9.27	8.47	11.49	8.57	11.7	6.97	11.41
Salinty (ppt)	0.2	0	0.4	0.1	0.8	0.03	1	0.2	0.2	0.03	0.1	0.23	23.07	17.1	2.2	0.5	10	8.3
DO (%)	96.7	69.2	122.07	80.67	105.27	71.73	107.97	95.37	109.2	90.17	97	103.98	148	147.8	125.1	166.57	160	191.8
DO (mg/L)	12.7	9.89	13.07	11.23	12.1	10.21	12.52	13.67	12.61	12.76	13.84	17.85	18.07	17.85	15.14	21.59	19.51	24.48
SO_4^{2-} (mg/L)	9.08	0.65	26.61	5.38	30.78	4.33	46.62	10.8	24.16	0.9	3.84	3.69	19602	12900	358.5	82.52	5642	5143
Cl^- (mg/L)	91.1	5.12	196.27	15.7	411.06	38.34	1066.3	95.4	94	2.92	27.35	36	3370.6	1590.1	893.7	215.51	1803.7	1341.9
Na^+ (mg/L)	41.8	2.52	123.11	22.6	189.74	20	395.64	44.2	48.65	2.58	22.76	29.7	10966	6957.5	726.4	172.6	3658.7	3142.56
K^+ (mg/L)	2.4	0.28	8.36	1.92	12.53	1.94	25	3.68	3.63	0.38	1.73	2.33	197.74	82.57	32.63	9.61	97.45	78.55
Mg^{2+} (mg/L)	8.71	0.62	16.07	1.78	42.59	4.02	106.83	9.5	9.35	0.39	0.33	0.79	223.55	109.21	30.59	10.6	86.55	95.26
Ca^{2+} (mg/L)	9.67	1.21	10.82	5.15	33.08	7.18	105.58	13.4	15.45	0.96	1.3	1.83	231.87	135.5	8.84	11.47	54.54	54.54

Bulk Organic Carbon, Nitrogen, and Sulfur Isotopes

Bulk $\delta^{13}C_{org}$ values for Hjorth Hill Coast microbial mats range from -8.50 ‰ to -14.88 ‰, averaging -10.77 ‰ (Table 3, Fig. 6). Bulk $\delta^{13}C_{org}$ values for microbial mats sampled from Garwood Valley have a greater range (-5.86 ‰ to -15.70 ‰), but almost the same average (-10.75 ‰) (Table 3, Fig. 6). Benthic microbial mats from both sites and both seasons are more depleted in ^{13}C compared to benthic organic matter (BOM) found in Taylor Valley streams and large, perennially ice-covered lakes, and ornithogenic organic matter, but have similar $\delta^{13}C$ values as BOM found in the moats surrounding the lakes in Taylor Valley and upland ponds (Fig. 7) (Lawson et al., 2004).

Microbial mats sampled from Hjorth Hill Coast have bulk $\delta^{15}N_{org}$ values ranging from 0.8 ‰ to 6.95 ‰, and averaging 3.62 ‰ (Table 3, Fig. 6). Bulk $\delta^{15}N_{org}$ values for microbial mats at Garwood Valley are more depleted in ^{15}N compared to Hjorth Hill Coast, ranging from 0.89 ‰ to -3.98 ‰ with an average of -1.13 ‰ (Table 3, Fig. 6). Although there is a difference in $\delta^{15}N_{org}$ values between mats at Hjorth Hill Coast and Garwood Valley during both seasons, both sites are slightly more enriched in ^{15}N compared to the majority of BOM from Taylor Valley streams, lakes, and moats (Fig. 7; Lawson et al., 2004). Overall, Hjorth Hill Coast and Garwood Valley mats are most similar isotopically to moat BOM. The $\delta^{15}N$ values distinctly separate Hjorth Hill Coast mats from other sources of organic matter.

TABLE 3. BULK CARBON, NITROGEN, AND SULFUR ISOTOPIC COMPOSITION OF MATS, HJORTH HILL COAST AND GARWOOD VALLEY

Hjorth Hill Coast	04-05 season						Garwood Valley	04-05 season					
Sample	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	% C	% N	% S	Sample	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	% C	% N	% S
Obelisk 1	-10.00	2.01	9.20	1.89	0.26	0.12	Marina 1	-7.89	-0.62	19.00	14.21	1.11	0.24
Obelisk 2	-8.89	3.79	8.20	2.26	0.19	0.19	Marina 2	-9.45	-2.54	19.50	7.80	0.98	0.33
Obelisk 3	-8.50	3.57	7.70	1.18	0.15	0.26	Marina 3	-8.28	-1.23	19.10	5.84	0.73	0.29
Tom 1	-9.32	1.38	7.50	1.09	0.18	0.10	Susan 1	-15.70	0.89	12.90	8.10	0.59	0.31
Tom 2	-9.24	2.75	3.88	1.29	0.14	0.11	Susan 2	-14.33	-1.51	13.00	16.27	1.68	0.19
Tom 3	-9.24	4.27	6.31	6.92	0.74	0.21	Susan 3	-12.45	-0.83	12.80	6.14	0.73	0.26
Skua 1	-11.22	2.79	6.30	2.78	0.31	0.09	Cody 1	-10.12	-1.24	17.40	3.96	0.53	0.45
Skua 2	-11.98	2.19	4.97	0.88	0.13	0.11	Cody 2	-10.90	-1.72	17.60	5.91	0.73	0.46
Skua 3	-9.32	1.83	5.60	6.49	0.76	0.11	Cody 3	-9.83	-1.72	17.40	11.02	1.13	0.45
Big Sister 1	-12.76	6.95	16.84	4.01	0.62	0.11	GV MET 1	-5.86	-3.98	15.40	1.37	0.20	0.08
Big Sister 2	-14.88	6.64	17.34	0.66	0.11	0.20	GV MET 2	-7.26	0.79	19.80	6.51	1.06	0.08
Little Sister 1	-13.26	4.07	4.24	0.78	0.11	0.19							
Little Sister 2	-13.20	6.26	6.95	1.34	0.22	0.17							
Minimum	-14.88	1.38	3.88	0.66	0.11	0.09	Minimum	-15.70	-3.98	12.80	1.37	0.20	0.08
Maximum	-8.5	6.95	17.34	6.92	0.76	0.26	Maximum	-5.86	0.89	19.80	16.27	1.68	0.46
Mean	-10.91	3.73	8.08	2.43	0.30	0.15	Mean	-10.19	-1.25	16.72	7.92	0.86	0.29
Std. Dev	2.09	1.87	4.28	2.12	0.24	0.05	Std. Dev	3.00	1.38	2.74	4.37	0.39	0.14
Number	13	13	13	13	13	13	Number	11	11	11	11	11	11

Hjorth Hill Coast 03-04 season							Garwood Valley 03-04 season						
Sample	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	% C	% N	% S	Sample	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	% C	% N	% S
Tom	-9.15	0.8	5.16	28.4	3.18	0.08	Marina	-13.75	-1.91	11.96	8.54	0.85	0.38
Big Sister	-13.54	4.99	17.5	4.13	0.63	0.11	Susan	-15.31	-0.37	13.22	15.3	1.6	0.18
							Cody	-12.21	0.23	16.7	6.5	1.02	0.12
Minimum	-9.15	0.8	5.16	4.13	0.63	0.08	Minimum	-15.31	-1.91	11.96	6.5	0.85	0.12
Maximum	-13.54	4.99	17.5	28.4	3.18	0.11	Maximum	-12.21	0.23	16.7	15.3	1.6	0.38
Mean	-11.35	2.90	11.33	16.27	1.91	0.10	Mean	-13.76	-0.68	13.96	10.11	1.16	0.23
Std. Dev	3.10	2.96	8.73	17.16	1.80	0.02	Std. Dev	1.55	1.10	2.46	4.61	0.39	0.14
Number	2	2	2	2	2	2	Number	3	3	3	3	3	3

