MICROBIAL ECOLOGY OF AN ANTARCTIC SUBGLACIAL ENVIRONMENT

by

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APPROVAL

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This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Jill Ann Mikucki

October 31, 2005

To Dr. David Boone for opening the door to the world

of microbial ecology and inviting me to step in;

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ABSTRACT

The research presented in this dissertation focused on the microbial ecology of the subglacial discharge from the Taylor Glacier in the McMurdo Dry Valleys, Antarctica. The major objectives of my research were to 1) define the biogeochemistry of the subglacial outflow 2) describe the microbial diversity of the subglacial outflow and 3) examine the impact of subglacial outflow on the geochemistry and biology of the west lobe of Lake Bonney, a lake that abuts the glacier.

The subglacial outflow from the Taylor Glacier is known as Blood Falls owing to a visible accumulation of iron-oxides at the point where it flows from the snout of the glacier. The subglacial reservoir is thought to originate from the Pliocene Epoch (~5 Mya) when the dry valleys were fjordlands. The episodic release of subglacial water at Blood Falls provides a sample of what is believed to be ancient seawater trapped in the upper Taylor Valley and eventually covered by the Taylor Glacier as it advanced. Biogeochemical measurements, culture-based techniques, and molecular analysis (based on 16S rDNA sequences), were used to characterize microbes and chemistry associated with the subglacial outflow. Culture and molecular-based techniques, along with geochemical data, indicate the presence of a diverse chemoautotrophic and heterotrophic bacterial assemblage that utilizes iron and sulfur minerals for growth. 16S rDNA clone library phylotypes and cultured isolates were related to organisms that inhabit permanently cold environments. The biological and geochemical component of the ancient outflow changes as it travels from the subglacial environment to the moat of the west lobe of Lake Bonney and eventually into the saline deep water in the lake proper. The bottom water of the west lobe of Lake Bonney is geochemically similar to ancient subglacial outflow, but the microbial diversity in the two systems is distinct, and ancient subglacial brine does not appear to provide the microbial seed for the deep water of Lake Bonney. Collectively, my data indicate that the habitat beneath the Taylor Glacier harbors a functional microbial ecosystem that utilizes chemosynthetic and heterotrophic activity to obtain carbon and energy in the absence of light.

CHAPTER 1

AN OVERVIEW OF ICY SYSTEMS

Organization of the Dissertation

Chapter 1 is an introduction to cold environments with a focus on the subglacial environment. This chapter establishes the background and significance for my dissertation entitled, Microbial Ecology of an Antarctic Subglacial Environment, as well as provides a site description and presents the major hypotheses addressed by my research.

The remainder of my dissertation focuses on the subglacial outflow of the Taylor Glacier (Blood Falls) and is presented as individual manuscripts that have been published (Chapter 2) or are in preparation for submission to scientific journals (Chapters 3, 4, and 5). Chapter 2, published in *Aquatic Geochemistry* (Mikucki et al., 2004), describes the detection of a metabolically active microbial assemblage associated with subglacial outflow from the Taylor Glacier. The data presented in this chapter resulted from an initial survey of Blood Falls and led to the hypotheses-driven research presented in the subsequent chapters. The remaining chapters were written as individual manuscripts and as such, repetition in the methods and background sections of the chapters exists. Chapter 3 illustrates the episodic nature of subglacial release events as revealed through biogeochemical analyses. Bacterial diversity associated with Blood Falls outflow is described in Chapter 4. Chapter 5 presents data on how this subglacial outflow

influences the west lobe of Lake Bonney, a proglacial lake that abuts the Taylor Glacier. The final chapter (Chapter 6) summarizes my major conclusions and makes recommendations for future research.

Several icy Antarctic systems were explored before this dissertation focused specifically on the subglacial environment below the Taylor Glacier. Appendix A describes this exploration through the collection and processing of samples from frozen environments including lake ice and deep glacial ice. Working with these deep glacial ice and lake ice samples helped establish protocols for sampling Blood Falls. Appendix A also presents decontamination protocols that I helped develop for samples from the deep glacier ice that were recently published (Christner et al., 2005). Public outreach became a synergistic goal with the research objectives of this dissertation and resulted in "Living Ice," a documentary film and laboratory curriculum designed to introduce high school students to the importance of Antarctic microbiology. The production of "Living Ice" is described in Appendix B.

Introduction

Cold Ecosystems

More than 80% of the Earth's biosphere is permanently cold (Russell, 1990) with much of it existing as ice. Cold, icy environments create a surprising diversity of niches for microbial life (Price, 2000, Deming, 2002, Priscu and Christner, 2004). Approximately 20% of our planet's soil ecosystem is permafrost and supports microorganisms capable of growth at temperatures well below freezing (Gilichinsky and Wagener, 1995, Bakermans et al, 2003). Sea ice at its maximum extent covers

approximately 5% of the Northern Hemisphere and 8% of the Southern Hemisphere by volume, (Psenner et al., 2002, Thomas and Dieckmann, 2002). Sea ice microbial communities are an important component of high latitude marine trophic food webs and the overall productivity of the polar oceans (Karl, 1993, Brown and Bowman, 2001, Mock and Thomas, 2005). The majority (70%) of the Earth's freshwater is in the form of ice, which resides in Antarctica. Liquid veins in deep poly-crystalline glacial ice have been hypothesized to be suitable microbial habitats (Price, 2000) and viable microbes have been isolated from deep glacier ice cores (Priscu et al., 1999, Christner et al., 2001). The terrestrial regions of high latitudes contain frozen soils, permanent lake ice and glacier ice, all of which provide a refuge for microbial growth in what would otherwise be considered inhospitable environments. The perennial lake ice and glaciers of the McMurdo Dry Valleys, for example, host microbial assemblages associated with sediment inclusions that carry out phototrophic primary production, nitrogen fixation and heterotrophic decomposition (Priscu et al, 1998; Pearl and Priscu, 1998). Sediment inclusions on glaciers, called cryoconites, are hotspots for microbial activity which significantly alter the geochemistry of the glacier (Wharton et al., 1985, Tranter et al., 2004). Recently, microbial activity has been detected in subglacial samples including subglacial outflow, glacier basal ice and basal sediments (Sharp et al., 1999, Skidmore et al., 2000, Foght et al., 2004, Mikucki et al., 2004). Such subglacial microbial metabolism can play an important role in subglacial weathering and carbon cycling on glacial-interglacial timescales (Sharp et al., 1999). Research on icy systems over the past decade has changed the way we view these systems. We now know that they harbor diverse microbial assemblages and play an important role in global processes.

Early Earth's History. Earth's icy systems are important in understanding the history of life on Earth. The geologic record strongly supports distinct global glaciations known as "Snowball Earth" events (Kirschvink, 1992, Kirschvink et al., 2000). Though it is argued that these events would have significantly curtailed biological activity (Hoffman et al., 1998, Kirshivink et al., 2000, Hoffman and Schrag, 2002), polar ecologists point out that even during a global glaciation, surface ice could create an important refuge for microbial life (Vincent et al., 2000, Vincent and Howard-Williams, 2000). Life within icy refugia might in fact experience enhanced evolutionary selection pressures similar to modern sea ice and cryoconite microbial communities (Vincent et al., 2000; Deming et al., 2002, Priscu and Christner, 2004). Kirschvink (2000) and colleagues argue that the Snowball Earth event hypothesized to have occured 2.4 billion years ago, forced the adaptation of microorganisms to increased concentrations of atmospheric oxygen. Another significant Snowball Earth event, 600-800 million years ago (Mya), corresponds with the first appearance of multicellular life at approximately 700-1,000 Mya (Hyde et al., 2000). These global glaciations coincide with major milestones in Earth's biological history, suggesting that life has had to pass through a number of "glacial" bottlenecks (Hoffman et al., 1998, Hyde et al., 2000). Organisms existing in ice and below ice sheets provide clues to biological evolution and survival throughout Earth's history.

Extraterrestrial Cold Ecosystems. The discovery of planetary satellites with possible ice-covered oceans has expanded our interpretation of the solar system's 'habitable zone' (Squyres et al., 1983, Jakosky and Shock, 1998). Research on the

moons of Jupiter, including Europa and Callisto, has shifted the interest of exobiologists to the study of life suited for growth at low temperatures (Reynolds et al., 1983, Chyba and Phillips, 2001, Zimmer et al., 2000). Current studies of Mars, our closest planetary neighbor, have revealed the presence of water and CO_2-ice at the poles and in near to deep subsurface ice deposits; with increasing evidence for mid- to low-latitude ice and permafrost features (i.e. Clifford et al., 2003, Kuzmin, 2005). The recent detection of methane on Mars (Formisano et al., 2004) is also of exobiological interest as the majority of methane (70%) in the Earth's atmosphere is biogenic in origin (White, 2000).

The McMurdo Dry Valleys of Antarctica provide the closest earthly analog to current climatic conditions on Mars. The glaciated and permafrost features of the dry valleys, in particular, are model systems for the study of physical and presumably biological Martian processes (Cameron et al., 1976, Priscu et al., 1998, Nealson, 1999, McClune et al., 2003, McKay, 1993, McKay et al., 2005) and may reveal potential Martian microbial niches.

The great expanse of our Universe has an average temperature of near absolute zero (Smoot et al., 1992). With the preponderance of life on our planet living in low temperature environments, life throughout the Universe might also be well-suited for cold systems, thus making the search for extraterrestrial life a search for psychrotolerant or psychrophilic organisms.

Microbial Diversity

"Biological diversity is the key to the maintenance of the world as we know it."

-E.O. Wilson, 1992

Biological diversity has been a central theme in ecology for more than a century (Magurran, 1988). Understanding the role of diversity in ecosystem function is critical for habitat management and the response of ecosystems to global environmental changes (NRC, 1999). Additionally, the promise of broad biotechnological applications including pharmaceuticals, biofuels, and raw materials may await in the biocompounds of undiscovered microorganisms (Bull, 2004). Biological diversity is important to the function of the Earth system, yet relatively little is know about microbial diversity and the vast majority of prokaryotic species remain undescribed. Despite the challenges of obtaining cultured representatives of this undescribed prokaryotic majority, such work is an ecologically and commercially important endeavor. Basic curiosity (Bull and Stach, 2004), surveys of the natural history of a geographical region (Floyd et al., 2005), understanding biogeochemical cycling and how life is possible in extreme niches (Tiedje, 1995) and bioprospecting, should drive microbial isolation initiatives. Since most microbes in nature remain uncultivated using traditional methods, molecular techniques have been developed to characterize community diversity at the level of genes encoding the small subunit ribosomal RNA (Pace, 1997). Many molecular methods, such as gene sequencing, still limit our ability to understand the ecological function of specific microorganisms in naturally occurring populations. Putative activity assignments can only be based on the functions attached to cultivated phylogenetic relatives and even these culture-based methods remain equivocal when it comes to determining *in situ*

activity. Therefore, any study of biodiversity of a particular habitat should use polyphasic approaches that combines cultivation, molecular characterization and microscopic approaches (Tiedje, 1995). Biodiversity studies in concert with biogeochemical measurements will provide important information on the role of diversity in ecosystem function; a relationship that currently remains largely unresolved.

Microbial Diversity in Extreme Ecosystems. Microorganisms are the dominant biota in permanently cold environments (Block, 1984) and therefore play an integral role in the ecology of the cold extremes. The Antarctic interior represents an entire continent dominated by microorganisms and is a paramount site for the study of the microbial diversity of frozen systems. Research in the polar regions, and in Antarctic specifically, has increased in the last decade (e.g. Deming, 2002, Priscu and Christner, 2004). However, less than five percent of the publications on Antarctica have focused on microbial ecology (Figure 1.1), and polar regions are currently the most underrepresented group (only 1.3%) of accessions in the American Type Culture Collection (Floyd et al., 2005). Answering important ecological questions such as the pattern and function of microbial diversity in the Antarctic requires additional research. A general decrease in plant and animal species diversity from the tropics towards the poles is an accepted ecological paradigm, (Rosenzweig, 1995) but does microbial diversity also decrease in the polar regions? An emerging story that will hopefully fuel future polar ecology studies is that Antarctic microbiota may be surprisingly diverse (Tindall, 2004).