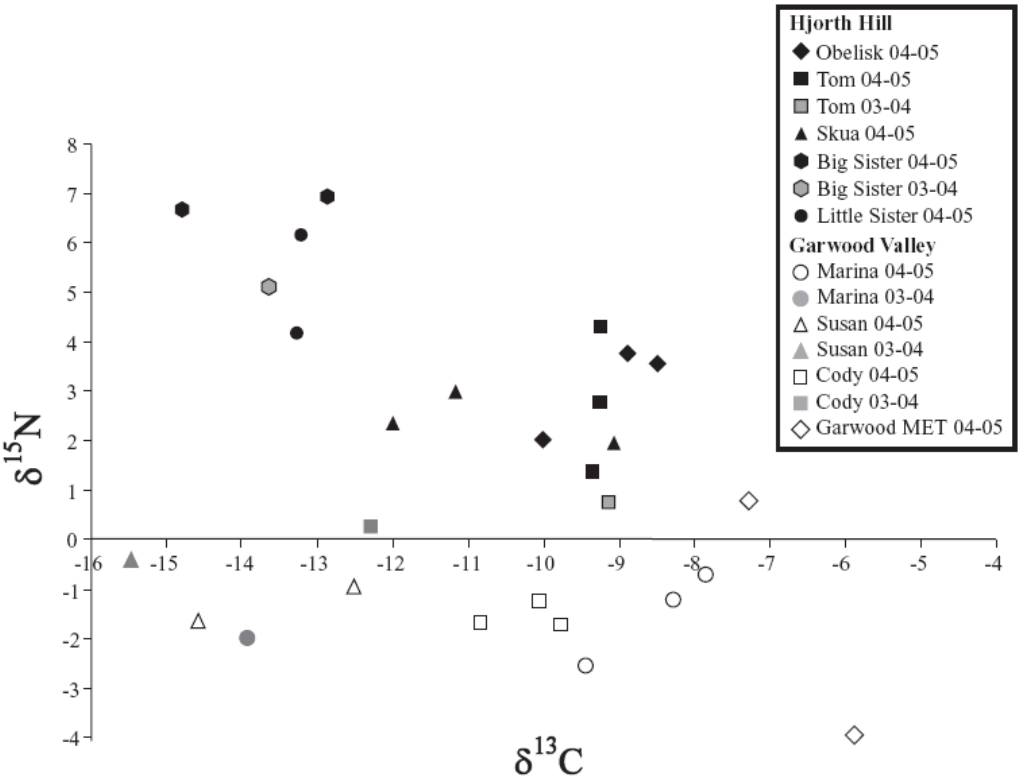

Figure 6. Bulk average $\delta^{13}C_{org}$ and bulk $\delta^{15}N_{org}$ values for benthic microbial mats, Hjorth Hill Coast and Garwood Valley.

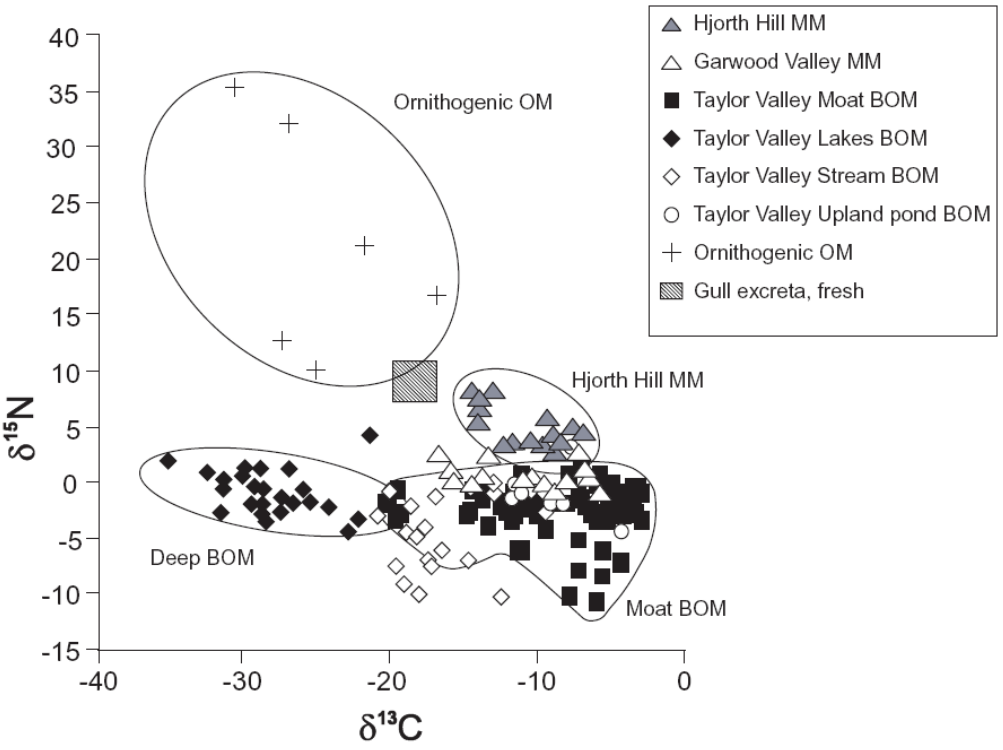

Figure 7. Bulk $\delta^{15}N_{org}$ and bulk $\delta^{13}C_{org}$ values for organic matter in the McMurdo Dry Valleys. MM = modern mat; BOM = benthic organic matter; OM = organic matter. Modified from Wada et al. (1975), Mizutani and Wada (1998), and Lawson et al. (2004).

Microbial mats at Hjorth Hill Coast have percent carbon ranging from 0.66 % to 28.4 %, percent nitrogen ranging from 0.11 % to 3.18 %, and percent sulfur ranging from 0.08 % to 0.26 %. Microbial mats at Garwood Valley have percent carbon ranging from 1.37 % to 19.80 %, percent nitrogen ranging from 0.2 % to 1.68 %, and a percent sulfur ranging from 0.08 % to 0.46 %.

Lipid Biomarkers

Microbial mats in all ponds contain a mixture of mid-branched saturated fatty acids (0.0 – 1.7 mol %), branched monounsaturated fatty acids (0.70 – 5.43 mol %), terminally branched saturated fatty acids (3.18 – 19.57 mol %), normal saturated fatty acids (15.61 – 28.59 mol %), polyunsaturated fatty acids (18.43 – 40.18 mol %), and monounsaturated fatty acids (20.33 – 50.41 mol %) (Fig. 8, Table 4).

Specific biomarkers with the highest concentrations from the mats at all sites during the 04-05 season are 16:1ω7c (ave. 19.25 ± 5.82 mol %), 16:0 (ave. 16.40 ± 4.23 mol %),18:1ω7c (ave. 10.31 ± 2.29 mol %), 18:3ω3 (ave. 10.06 ± 4.50 mol %), and 18:1ω9c (ave. 7.23 ± 2.34 mol %) (Fig. 9). 16:1ω7c, 18:1ω7c and 18:1ω9c are indicative of aerobic prokaryotes and eukaryotes (Findlay and Dobbs, 1993). 18:3ω3 may indicate the presence of photoautotrophic microeukaryotes (Findlay and Dobbs, 1993) or algae (Boschker and Middelburg, 2002). 16:0 is fairly common in many organisms, but may be used to indicate the presence of sulfur-reducing bacteria or diatoms (Findlay and Dobbs, 1993). Biomarkers for sulfur-reducing bacteria (SRB) are present in all microbial mats at