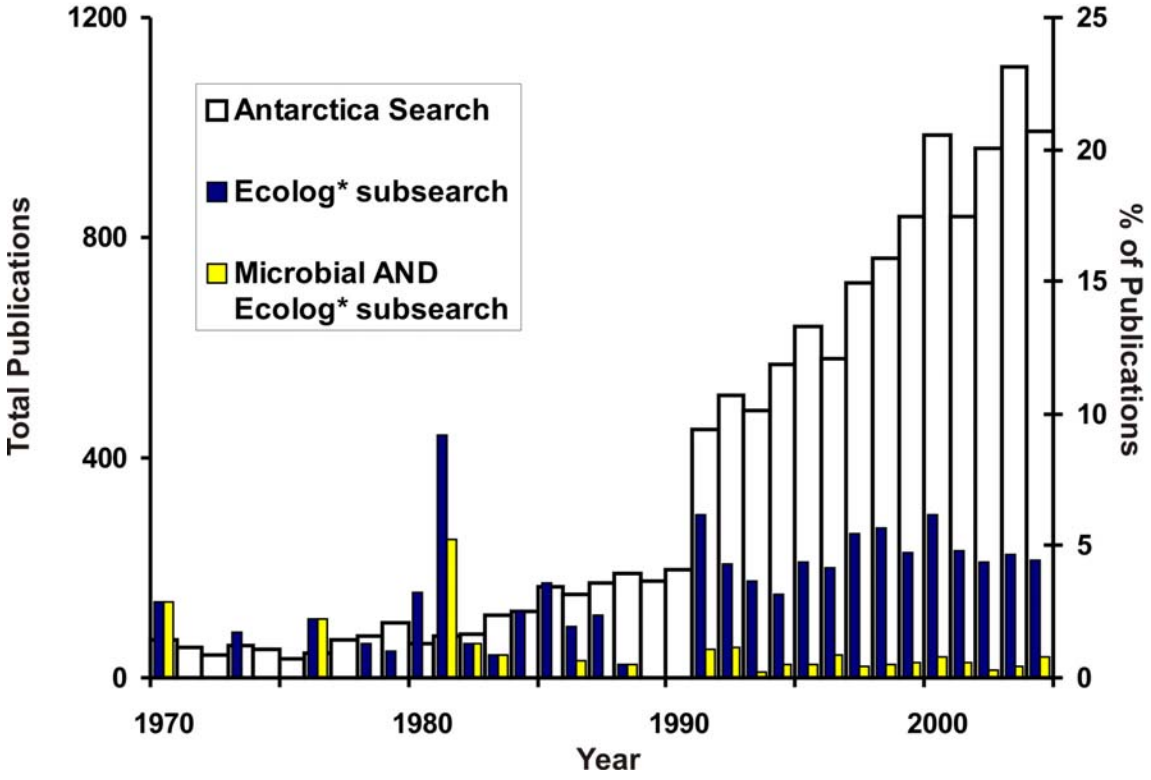

Figure 1.1. Number of publications returned for a Web of Science search (http://portal01.isiknowledge.com/portal.cgi) using "Antarctica" as the search term. Subsearches within all Antarctic entries using "Ecolog*" and "Microbial AND Ecolog*" are graphed as the percent of total entries on the secondary axis.

Diversity Studies in Antarctica. Antarctica is the highest, driest and coldest place on Earth. It comprises 10% of the Earth's land surface with only 2% of the continent being ice free; the remaining portion is covered by an average ice depth of 2.5 km (Walton, 1984, Priscu and Christner, 2004). The terrestrial flora of Antarctica is dominated by cryptogams, or small, encrusting plant forms; while the fauna contains a limited number of animals such as rotifers, nematodes and tardigrades (Block, 1984; Moss, 1988). Plant and animal life is primarily confined to the peninsula and maritime

western areas north of 70ºS latitude (Smith, 1984). The remainder and vast majority of Antarctic continental residents are microorganisms.

Microbiological studies in the Antarctic began with the first expeditions to the southernmost continent during the early 1900's. Erik Ekelöf, a bacteriologist on the Swedish 1901-1903 expedition, and the first Antarctic microbial ecologist, monitored the temporal changes in the soil microbiota and obtained numerous isolates of bacteria and yeasts (Block, 1984). The Viking missions to Mars, in the 1960's and 1970's, played an important role in motivating microbial ecology studies in the McMurdo Dry Valleys (Vishniac et al., 1966). The dry valleys were used as a living laboratory for both planning and testing life detection systems for these missions and interpreting the results of experiments conducted on Martian soils (Cameron et al., 1976, Nealson, 1999). In recent years, the discovery of subglacial lakes below the East Antarctic Ice Sheet has brought increased attention to the microbiology of the interior glacier ice and what lies below (Abyzov et al., 1998, Priscu et al., 1999; Karl et al., 1999; Christner et al., 2001, Priscu and Christner, 2004, Christner et al., 2005). However, most studies of Antarctic terrestrial ecology have focused primarily on the ice free oases around the continental margins, due largely to the inaccessibility of the interior continent (Walton, 1984).

The McMurdo Dry Valleys. The McMurdo Dry Valleys, located in southern Victoria Land, comprise the largest ice-free expanse on the Antarctic continent (Priscu, 1998). The exposed terrestrial systems of the dry valleys are ancient landscapes with deeply imprinted ecological legacies thus providing an excellent milieu for studying the links between paleohistory, chemistry and microbial diversity. The dry valleys are cold

polar deserts with striking landscape features, including large alpine glaciers, permanently ice-covered lakes, and arid soils. Extensive studies of the dry valley lakes, glaciers and soils began in the early 1960's with the work of Angino and colleagues, and have continued to the present day (Angino et al., 1964, Goldman et al., 1967, Hendy et al., 1977, Vincent, 1988, Priscu, 1998). The establishment of the McMurdo Dry Valleys as a National Science Foundation Long Term Monitoring Research (LTER) and Microbial Observatories (MO) site has exponentially increased the synergistic, multidisciplinary studies within the dry valleys ecosystems (Priscu et al., 1999, Lyons et al., 2001, Doran et al., 2002).

The ice-covered lake systems harbor hardy microbial communities where protozoans represent the highest trophic level (Priscu, 1997, Priscu et al., 1999). Microbial mats on the lake margins are among the most visible biota in the valleys, and have yielded many novel isolates (Vincent et al., 1993; Brambilla et al., 2001). The soil communities are considered among the simplest on Earth (Freckman and Virginia, 1997) and must contend with extreme conditions of desiccation, salinity and freezing (Vincent, 1988). Even rock formations in the dry valleys harbor novel communities of photosynthetic organisms, known as cryptoendoliths (Vincent, 1988, Nienow and Friedmann, 1993). Recently, studies of the glacier ice and lake ice in the dry valley regions have also revealed active microbial assemblages (Priscu et al., 1998, Tranter et al., 2004, Priscu et al., 2005). An area within the ice-free oases that has not been extensively studied, due largely to logistical constraints, is the subglacial environment.

The Subglacial Ecosystem

Subglacial processes are the most poorly understood of all glacier components; an obvious reason is that this is also the least accessible portion of the glacier (Benn and Evans, 1998). The interaction of glacial basal ice with the underlying substratum creates the subglacial environment (Knight, 1998), with the structure of this environment being controlled by glacial hydrology, bedrock geomorphology, and the legacy ecosystem overridden by the glacier. Examples of subglacial features include terrestrial substratum (Benn and Evans, 1998), large subglacial lakes below the East Antarctic Ice Sheet (Siegert, 2000, Priscu et al., 2003) and a subglacial volcanic crater lake (Gaidos et al., 2004).

Previously, subglacial weathering reactions were believed to be strictly abiotic, occurring below the glacier under oxic conditions (Raiswell, 1984 and Chillrud et al., 1994). New reports have established that glacier beds are in fact inhabited by microorganisms (Sharp et al., 1999, Skidmore et al., 2000, Foght et al., 2004, Mikucki et al., 2004). The extent to which microbes that colonize the subglacial environment are involved in geochemical processes such as the dissolution of underlying bedrock material and the processing of legacy or preglaciation carbon is still unclear (Tranter et al., 2005). The microbial communities present in subglacial environments might be distinct from one another, reflecting the pre-glaciation ecology. Alternatively, subglacial environments may universally select for particular traits and metabolic regimes resulting in uniform subglacial community structures. Unfortunately, studies on the microbial diversity of the subglacial environment are few. Detailed studies of the microbial diversity associated

with a broad distribution of subglacial environments are required to predict the ecologic function of the subglacial niche.

The contribution of subglacial environments to biological productivity and the release of biologically produced greenhouse gasses such as CO_2 and CH_4 are poorly understood. However, the extent of land covered by the last glaciation (~10,000 years ago) now contains 25% of the world's soil carbon or 400 x 10^{15} g C (Schlesinger, 1997). Knowing the fate of subglacial carbon could help reconcile global carbon budgets (Sharp et al., 1999). If the subglacial environment harbors microbial communities from pre-glacial periods in a permanently or temporarily sealed environment, the organisms should be physiologically capable of growth in permanent darkness and low temperatures. Subglacial systems in the polar regions may be sealed off from the atmosphere for extended periods of time. Lake Vostok, for example, has remained isolated for perhaps as long as 20-25 million years (Naish et al., 2001) and may contain unique, independently evolved organisms (Priscu and Christner, 2004). If microbial assemblages on Earth remain active in the subglacial environment for millions of years, protected from often inhospitable surface conditions; subglacial systems on other cold planets might also be refugia for life.

Significance of My Research on Subglacial Taylor Glacier

My dissertation investigates the microbial ecology of Blood Falls, the outflow from a subglacial system below the Taylor Glacier in the McMurdo Dry Valleys, Antarctica. Blood Falls is a unique feature in the dry valleys that provides information on the subglacial processes and the ecological history of the McMurdo Dry Valleys. The

Taylor Glacier overlies an ancient marine brine, the origin of which may be as old as the Pliocene, ~ 3-5 Mya (Elston and Bressler, 1981). I applied geochemical and microbiological techniques to samples of Blood Falls outflow to provide a first description of the structure of the ecological habitat below the Taylor Glacier. The influence of this subglacial outflow on the west lobe of Lake Bonney, which receives the flow, was also addressed to gain a more thorough understanding of the microbial diversity and biogeochemistry of this proglacial lake (Priscu, 1997, Ward and Priscu 1997).

Site Description

The McMurdo Dry Valleys, located at 76º30'-78º30'S, 160-164ºE, form the largest ice-free region on the Antarctic continent. The dry valleys form the coldest, driest desert on Earth and contain a mosaic of permanently ice-covered lakes, ephemeral streams, arid soils, exposed bedrock and large alpine glaciers (Priscu et al., 1998). The Taylor Glacier, located at the western end of the Taylor Valley (Figure 1.2) is an outlet glacier of the East Antarctic Ice Sheet and terminates into the west lobe of Lake Bonney (WLB) (Fountain et al., 1999). The Taylor Glacier has three major outflows into Lake Bonney: (1) the Santa Fe stream to the north, (2) Lyons stream to the south, both generated from supraglacial flow, and (3) a subglacial stream at the northern end of the glacier terminus. Taylor Glacier's subglacial outflow leaves a distinct red coloration on the glacier surface, resulting from iron oxides, and has consequently been named Blood Falls (Figure 1.2). The subglacial outflow at Blood Falls is a cold (-6º C) brine, high in dissolved iron and sodium chloride with a conductivity approximately 2.5 times seawater

(105 mS cm^{-1}). When this anaerobic water meets the atmosphere at the surface, iron and salts rapidly oxidize and precipitate forming a salt cone or crust. As the austral summer progresses, fresh surface glacial melt and stream flow from the Santa Fe Creek, which converges with Blood Falls at the moat of the west lobe of Lake Bonney, acts to dissolve the salt cone and dilute the subglacial flow.

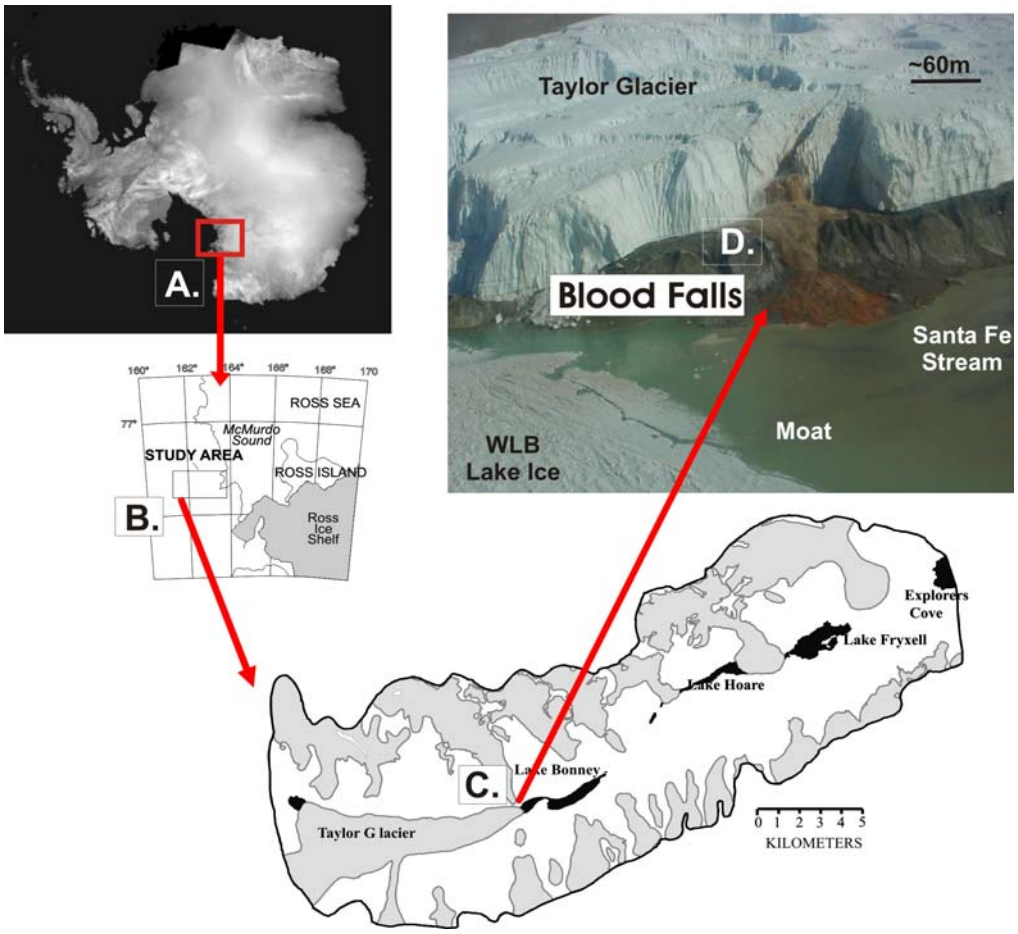

Figure 1.2. Location of Blood Falls. The McMurdo Dry Valleys are located in Eastern Antarctica (A) west of the McMurdo Sound (B). The Taylor Glacier is located in the western end of the Taylor Valley (C), photograph showing subglacial outflow at Blood Falls, which occurs at the northern end of the Taylor Glacier terminus (D).

Hypotheses and Objectives

The overarching hypothesis of my research is that the subglacial environment below the Taylor Glacier harbors a viable microbial-based ecosystem. Specific testable hypotheses that address various aspects of this theme follow. Each hypothesis is accompanied by a series of objectives that address each hypothesis.

H_1: Blood Falls harbors a diverse, metabolically active microbial assemblage.

Rationale. I believe that life will take hold in any environment where abundant energy sources exist in the presence of a liquid medium. Blood Falls contains elevated concentrations of reduced iron and organic carbon which could provide potential chemical energy sources for metabolism. The source of Blood Falls remains enigmatic; however, geochemical data including ion concentrations and chloride isotopes strongly imply that the origin of Blood Falls is marine (Lyons et al., 1998). If ancient marine waters exist below the Taylor Glacier, microorganisms that were once present in the water and underlying sediments before glaciation could have supplied the microbial seed to the subglacial system.