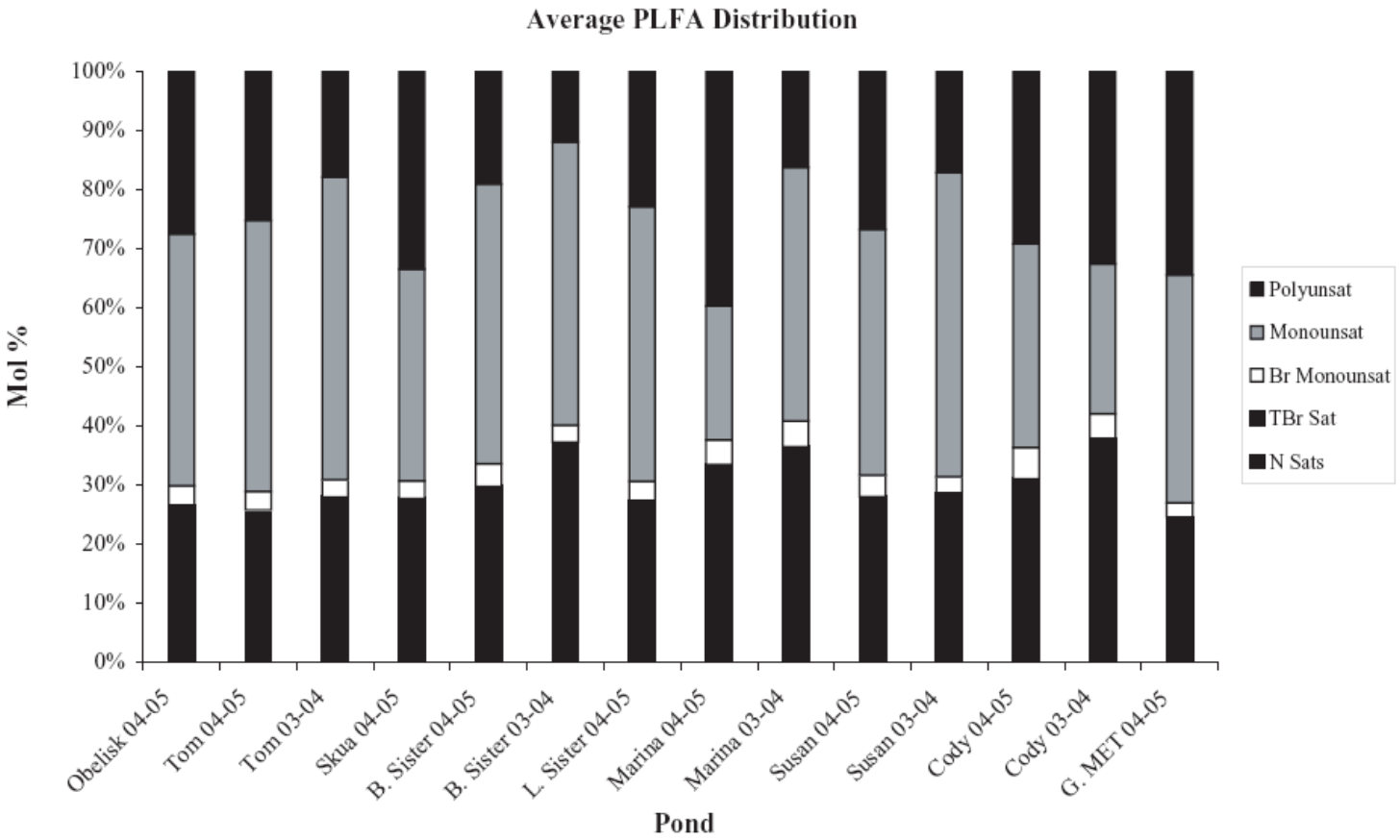

Figure 8. Average PLFA distribution of all microbial mats, Hjorth Hill Coast and Garwood Valley. Obelisk, Tom, Skua, Big Sister, and Little Sister Ponds are located at Hjorth Hill. Marina, Susan, Cody, and G. MET are located at Garwood Valley.

TABLE 4. COMMUNITY COMPOSITION OF MICROBIAL MATS (AVERAGE % OF PLFA BIOMARKERS FROM EACH POND ± STANDARD DEVIATION)

HJORTH HILL								
PLFA Biomarkers	Group of Organisms	Obelisk 04-05	Tom 04-05	Tom 03-04	Skua 04-05	Big Sister 04-05	Big Sister 03-04	Little Sister 04-05
Normal saturates	All genera	16.86 ± 1.17	17.17 ± 1.93	19.5	19.32 ± 1.87	16.49 ± 0.92	16.6	16.56 ± 0.62
Midchain branched saturates	Anaerboes	0.26 ± 0.06	0.19 ± 0.20	1.6	0.22 ± 0.07	0.06 ± 0.08	1.7	0.22 ± 0.05
Terminally branched saturates	Gram positives	9.61 ± 1.58	8.48 ± 2.75	7.6	8.25 ± 0.92	13.19 ± 0.07	19.7	10.73 ± 1.38
Branched unsaturates	Anaerobes	3.27 ± 0.48	3.12 ± 1.32	2.9	3.01 ± 0.34	3.86 ± 0.33	2.8	3.24 ± 0.11
Monounsaturates	Gram negatives	42.60 ± 0.81	45.95 ± 0.46	50.0	35.84 ± 0.74	47.38 ± 0.41	46.8	46.44 ± 0.03
Polyunsaturates	Microeukaryoes	27.40 ± 2.45	25.09 ± 2.68	17.4	33.37 ± 2.41	19.01 ± 0.82	11.5	22.81 ± 0.42

GARWOOD VALLEY								
PLFA Biomarkers	Group of Organisms	Marina 04-05	Marina 03-04	Susan 04-05	Susan 03-04	Cody 04-05	Cody 03-04	Garwood MET 04-05
Normal saturates	All genera	25.5 ± 1.11	24.52	18.33 ± 0.85	16.83	20.47 ± 2.58	28.89	17.78 ± 0.24
Midchain branched saturates	Anaerboes	0.00 ± 0.00	0.07	0.11 ± 0.19	1.52	0.40 ± 0.10	0.09	0.13 ± 0.19
Terminally branched saturates	Gram positives	7.94 ± 0.56	11.13	9.61 ± 2.00	11.13	10.39 ± 1.59	9.21	6.68 ± 4.95
Branched unsaturates	Anaerobes	4.18 ± 0.16	4.26	3.61 ± 0.57	2.66	5.32 ± 0.11	4.19	2.41 ± 2.42
Monounsaturates	Gram negatives	22.69 ± 2.10	42.08	41.63 ± 3.31	50.41	34.41 ± 7.99	25.40	38.59 ± 9.92
Polyunsaturates	Microeukaryoes	39.70 ± 0.42	15.85	26.70 ± 3.82	16.68	29.02 ± 5.03	32.52	34.41 ± 17.24