Objectives addressing H_1:

1. Determine the presence/absence of microbial cells
2. Test for viability and metabolic activity
3. Cultivate isolates from the outflow

H_2: The contemporary ecosystem below Taylor Glacier hosts autotrophic lifestyles.

Rationale. The subglacial source (brine) of Blood Falls (outflow) is isolated from the surface and microorganisms living in this brine must function in the complete absence of sunlight. The maintenance of this subglacial ecosystem would require the production of organic carbon via chemoautotrophic metabolism.

Objectives addressing H_2:

1. Assay for dark CO_2 incorporation by the natural assemblage under *in situ* conditions
2. Cultivate isolates using enrichment media selective for microbes capable of chemoautotrophic metabolism

H_3: Subglacial microbial diversity is structured by the geochemical and physical conditions below the glacier.

Rationale. Blood Falls outflow contains high concentrations of salts and iron, is permanently cold (temperature = -6º C), anoxic, and is slightly acidic (pH ~ 6.2). The Taylor Glacier overrides both ancient lacustrine carbonates and iron-rich marble. I contend that phylogenetic analysis of clones from Blood Falls will reveal organisms closely related to known iron metabolizers, with anaerobic lifestyles, that are acclimated to cold environments.

Objectives addressing H_3:

1. Collect detailed biogeochemical data on outflow water samples
2. Construct a 16S rDNA clone library from Blood Falls outflow
3. Perform phylogenetic analysis on cultivated isolates
4. Compare the 16S rRNA gene data of the clone library and isolates with published sequence and physiology data.

H_4: The biological assemblage in Blood Falls is similar to the assemblage found in the bottom waters of the west lobe of Lake Bonney.

Rationale. Outflow from below the Taylor Glacier flows directly into the west lobe of Lake Bonney and the geochemistry of the bottom saline waters of the west lobe of Lake Bonney resembles that of the outflow at Blood Falls (Lyons et al., 2005). I contend that the subglacial outflow from Taylor Glacier supplies viable microbes to the west lobe of Lake Bonney that can grow in the conditions present below the lake's chemocline.

Objectives focusing on H_4:

1. Measure biogeochemical parameters along a transect from Blood Falls into the west lobe of Lake Bonney
2. Determine phylogenetic diversity across the transect
3. Compare the similarity between diversity and geochemical data for each sample collected along the transect

CHAPTER 2

GEOMICROBIOLOGY OF BLOOD FALLS: AN IRON-RICH SALINE DISCHARGE AT THE TERMINUS OF THE TAYLOR GLACIER, ANTARCTICA

Adapted from: Jill A. Mikucki, Christine M. Foreman, Birgit Sattler, W. Berry Lyons, and John C. Priscu (2004) Geomicrobiology of Blood Falls: An iron-rich saline discharge at the terminus of the Taylor Glacier, Antarctica. *Aquatic Geochemistry* **10:** 199-220.

Abstract

Blood Falls, a subglacial discharge from the Taylor Glacier, Antarctica provides an example of the diverse physical and chemical habitats available for life in the polar desert of the McMurdo Dry Valleys. Geochemical analysis shows that Blood Falls outflow resembles concentrated seawater remnant from the Pliocene intrusion of marine waters combined with products of weathering. The result is an iron-rich, salty seep at the terminus of Taylor Glacier, which is subject to episodic releases into permanently ice-covered Lake Bonney. Blood Falls influences the geochemistry of Lake Bonney, and provides organic carbon and viable microbes to the lake system. Here I present the first data on the geobiology of Blood Falls, and relate it to the evolutionary history of this unique environment. The novel geological evolution of this subglacial environment makes Blood Falls an important site for the study of metabolic strategies in subglacial environments, and the impact of subglacial efflux on associated lake ecosystems.

Introduction

The subglacial environment has long been studied for its role in chemical weathering and its influences on glacier stability, sediment erosion, transport and deposition (Benn and Evans, 1998). Subglacial chemical weathering was originally thought to consist of strictly inorganic proton- and hydroxyl-promoted dissolution reactions (Raiswell, 1984, Chillrud et al., 1994). However, Sharp et al., (1999) challenged this idea with evidence for active subglacial microbial populations. Sharp and colleagues measured sulfide oxidation and cell division in basal ice samples from Arctic glaciers. Skidmore et al. (2000), studying the subglacial environment of Canadian glaciers, detected nitrate reduction, sulfate reduction and methanogenesis. Microbially mediated oxidation/reduction reactions would provide important proton sources that could enhance subglacial silicate weathering. The metabolic by-products of some microorganisms, such as organic acids or extracellular polysaccharides, might also contribute to subglacial weathering via ligand-promoted dissolution. Welch and Ullman (1999) found that ligand-promoted weathering reactions were particularly effective at feldspar dissolution at low temperatures (between 5º and 35º C). In a subglacial setting, the source of organic carbon for microbial life could be sedimentary material overridden by the advancing glacier and finely ground by subsequent abrasion processes. In most cases, this sedimentary material is permafrost soil (Sharp et al., 1999, Skidmore et al., 2000). There is a growing consensus that life plays an important role in earth surface processes. However, there is still limited data on the role of microbes in rock weathering, and specifically subglacial processes (Lüttge and Conrad, 2004).

Blood Falls is thought to be a remnant marine feature originating below the Taylor Glacier in the McMurdo Dry Valleys, southern Victoria Land, Antarctica. Blood Falls is arguably the oldest liquid water feature in the Taylor Valley, dating back to a time when the valley network was fjordlands during the Pliocene Epoch (3-5 million years ago). The Earth was warmer during the Pliocene than today (Kennett and Hodell, 1993) and this warming resulted in the incursion of marine waters from the Ross Sea embayment (Denton et al., 1993). When these Pliocene waters retreated from the valley network, a remnant sea remained, near the current Taylor Glacier terminus.

The present major ion chemistry of the Blood Falls outflow records the signature of its geological history. Data from chloride isotopes strongly support a marine origin of Blood Falls, with its bulk chemistry resembling that of cryoconcentrated seawater (Lyons et al., 1999, Lyons et al., 2001). A modest expansion of the Taylor Glacier during the late Pliocene or Pleistocene (Marchant et al., 1993) likely covered this concentrated sea, leaving a liquid water remnant of Pliocene marine waters sealed beneath the Taylor Glacier.

The subglacial outflow of Blood Falls provides a surface feature where samples representative of the subglacial environment can be obtained. The presence of metabolically active, subglacial microbial communities could significantly affect biogeochemical cycling on glacial and interglacial timescales (Sharp et al., 1999). This ancient saline subglacial system also provides an important site for exobiological studies, as similar environments may exist under the Martian polar caps or subsurface terrain today. Here I present the first biological data on Blood Falls, and relate it to *in situ* geochemistry.