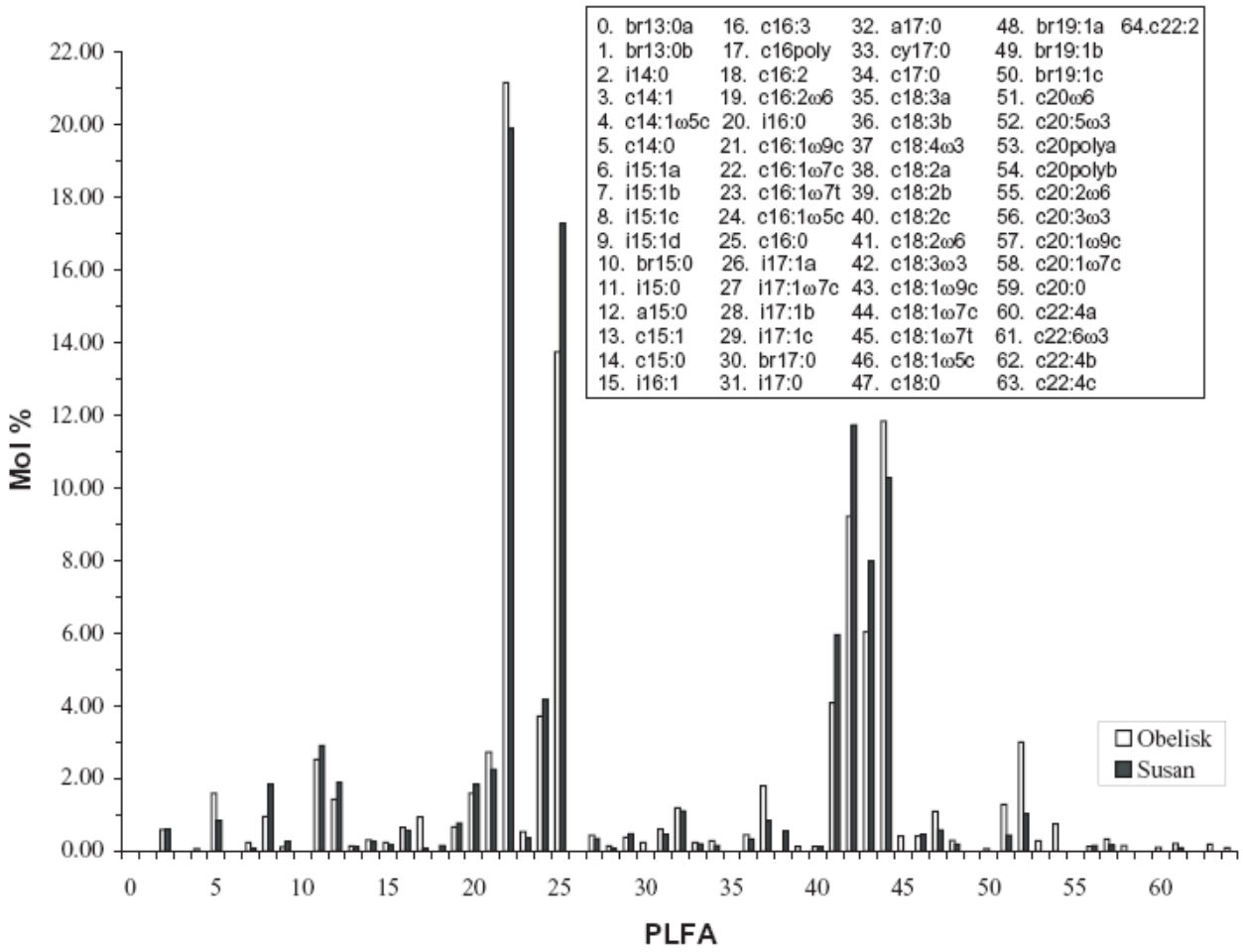

Figure 9. Typical PLFA profiles from Hjorth Hill Coast (Obelisk Pond) and Garwood Valley (Susan Pond) from the 04-05 season.

both sites, though not always abundant. On average, the total concentration of SRB biomarkers (a15:0, i15:0, a17:0, and i17:0) is 7.26 mol % for both sites during the 04-05 season.

For all microbial mats, the ratios indicative of stress-induced lipid loss (t/c 16:1ω7c, t/c 18:1ω7c, and cy 17/16:1ω7c) range from 0.01 to 0.06, indicating non-stressed communities (Fig. 10). The abundance of cy17:0 is typically below detection limits in most samples, but was detected in some at 1.43 mol %, whereas cy19:0 was not present in any sample.

Hjorth Hill Coast microbial mat biomass during the 04-05 season ranged from 2.38 x 10^4 pmol/g to 3.96 x 10^5 pmol/g, averaging 9.01 x 10^4 pmol/g. Garwood Vally microbial mat biomass during the 04-05 season ranged from 1.53 x $l0^4$ pmol/g to 5.97 x 10^5 pmol/g, averaging 1.53 x 10^5 (Fig. 11).

Compound-Specific Isotope Analysis of Lipid Biomarkers

The carbon isotopic composition of FAMES from a typical modern mat sample (Cody Pond 3) range from -8.44 ‰ to -30.75 ‰ (Fig 12; Table 5). The PLFAs are depleted in ^{13}C relative to the bulk $\delta^{13}C$ value (-9.83 ‰) anywhere from 2.19 ‰ to 20.92 ‰ with the exception of cy17:0, which has a $\delta^{13}C$ value of -8.44 ‰ and is thus enriched in ^{13}C by 1.39 ‰ (Table 5). The Gram-negative lipid biomarkers have $\delta^{13}C$ values ranging from -8.43 ‰ to -21.31 ‰, averaging -12.06 ‰ (Table 5). The microeukaryotes lipid biomarkers have $\delta^{13}C$ values ranging from -22.08 ‰ to -30.75 ‰, averaging -26.38 ‰ (Table 5).

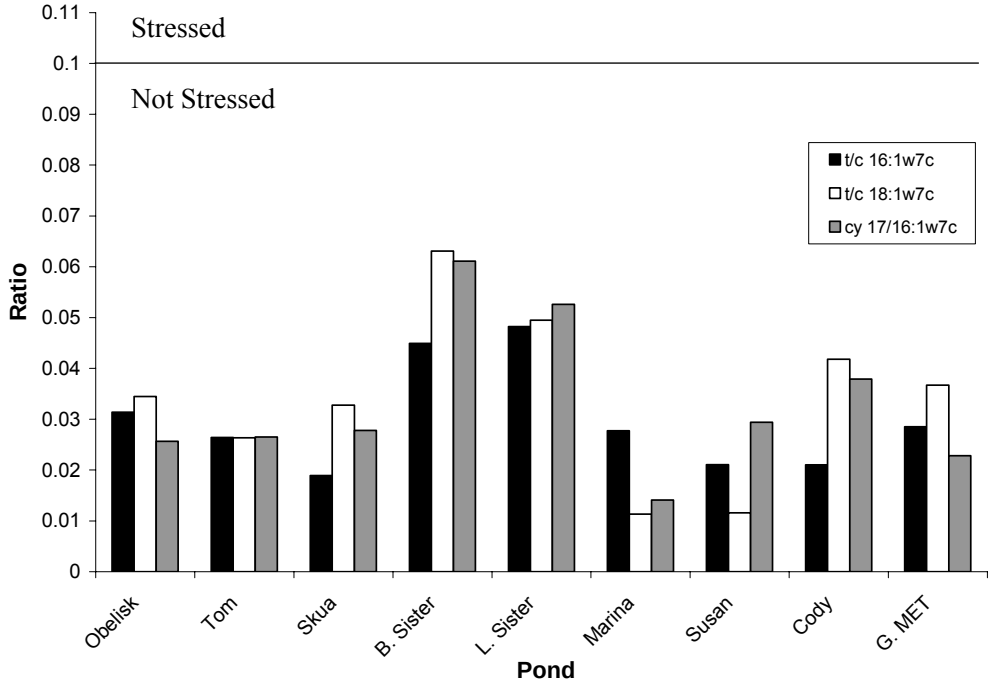

Figure 10. PLFA stress-indicating ratio values for microbial mats collected from Hjorth Hill Coast (Obelisk, Tom, Skua, B. Sister, and L. Sister Ponds) and Garwood Valley (Marina, Susan, Cody, G. MET Ponds).

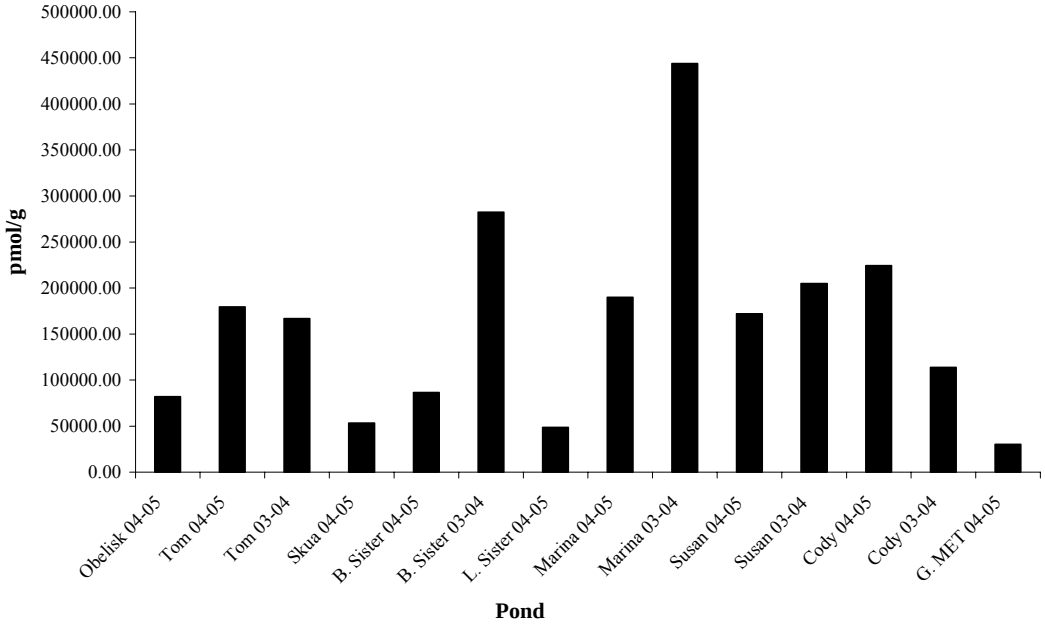

Figure 11. Average biomass for microbial mats from Hjorth Hill Coast (Obelisk, Tom, Skua, B. Sister, and L. Sister Ponds) and Garwood Valley (Marina, Susan, Cody, and G. MET Ponds). Values based on sum of PLFAs from each sample.