Study Area

The McMurdo Dry Valleys occupy the largest ice-free region on the Antarctic continent (approximately 4,800 km^2, located at 76°30'-78°30' S, 160-164° E), composing the coldest, driest desert on Earth. The Transantarctic Mountain range prevents the flow of the East Antarctic Ice Sheet from the Polar Plateau into the dry valleys, maintaining ice-free conditions. Mean annual temperatures in the Taylor Valley range from -16 to -21°C, and precipitation is less than 5mm water equivalents annually (Fountain et al., 1999). This cold polar desert is characterized by a mosaic of permanently ice-covered lakes, ephemeral streams, arid soils, exposed bedrock, and large alpine glaciers (Priscu, 1998). Taylor Glacier flows into the valley from the west as an outlet glacier of the East Antarctic Ice Sheet and terminates in the Taylor Valley against the western lobe of Lake Bonney (Fountain et al., 1999). The Taylor Glacier also provides the largest contribution (over 60%) of stream flow into Lake Bonney (Chinn, 1993, Fountain et al., 1999).

Blood Falls is a highly visible, iron-oxide-rich, saline discharge at the northern end of the Taylor Glacier terminus. Although Blood Falls was first observed as early as 1911 (Taylor, 1922), researchers only began documenting the nature of the iron-rich, saline outflow 50 years later (Hamilton, 1962). Taylor (1922) was the first to describe this feature, writing "bright red alga lent an unusual touch of colour [at the snout of the glacier]." Geochemical analysis would later reveal that iron oxides, not algae were responsible for the red color (Angino et al., 1964, Black et al., 1965, Keys, 1979, Lyons et al., 1998). Black et al., (1965) described Blood Falls as a "striking reddish-yellow icecone built from a saline discharge at the terminus of the Taylor Glacier." Keys (1979)

later documented that the iron-oxides were not visible in fresh releases from Blood Falls; iron released from Blood Falls was reduced, and only after contact with the air did the discharge become oxidized to its rust-red color.

The prominent coloration of Blood Falls holds true today, though release events appear to be episodic. The volume and physical extent of Blood Falls outflow from the glacier terminus has varied over time, as has the concentration of ions present in the outflow (Black et al., 1965, Keys, 1979, Lyons et al., 2005). Black and Bowser (1968) documented an advance of the Taylor Glacier between 1965 and 1966 of "a few meters", an event which caused slumping of a portion of the glacier terminus into Lake Bonney. The authors further reported, "The advance produced the fractures at the northeast corner of the glacier through which the saline discharge took place." Other investigators made similar observations of an "upwelling of highly saline waters" at the Taylor Glacier snout in the early 1960's (Hamilton et al., 1962, Black et al., 1965). The last known major discharge occurred between February and August 1991 and flowed several hundred meters over the surface of the Lake Bonney ice cover (Priscu, personal observation). Discharge from Blood Falls in 1991 was so large that by August, a reddish-orange slush covered extensive areas of shoreline next to the glacier and water samples collected between 20 and 25 m in the west lobe during October contained such high iron levels that a noticeable iron oxide precipitate developed when exposed to air (the iron was reduced under *in situ* lake conditions) (Priscu, personal observation). A similar reddish-brown residue is also observed on filters used to collect water samples from depths below 20 m in the west lobe of Lake Bonney today.

Methods

Sample Collection.

Samples were collected from subglacial outflow waters associated with Blood Falls discharge during austral summers from 1999-2003. Samples near the outlet of Blood Falls were collected for enumeration and bacterial activity in December of 2000 and 2001. A transect of samples (TransX) was collected in December (see Figure 1), 2002 to capture a profile of releases from Blood Falls into Lake Bonney, starting at the source of the outflow (Trans1) and out onto the moat ice surrounding the lake with increasing distance from the source (Trans2-5). Trans2 was collected from basal ice below the source, Trans3 was collected from waters at the shoreline where Blood Falls meets the moat ice, Trans4 was collected 5 m from the shoreline just below moat ice that melted and refroze daily, and Trans5 was collected under 0.4 m of moat ice approximately 70 m from the shoreline before the moat ice meets the permanent ice cover of Lake Bonney. Autoclaved metal spatulas were used to scrape ice samples into sterile plastic bags (Whirl-Pak) or sterile plastic conical tubes (Falcon). Subsequent manipulations were carried out aseptically in a positive flow hood. Subglacial flow water was collected in clean Nalgene bottles for geochemical analysis. All ice samples were melted slowly at 2-4 °C in sterile containers before analysis. Samples for analysis of dissolved organic carbon were collected in acid-washed, combusted (450 °C for > 4h) amber glass bottles. Dissolved inorganic carbon samples were collected in gas tight glass serum vials. Samples collected for bacterial enumeration were treated with formalin (2%

final concentration) to prevent a change in bacterial numbers during storage (~ 2 weeks) before counting.

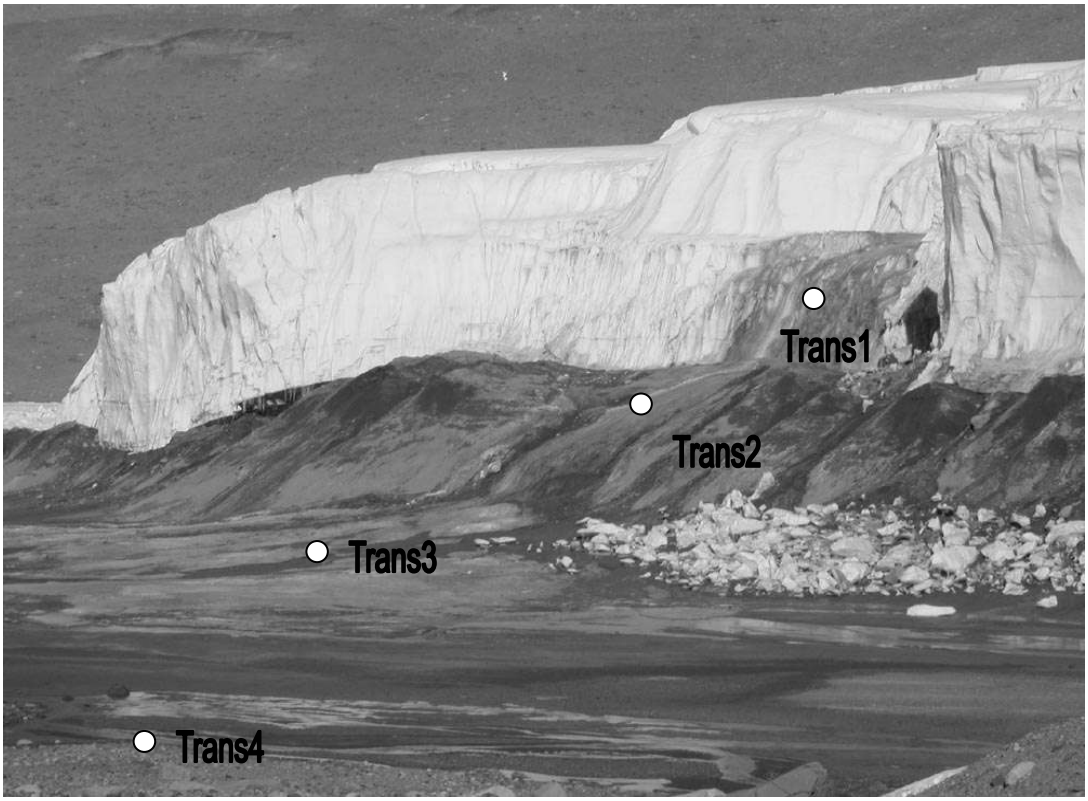

Figure 2.1. Blood Falls at the terminus of the Taylor Glacier in December 2002. Transect sample collection points are marked. Trans5 is not visible in this image.