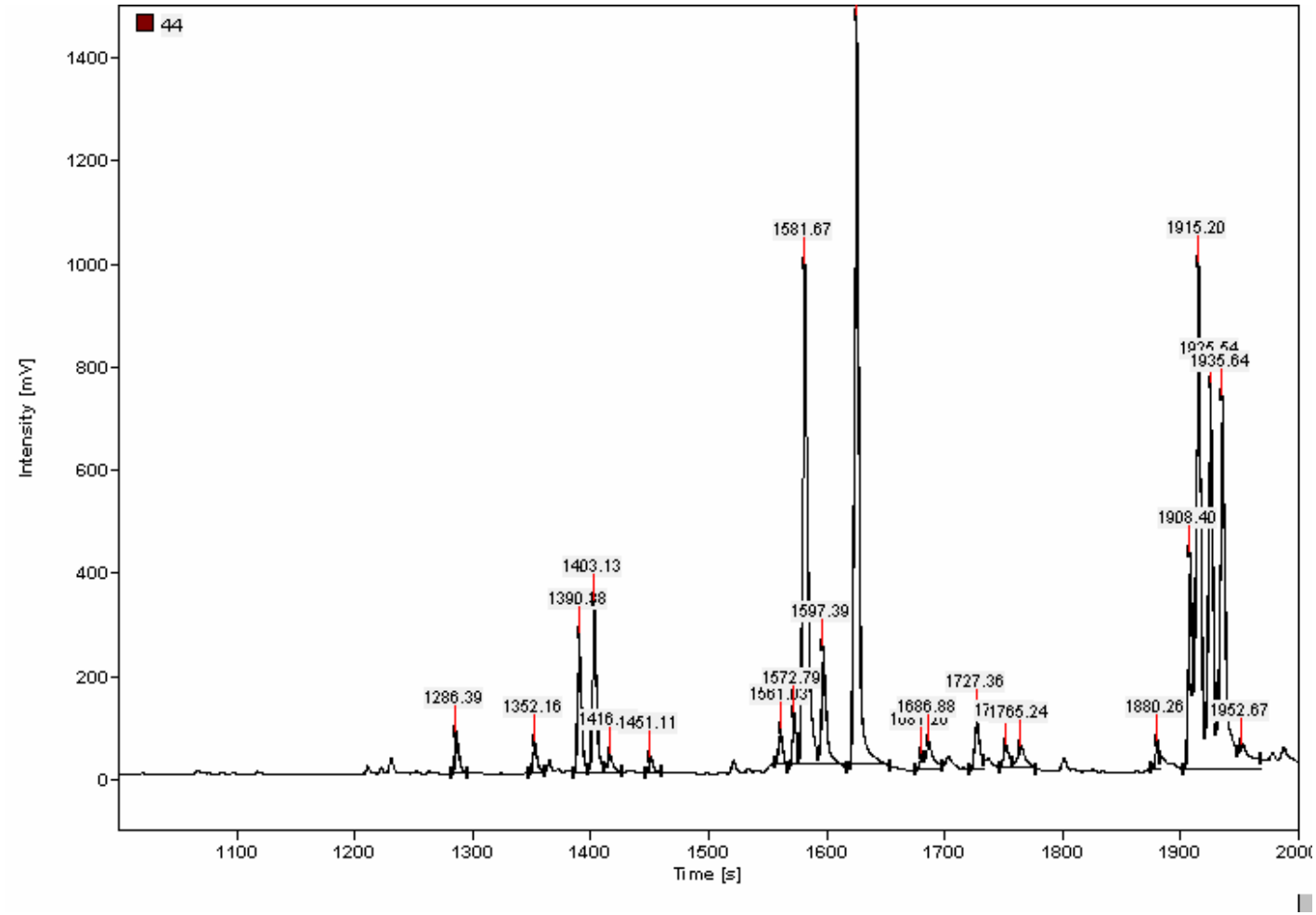

Figure 12. Typical GC/IRMS chromatogram from Cody Pond #3.

TABLE 5. CARBON ISOTOPIC COMPOSITION OF FAMES EXTRACTED FROM A MODERN MICROBIAL MAT SAMPLE, CODY POND

Cody Pond #3 04-05 season			
Retention Time (sec)	$\delta^{13}C$ (‰)	PLFA	Group of Organisms
1286.39	-17.26	i14:0	Gram-positives
1352.16	-15.60	14:0	All genera
1390.18	-18.96	i15:0	Gram-positives
1403.12	-20.23	a15:0	Gram-positives
1416.40	-12.99	15:1	Gram-negatives
1451.11	-14.29	15:0	All genera
1561.03	-16.18	i16:0	Gram-positives
1572.79	-17.62	16:1w9c	Gram-negatives
1581.67	-19.78	16:1w7c	Gram-negatives
1597.39	-12.02	16:1w5c	Gram-negatives
1625.50	-22.36	16:0	All genera
1681.30	-28.66	i17:1	Anaerobes
1686.88	-17.66	i17:1w7c	Anaerobes
1751.90	-21.58	a17:0	Gram-positives
1765.24	-8.44	cy17:0	Gram-negatives
1880.26	-30.75	18:4w3	Microeukaryotes
1908.40	-28.69	18:2w6	Microeukaryotes
1915.20	-24.08	18:3w3	Microeukaryotes
1925.54	-22.02	18:1w9c	Microeukaryotes
1935.64	-21.31	18:1w7c	Microeukaryotes

Microbial Mats

Based on visual observation, mats at Hjorth Hill Coast and Garwood Valley have surface layers rich in carotenoid pigment, producing an orange-brown color. Unlike the mats at Hjorth Hill, the mats at Garwood Valley also contain a distinct green layer beneath the surface layer that, based on visual observation and comparison to analogous mats, is rich in chlorophylls and phycocyanin. In analogous mats from other shallow, freshwater Antarctic environments, it has been shown that this green layer only receives approximately 5% of incident radiation, but is responsible for most of the autotrophic production (Hawes and Schwarz, 1999; Hawes and Schwarz, 2000).

Statistics

The Kruskal-Wallis test can be applied in the one-factor ANOVA case. It is a non-parametric test for the situation where the ANOVA normality assumptions may not apply. Because the Kruskal-Wallis statistical test requires at least five samples for the approximation to be valid, the only statistically sound comparison that can be made in this study is between the data collected at Hjorth Hill Coast during the 04-05 season and at Garwood Valley during the 04-05 season. During the 03-04 season, the Hjorth Hill Coast sample size is only two and the Garwood Valley sample size is only three. There is a statistical difference between Hjorth Hill Coast and Garwood Valley during the 04-05 season with regard to normal saturates, branched unsaturates, monounsaturates, temperature, salinity, dissolved oxygen, percent carbon, percent nitrogen, percent sulfur, $\delta^{15}N$, $\delta^{34}S$, $[SO_4^{2-}]$, $[Cl^-$, $[Na^+]$, $[K^+]$, $[Mg^{2+}]$, and $[Ca^{2+}]$ ($p \leq 0.1$) (Table 6).

TABLE 6. KRUSKAL-WALLIS STATISTICAL RESULTS

	Hjorth Hill 04-05 vs Garwood Valley 04-05		
	p-value	n (Hjorth Hill 04-05)	n (Garwood Valley 04-05)
Biomass (pmol/g)	0.275	13	11
Normal saturates (mol %)	**0.003**	13	11
Midchain branched saturates (mol %)	0.310	13	11
Terminally branched saturates (mol %)	0.691	13	11
Branched unsaturates (mol %)	**0.008**	13	11
Monounsaturates (mol %)	**0.010**	13	11
Polyunsaturates (mol %)	0.150	13	11
Temperature (°C)	**0.060**	13	11
pH	0.600	13	11
Salinity (ppt)	**0.001**	13	11
DO (mg/L)	**0.000**	13	11
% Carbon	**0.002**	13	11
% Nitrogen	**0.002**	13	11
$\delta^{13}C$	0.896	13	11
$\delta^{15}N$	**0.001**	13	11
% Sulfur	**0.013**	13	11
$\delta^{34}S$	**0.000**	13	11
SO_4 (mg/L)	**0.001**	5	4
Cl (mg/L)	**0.003**	5	4
Na (mg/L)	**0.001**	5	4
K (mg/L)	**0.001**	5	4
Mg (mg/L)	**0.036**	5	4
Ca (mg/L)	0.314	5	4

CHAPTER VI

DISCUSSION

Pond Water Chemistry

Garwood Valley microbial mats from the 04-05 season have higher biomass (154,154 pmol/g) than mats from Hjorth Hill Coast (90,059 pmol/g), greater % C and % N, and the ponds from Garwood Valley have higher concentrations of dissolved oxygen (15.69 mg/L) than ponds from Hjorth Hill Coast (12.59 mg/L). At Garwood Valley, the mats are thicker overall and more distinctly laminated than the mats at Hjorth Hill. Hjorth Hill Coast mats consist of a single orange layer, approximately 1-2 mm thick. Garwood Valley mats consist of an upper orange layer 1-2 mm thick, and a lower layer 2-3 mm thick of alternating light and dark green laminations. All of this supports greater productivity at Garwood Valley than at Hjorth Hill, which may be due to nutrient loading from greater water influx. The ponds at Garwood Valley receive more water than ponds at Hjorth Hill Coast as a result of the geomorphic setting of each site. Garwood Valley ponds are located in a U-shaped valley bounded by steep, snow-covered slopes, and the Garwood Valley River flows from the Garwood Glacier located at the head of the valley down to the sea ice. Increased water influx allows for increased nutrient loading, allowing the mats to be more productive and accumulate more biomass.

In addition to water influx, pond chemistry is also affected by evaporative/freeze concentrating mechanisms. During the freeze/evaporative concentration processes of the ponds, major ionic components may be removed from the aqueous phase into the solid phase as mirabilite ($Na_2SO_4 \bullet 10H_2O$). The greatest number of mirabilite deposits lie within a five-kilometer square area of stagnant, ice-cored moraine between the terminus of Hobbs Glacier and the coastline, which is only approximately 15 km north of Garwood Valley (Bowser et al., 1970). A study by Takamatsu et al. (1998) from the McMurdo Dry Valleys indicates that the salts in more saline, inland ponds are mainly derived from deep ground waters influenced by saline water-rock interaction, and salts in freshwater ponds are attributed to atmospheric deposition. An earlier study by Bowser et al. (1970) on coastal ponds near Hobbs Glacier indicates that the δD and $\delta^{18}O$ composition of mirabilite structural water is consistent with a mechanism involving crystallization from ponds whose water isotopic compositions is derived from glacial meltwater, not precipitation caused by freezing of seawater. However, stable isotope data for sulfate oxygen and sulfur indicate an ultimate marine source for the mirabilite sulfate (Bowser et al., 1970). Thus, it appears as though the salts are derived from a landward source (glaciers) which itself is derived from seawater (Bowser et al., 1970).

Garwood Valley has a substantially greater concentration of mirabilite than Hjorth Hill, which is consistent with greater anion and cation concentrations at Garwood Valley compared to Hjorth Hill, especially for SO_4^{-2} and Na^+, and suggests increased water influx at Garwood Valley. With increased water influx,

one would expect more salts to be dissolved and transported into the ponds. Marina and Cody Ponds fall directly on a mixing curve between mirabilite as and Garwood MET pond as the other end member, suggesting that their [Na^+] and [SO_4^{2-}] is consistent with the dissolution of mirabilite (Fig. 13). Garwood MET pond was selected as an end member because it has the lowest anion and cation concentration of all the ponds and represents the pond closest to freshwater.

Bulk Organic Carbon, Nitrogen, and Sulfur Isotopes

Bulk $\delta^{13}C_{org}$ values are useful for distinguishing Antarctic marine phytoplankton (MDOM -15 ‰ to -20 ‰), Taylor Valley lakes' deep benthic microbial mats (LDOM -20 ‰ to -35 ‰), and benthic microbial mats from moats surrounding the lakes (-2.7 ‰ to -19.6 ‰) (Lawson et al., 2004). Even though the CPDOM from Hjorth Hill Coast and Garwood Valley have similar $\delta^{13}C_{org}$ compositions to the moat BOM, bulk $\delta^{13}C_{org}$ can still be used to distinguish CPDOM from MDOM, LDOM and OOM. The coastal ponds partially or completely freeze to the bottom during winter, which likely results in decreased dissolved CO_2 concentrations. High summer growth rates and low CO_2 availability potentially results in a CO_2 limited ecosystem, which decreases carbon isotope fractionation with continued growth. Subsequently, bulk carbon isotopes become enriched in ^{13}C (Schouten et al., 2001). Also, in aquatic environments with pH between 6.5 and 10.3, such as the coastal ponds, the majority of the dissolved inorganic carbon (DIC) is in the form of bicarbonate (HCO_3^-), not CO_2. In this environment, microorganisms use both the enzyme

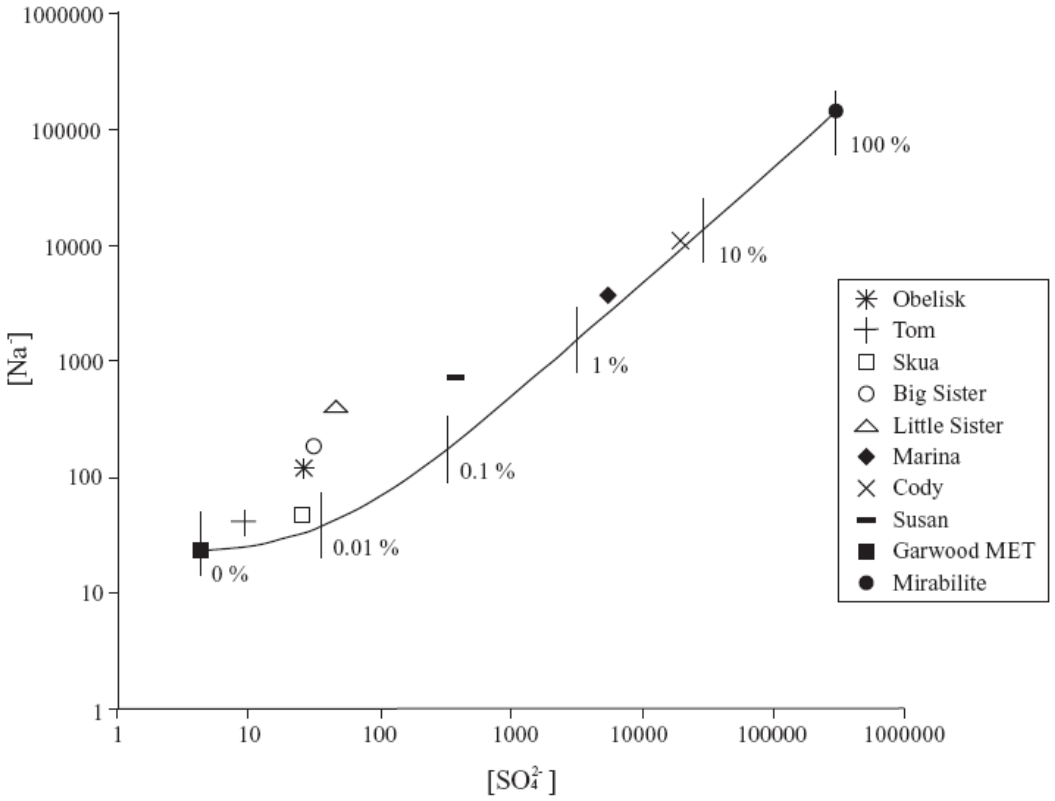

Figure 13. $[Na^+]$ vs $[SO_4^{2-}]$ for ponds at Hjorth Hill Coast and Garwood Valley. Curve represents mixing model with mirabilite and Garwood MET Pond as its end members.