Geochemistry

The pH was measured with a calibrated Beckman Φ12 pH meter. E_h was measured using a Hach ORP combination electrode (Hach Company, Loveland, CO) connected to a Beckman Φ12 digital voltmeter, with corrections for the reference electrode made in accordance with the ORP electrode instruction manual. Dissolved oxygen (DO) was determined by Winkler titration (Standard Methods, 1992). Dissolved inorganic carbon (DIC) was measured by infrared gas analysis on acid sparged samples.

Dissolved organic carbon (DOC) was measured on filtered (25mm Whatman GF/F), acidified (HCl to pH~2-3) samples with a Shimadzu 5000 TOC analyzer. Nitrate (NO_3^-) and nitrite (NO_2^-) concentrations in water samples were measured with a Lachat autoanalyzer according to Standard Methods (1992). Nitrite levels were determined by diazotization, with sulfanilamide, and then coupled with a diamine to produce a pink dye, analyzed spectrophometrically on a Lachat autoanalyzer. Nitrate was reduced to nitrite by passing the sample through a copperized cadmium column and analyzing for nitrite, as above, giving a nitrate plus nitrite concentration. The nitrate concentration was determined by subtracting the nitrite concentration from the total (nitrate + nitrite) concentration. Ammonium (NH_4^+) concentrations were measured separately by reaction with alkaline phenol followed by sodium hypochlorite, forming indophenol blue. Sodium nitroprusside was added to enhance sensitivity and the sample was analyzed spectrophometrically on a Lachat autoanalyzer. Dissolved inorganic nitrogen (DIN) was reported as the summation of nitrate, nitrite and ammonium ($DIN = NO_3^- + NO_2^- + NH_4^+$). Soluble reactive phosphorus (SRP) was determined on 10 ml samples with the antimony-molybdate method (Standard Methods, 1992), absorbance was read in a 10 cm cell with a Beckman DU-640 spectrophotometer. Chlorophyll-*a* (CHL) was extracted into 90% acetone for 24 h at <0 °C in the dark from particles collected on a Whatman GF/F filter, and the subsequent concentration was determined fluorometrically.

Conductivity was measured at 1-meter depth intervals in the west lobe of Lake Bonney using a Seabird SBE-25 conductivity temperature depth (CTD) sensor (Sea-Bird Electronics, Inc., Bellevue, Washington). Salinity was calculated using the UNESCO salinity algorithm available in the Seabird data acquisition software package (Sea-Bird

Electronics, 1998). Modifications for the high salinity concentrations found below the halocline of Lake Bonney are described in Spigel and Priscu (1996). Total iron was determined using the ferrozine assay (Fulton et al., 2004).

Microbiology

Heterotrophic marine agar (Bacto) and oligotrophic R2A (Difco) agar plates were prepared to examine the presence of colony forming units (CFUs) in transect samples. Plates were preincubated at room temperature for 48 hours to test for contamination, and then pre-chilled to 2 °C before inoculation. Aliquots (100 μL) of Blood Falls outflow from each transect collection were serially diluted, plated and incubated at 2 °C for three weeks. All transfers were done on ice in a positive-flow hood. Plate counts on both media types were made after a three-week incubation. Environmental (*in situ*) cell abundance was determined on 0.2 μm-filtered samples as described by Lisle and Priscu (2004). Samples collected in 2001 were stained with 4′,6-diamidino-2-phyenylindole (DAPI) DNA stain (Sigma). Samples collected in December 2002 were enumerated with Sybr Gold DNA stain (Molecular Probes).

Bacterial Activity

Heterotrophic bacterial activity was measured using ^{3}H-thymidine (20 nM) and ^{3}H-leucine (20 nM) incorporation according to the methods described in Takacs and Priscu (1998). Formalin (5% final concentration)-treated replicates served as killed controls. Samples were incubated in the dark at 0-1 °C. Incubation was terminated by the addition of cold trichloroacetic acid (TCA) to a final concentration of 5%. The effect of temperature on the productivity of the natural bacterial assemblages from Blood Falls

outflow was determined by incubating triplicate samples (10 mL) with 10 nM ^{3}H-leucine for 24 h at varying temperatures using the methods described above. Doubling rates were calculated from the ^{3}H-leucine incorporation data (Takacs and Priscu, 1998)

Results

The episodic discharge of subglacial waters from Blood Falls makes sample-to-sample comparisons and determination of definitive trends challenging. Consequently, results reflect conditions at the specific period of sampling and may not reflect annual trends.

Table 2.1. Summary of biogeochemical analysis performed on each transect point during the 2002 austral summer. ND= not detected, TN = too numerous to count, na = not available.

Sample Location	pH	DO (µM O_2)	Eh (mV)	DIC (µM C)	DIN (µM)	SRP (µM)	DIN: SRP	DOC (µM C)	CHL (µg L^{-1})	Bacteria (10^4cells mL^{-1})	Colony Growth			
											R2A		Marine	
											10^{-1}	10^{-3}	10^{-1}	10^{-3}
Trans1	8.16	na	230	na	0.22	0.09	2.4	771	1.4	1.08	32	1	TN	0
Trans2	8.00	na	247	na	0.26	0.38	0.7	211	0.2	0.80	75	0	TN	0
Trans3	8.80	215	250	476	9.98	0.05	200	47.5	15.0	0.31	TN	0	241	na
Trans4	9.28	220	250	165	9.40	0.08	118	52.0	18.5	1.10	TN	0	TN	0
Trans5	9.32	140	252	71	8.37	0.08	105	ND	11.8	1.07	TN	0	30	0

Biogeochemistry

The outflow from Blood Falls, during 2002, was basic, all points along the transect had a pH of 8.0 or higher (Table 2.1). The highest pH, 9.32, was recorded at the sample point furthest from the source (Trans5). Dissolved oxygen was between 140 and 220 μM O_2 for the three measured transect points (Trans3-5), however, mixing with surface air occurred once the discharge entered the atmosphere so these data are presumably higher than actual subglacial conditions. Two Eh values for each transect sampling point were collected and averaged. The average Eh value for Trans1 is 230 mV, Trans2 was higher (247 mV) and both Trans3 and Trans4 were about 250 mV. Trans5 was slightly higher at 252 mV. DIC values were obtained for Trans3-5 and were lowest at the sample site furthest from the source (Trans5). The DIC concentration of Trans3 was 476 μM C, while Trans5 was 70.8 μM C. DOC at the source (Trans1) of Blood Falls was high (9.25 mg C L^{-1} or 771 μM) when compared to values typical of marine waters (40-80 μM) (Williams, 2000). Blood Falls DOC concentration is more comparable with that of systems receiving allochthonous inputs, such as black water stream flow systems ranging from 250 – 3480 μM C (Meyers and Edwards, 1990). DOC quickly decreases to below the detection limits of our methods (40 μM C) by Trans5 (Table 2.1). SRP was highest (0.38 μM) in Trans2, which is basal ice directly below the source. All other transect samples had SRP values below 0.1 μM and this may be a result of abiotic losses of phosphorus via adsorption to iron hydroxides. DIN values in Trans1 and 2 were 0.22 and 0.26 μM, which are lower than values reported for the perennial ice cover of east lobe Lake Bonney (between 2 and 10 μM) (Priscu et al., 1998). DIN in