ribulose 1,5-bisphosphate carbonylase-oxygenase (Rubisco), which utilizes CO_2, and the enzyme phosphoenolpyruvate-carboxylase (PEPC), which utilizes HCO_3^-, for carbon fixation (Goericke et al., 1994). The carbon isotope ratio of HCO_3^- is ~8 ‰ more positive than that of dissolved CO_2 (Fogel and Cifuentes, 1993). Thus, it is not surprising that the modern microbial mats found in the coastal ponds are enriched in ^{13}C (-11.26 ‰ at Hjorth Hill Coast; -9.89 ‰ at Garwood Valley) compared to other microbial mats found in the dry valleys. CPDOM and moat BOM are expected to be isotopically similar because they are both shallow ecosystems that freeze solid in the winter, thaw by mid-summer, and have high summer growth rates.

About one half of the examine Hjorth Hill Coast mats have greater $\delta^{15}N$ values than mats found in Garwood Valley, as well as mats from Taylor Valley lakes, moats, and streams. Although not all of the Hjorth Hill Coast mats have $\delta^{15}N$ as enriched in ^{15}N as the fresh gull excreta, mats from Big Sister and Little Sister Ponds have $\delta^{15}N$ values only 1-2 ‰ off from the lower end of the fresh gull excreta range (Fig. 7) (Mizutani and Wada, 1998). Hjorth Hill Coast is a nesting site for Antarctic skua (*Catharacta maccormicki*), which excrete waste directly into the ponds. These birds are especially prominent in and around Big Sister and Little Sister Ponds and might represent plausible explanation for the elevated $\delta^{15}N$ values in some samples. ^{15}N-enriched isotopic compositions from ornithogenic sources result from ^{15}N enrichment through marine trophic levels and secondary ammonia volitization. This source of organic matter is not present at Garwood Valley or Taylor Valley since skuas are not present.

The $\delta^{34}S$ values do not identify a source; however, since there is no overlap in the $\delta^{34}S$ values, they can be used to distinguish CPOM from Hjorth Hill Coast and Garwood Valley. With the exception of Big Sister Pond, the $\delta^{34}S$ values at Hjorth Hill Coast range from 4.24 ‰ to 9.20 ‰; and $\delta^{34}S$ values at Garwood Valley range from 11.96 ‰ to 19.80 ‰. The reason for the differences in $\delta^{34}S$ values is unclear. The two sites have similar geologic settings, they are about the same distance from the Ross Sea, they are about the same distance from Mt. Erebus, the nearby active volcano, and they both have similar microbial mat community compositions.

Lipid Biomarkers

With the exception of microbial mats from Marina Pond from the 04-05 season and Cody Pond from the 03-04 season, all mats are dominated by monounsaturated fatty acids, which are indicative of gram-negative bacteria, aerobic prokaryotes and eukaryotes (Findlay and Dobbs, 1993; Navarrete et al., 2000). Gram-negative bacteria have cell walls that contain small amounts of peptidoglycan and, characteristically, lipopolysaccharides, as opposed to gram-positive bacteria that have cells walls that contain large amounts of peptidoglycan and no lipopolysaccharides. Common examples of gram-negative bacteria include cyanobacteria, green sulfur bacteria, and green nonsulfur bacteria (Madigan et al., 2003). The ω9 monounsaturated PLFAs result from the aerobic desaturase pathway common to all cells, and the ω7 PLFAs result from the anaerobic desaturase pathway, which is most commonly a prokaryotic biochemical pathway (Navarrete et al., 2000). The second most prevalent group

of fatty acids for all ponds, which is also the most prevalent group at Marina Pond 04-05 and Cody Pond 03-04, is polyunsaturates. These fatty acids are indicative of microeukaryotes. Although the branched unsaturated fatty acids are not a dominant group in the coastal pond microbial mats (3.01 to 5.32 mol %), they may be important biomarkers since they are common in the anaerobic *Desulfovibrio*-type sulfate-reducing bacteria (Navarrete et al., 2000).

Unsaturated PLFAs 16:1ω7 and 18:1ω7 are increasingly converted to the cyclopropyl fatty acids cy17:0 and cy19:0, respectively, in Gram-negative bacteria as the microbes move from a logarithmic to a stationary phase of growth (growth without cell division). This shift can occur in microbes when carbon source(s) and terminal electron acceptors are present but some essential nutrients are not available (Navarrete et al., 2000). Since the abundance of cy17:0 is extremely low and the there is no cy19:0 present in any of the microbial mat samples from Hjorth Hill Coast or Garwood Valley, it is likely that the microbial mats are not lacking in any essential nutrients and remain in a stationary logarithmic growth phase for the duration of the austral summer growing season. This conclusion is supported by the low (< 0.1) *trans*/*cis* ratios, since ratios > 0.1 indicate metabolic stress (e.g., toxicity, starvation) (Navarrete et al., 2000).

Benthic microbial mats, whose uppermost layers are dominantly cyanobacteria, are ubiquitous in most melt-water environments of Antarctica, including coastal ponds, streams, and moats surrounding the large, perennially ice-covered lakes found in the dry valleys (Parker et al., 1981; Hawes, 1993; Hawes et al., 1999; Hawes et al., 2001; de los Rios et al., 2003; Lawson et al.

2004; Jungblut et al., 2005). Microbial mats in similar ponds found on the McMurdo Ice Shelf near Bratina Island are dominated by cyanobacteria belonging to the filamentous order Oscillatoriales (*Phormidium* sp., *Oscillatoria* sp. and *Lyngbya* sp.) and Nostocales (*Nostoc* sp. and *Nodularia* sp.) (Hawes et al., 1999; Jungblut et al., 2005). During most austral summers, the edges of large, perennially ice-covered lakes found in the McMurdo Dry Valleys melt, creating ice-free moats that can be up to 3 % of the lake surface area. These moats contain benthic microbial mats with a variety of cyanobacterial species, dominantly *Phormidium*, *Oscillatoria*, *Plectonema*, and *Nostoc* (Lawson et al., 2004).

Compound-Specific Isotope Analysis of Lipid Biomarkers

Typically, compound-specific isotope work is utilized in tracer studies and studied designed to elucidate specific biogeochemical processes in microbial populations; however, these studies all involve the ^{13}C-labeling of biomarkers or other compounds (Boscker et al., 1998). This study only examined the $\delta^{13}C$ of biomarkers with natural abundances. All of the modern microbial mat PLFAs in the study are depleted in ^{13}C compared to the bulk biomass, except for cy17:0 which is enriched by 1.39 ‰. The relationship of lipids being depleted is ^{13}C relative to bulk biomass is well established in the literature and is attributed to the discrimination of carbon isotopes during the oxidation of pyruvate to acetyl coenzyme A just prior to the start of the citric acid cycle (Abraham et al., 1998; DeNiro and Epstein, 1977). The biomarkers indicative of gram-negative bacteria, such as cyanobacteria, range from -8.43 ‰ to -21.31 ‰; and the biomarkers indicative of microeukaryotes range from -22.02 ‰ to -30.75 ‰. The maximum

fractionation in cyanobacteria is expected to be lower than that of eukaryotes because the isotope effect associated with cyanobacterial forms of rubisco is smaller (Sakata et al., 1997).

Year-to-Year Comparison

Samples were collected approximately one month earlier in the austral summer during the 03-04 season compared to the 04-05 season in order to determine the degree of variability within and between the ponds. Ponds were sampled at Hjorth Hill Coast in early December 2003 and late December 2004 through early January 2005. Ponds were sampled at Garwood Valley in late December 2003 through early January 2004 and January 2005. During the 03-04 season, ponds at Hjorth Hill Coast were still partially frozen. Thus, in order to collect samples and *in situ* measurements, a hole was made in the pond ice. By the time ponds were sampled at Garwood Valley during the 03-04 season, they were completely thawed. During the 04-05 season, all of the ponds at both sites, with the exception of Garwood MET Pond, were completely thawed when sampled.

The data collected from the 03-04 season should be considered preliminary since only five of the nine selected ponds contained microbial mats, some of the ponds were still frozen when sampled, and only one microbial mat sample from each pond was collected. Also, the microbial mat samples from Hjorth Hill Coast (Tom and Big Sister Ponds) collected during the 03-04 season are not viable benthic microbial mats since the pond was too deep to collect benthic samples at the center of the pond where the hole was made in the ice.

Those samples are most likely benthic microbial mats that lifted off the bottom due to gas bubbles and had been floating in the water column for some unknown amount of time.

At Hjorth Hill Coast, microbial mats from the 03-04 season, in comparison to the 04-05 season, had greater biomass, greater percent of normal saturated and monosaturated fatty acids, lower percent of polyunsaturated fatty acids, were depleted in ^{13}C and ^{15}N, were enriched in ^{34}S, had a greater % C and % N, and had a lower % S. The Hjorth Hill Coast pond water in the 03-04 season had lower temperatures, pH, salinity, dissolved oxygen, $[SO_4^{-2}]$, $[Cl^-]$, $[Na^+]$, $[K^+]$, $[Mg^{+2}]$, and $[Ca^{+2}]$ compared to the 04-05 season. At Garwood Valley, microbial mats from the 03-04 season, in comparison to the 04-05 season, had greater biomass, greater percent of monosaturated fatty acids, lower percent of polyunsaturated fatty acids, were enriched in ^{15}N and ^{34}S, were depleted in ^{13}C, had a greater % C and % N, and had a lower % S. The Garwood Valley pond water in the 03-04 season had lower temperatures, salinity, $[SO_4^{-2}]$, $[Cl^-]$, $[Na^+]$, $[K^+]$, $[Mg^{+2}]$, and $[Ca^{+2}]$ compared to the 04-05 season, but had higher pH and dissolved oxygen.

The microbial mats during the 03-04 season are composed of a single, thin orange layer. Since the top layer of most microbial mats is dominated by cyanobacteria, it is not surprising to find a greater percent of monounsaturated fatty acids, which are indicative of Gram-negative bacteria, such as cyanobacteria, during the 03-04 season compared to the 04-05 season. Since the ponds were still partially frozen, or had just thawed when samples were collected during the 03-04

season, it is likely that the mats did not have enough time to develop thicker, more laminated mats, like they did before they were collected during the 04-05 season. Compared to the 03-04 season, mats had more time in more ideal growing conditions (i.e. warmer temperatures and sunlight) before they were collected during the 04-05 season.

The assumption was made before the data was analyzed that the mats would be more productive, and thus have greater biomass and percent carbon and nitrogen, during the second season when the mats had more time in favorable growing conditions than during the first season. Also, the mats would fractionate more since more carbon and nitrogen would be present in the non-frozen ponds. However, what was found was the mats had greater biomass, greater percent carbon and nitrogen, and were more depleted in ^{13}C in the 03-04 season compared to the 04-05 season. It may be that the fully developed mats are getting frozen at the end of the austral summer season and remain in some form of stasis, so that when the ponds thaw the next austral summer, new mats are being established on older mats that already have a great deal of biomass produced. This trend may also be explained by a not yet determined mechanism, and more detailed sampling throughout the course of an entire year is required to fully understand this complex ecosystem.

There are numerous variables that control the biogeochemistry of the coastal pond environment and, based on the data collected over two years at two different sites, there is a great deal of variability within this ecosystem. The landscape surrounding the coastal ponds is constantly changing. There are spatial

and temporal changes in temperature, amount of snow melt, amount and location of ground ice melt, amount and source of run-off, etc. that all affect the coastal pond environment. Despite the ranges seen in the microbial mat isotopic signatures and lipid profiles and in the aqueous chemistry of the pond water, the coast pond environment and the CPOM is distinct from other environments within the McMurdo Dry Valleys (i.e. large ice-covered lakes, streams, soils).

CHAPTER VII

CONCLUSIONS

Ponds at Garwood Valley have significantly greater salinity, dissolved oxygen, $[SO_4^{2-}]$, $[Cl^-]$, $[Na^+]$, $[K^+]$, $[Mg^{2+}]$, and $[Ca^{2+}]$ ($p \leq 0.05$; except for $p \leq 0.1$ for $[Ca^{2+}]$) than ponds at Hjorth Hill. However, difference is salt content does not seem to affect primary productivity. Ponds at both sites are supersaturated with respect to dissolved oxygen. All pond water at both sites was slightly basic, resulting in HCO_3^- being the dominant DIC species, not $CO_{2\ (aq)}$, which is reflected in the $\delta^{13}C$ values being enriched in ^{13}C compared to other aquatic environments within the McMurdo Dry Valleys.

The carbon and nitrogen isotopic composition of Hjorth Hill Coast benthic microbial mats range from -8.5 ‰ to -14.88 ‰ (average -10.91 ‰) for $\delta^{13}C$ and 0.8 ‰ to 6.95 ‰ (average 3.62 ‰) for $\delta^{15}N$. At Garwood Valley, $\delta^{13}C$ values for benthic microbial mats range from -5.86 ‰ to – 15.70 ‰ (average -10.19 ‰), and $\delta^{15}N$ values range from -3.98 to 0.89 (average -1.25 ‰). $\delta^{13}C$ values are enriched in ^{13}C compared to other sources of organic matter in the dry valleys as a result of low CO_2 concentrations due to ponds completely freezing in winter and to high summer benthic microbial mat growth rates. ^{13}C enrichment is also due to HCO_3^-, which is ~8‰ more positive than dissolved CO_2, being the dominant component of the pond DIC due to the alkalinity of the pond water. While the CPDOM is similar to the Taylor Valley large, permanently ice-covered lake moats' BOM,

bulk $\delta^{13}C_{org}$ and bulk $\delta^{15}N_{org}$ can be used to distinguish CPDOM from other sources of organic matter in the dry valleys. Bulk $\delta^{15}N_{org}$ can also be used to distinguish CPDOM from different coastal ponds, depending on the ornithogenic influence at the site. These results agree with Burkins et al. (2000) that $\delta^{15}N$ is a better tracer of organic matter in the McMurdo Dry Valleys than $\delta^{13}C$.

Microbial mats from both Hjorth Hill Coast and Garwood Valley are dominated by monounsaturated PLFAs, which are indicative of gram negative bacteria, and polyunsaturated PLFAs, which are indicative of microeukaryotes. The $\delta^{13}C$ of the PLFAs are depleted in ^{13}C compared to the bulk biomass. All microbial mats have stress-induced lipid loss ratios of less than 0.1, indicating healthy, non-stressed microbial communities are present. Although the community structure is similar at both sites, the microbial mats from Garwood Valley are more productive than mats from Hjorth Hill. This is supported by the mats from Garwood Valley having higher biomass, greater % C and % N, and being thicker and more laminated than mats from Hjorth Hill, and the ponds from Garwood Valley having a greater concentration of dissolved oxygen than the ponds from Hjorth Hill.

There is spatial and temporal variability within the coastal pond environment, with regard to the isotopic composition and community structure of the modern microbial mats and the aquatic chemistry of the pond water. Despite the ranges seen is each of the variables pond to pond and year to year, the coastal pond environment and the CPOM are distinct from other ecosystems in the McMurdo Dry Valleys.

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