Trans3-5 was between 8.4 and 10.0 μM, which are similar to values found in the lake ice covers. 100 percent of the DIN in Trans1-2 was in the reduced form (NH_4^+), whereas DIN in transects further from the source were comprised mainly (>60%) of nitrate (NO_3^-) the most oxidized form of N. Increases in DIN may be a result of the biological fixation of atmospheric nitrogen by diazotrophic cyanobacteria. The increase in the fraction of DIN in the oxidized form has a strong correlation ($r = 0.97$, $p = 0.006$) with an increase in Chlorophyll-*a* (CHL). Farther downstream from the source of Blood Falls outflow, CHL increases from 1.4 μg L^{-1} (Trans1) to 18.5 and 11.8 μg L^{-1} (for Trans4 and 5 respectively). This increase in CHL correlates weakly with a decrease in DIC but is not significant, ($r = 0.25$, $p = 0.849$), implying that photoautotrophs may be colonizing the outflow as nutrients are supplied by Blood Falls. However, it is more likely that the decrease in DIC is due to equilibration with the atmosphere. DIN:SRP increased from 0.7 in the upper transect points (Trans1-2) to 200 in the lower transect points (Trans3-5). These ratios imply phosphorus deficiency in the flow where it enters Lake Bonney. Phosphorus deficiency is also a feature of the upper water column of Lake Bonney (Dore and Priscu, 2001).

Microbiology

Prokaryotic cell morphologies included rods, cocci, dipplococci and diplorod cell types. Cells ranged in length, width and volume between 0.59-0.91 μm, 0.44-0.45 μm and 0.11-0.16 μm^3 respectively. Based on this range of computed cell volumes a total bacterial biovolume in Blood Falls ranged from $1.7x10^3$ to $9.5x10^3$ μm^3 ml^{-1}. The average density of cells from Blood Falls outflow was 10^4 cells ml^{-1} (range = $0.3x10^4$ to

$1.1x10^4$ cells ml^{-1}). Bacterial colonies grew on both low nutrient R2A plates and high nutrient marine agar plates (Table 2.1). Colonies appeared within two weeks of incubation at 2 °C. Percent recovery, relative to direct microscopic counts, from the two heterotrophic media types (R2A and marine agar) was generally low. Inoculum from Trans1 and Trans5 demonstrated a 2.9% and 2.8% recovery, respectively, based on total cell counts when grown on R2A media. Trans3 had an unusually high plate recovery (78%) when microbes were grown on marine agar. Subsequent isolation and transfers indicated that most colonies were capable of growth at 15 °C. Pigmented pink, yellow and orange colonies, as well as opaque and translucent colonies, grew on both media preparations incubated at 2 °C. Growth also occurred on plates incubated at room temperature (22 °C) but colonies were less dense and usually non-pigmented.

Bacterial Activity

Bacteria at the source of Blood Falls incorporated thymidine into TCA-insoluble products at an average rate of 1.5×10^{-3} nM d^{-1}. There was considerable variation between the samples, with rates ranging from 0.4 to 3.0 x 10^{-3} nM d^{-1} (SD = 1.1×10^{-3}) (Table 2.2). Thymidine incorporation experiments were used to compute cell doubling times ranging from 37 to 54 days at 0 °C. The effect of temperature on leucine incorporation was determined at 0 °C, 5 °C, 10 °C, 15 °C, 20 °C and 30 °C (Figure 2.3). The activation energy (E_a) over the range of temperatures tested, and the Q_{10} for metabolic activity between 0 and 20 °C were 8764 kcal mol^{-1} and 1.74, respectively.

Table 2.2. Summary of biological activity data collected at the source of Blood Falls during the austral summers of 2000 and 2001.

Date	Temperature (°C)	^{3}H-labeled Thymidine incorporation (nM d^{-1} x 10^{-3})	^{3}H-labeled Leucine incorporation (nM d^{-1})	Doubling time (d)
Nov 2000	0	1.02	0.24	37
Dec 2000	0	0.40	0.12	54
Dec 2001	2	3.00		

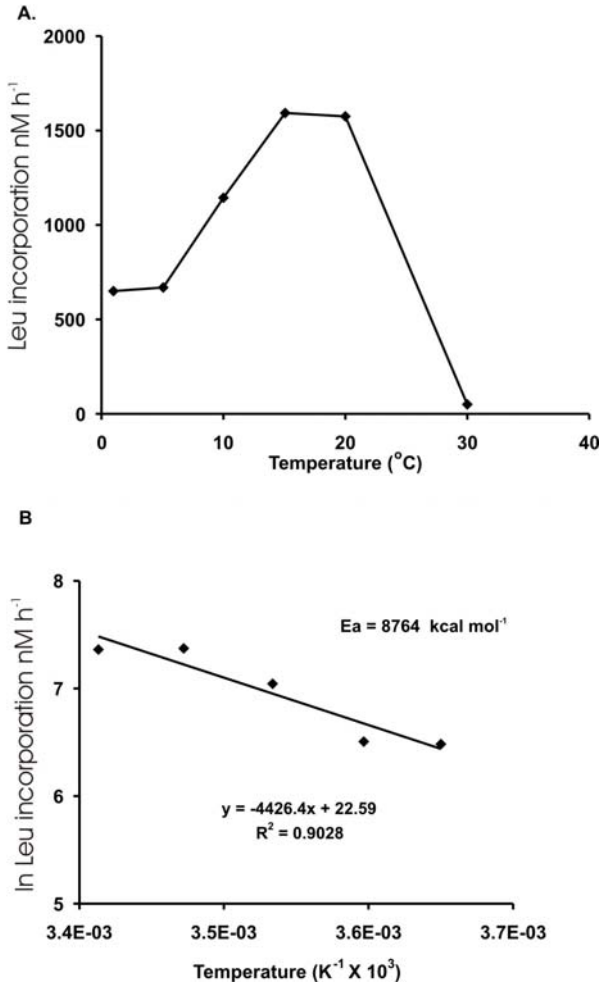

Figure 2.2. Effect of temperature on Blood Falls outflow plotted arithmetically (A) and with the Arrhenius equation (B).

Discussion

Biogeochemical Data. Dissolved organic carbon (DOC) and inorganic nutrient levels in Blood Falls are sufficient to support both subglacial microbial growth and provide nutrients to the west lobe of Lake Bonney (Table 2.1). The pH of Blood Falls outflow was alkaline (8.00-9.32), which results in part from the high DIC (HCO_3^- + $CO_3^=$) values of these waters. DOC at the source of Blood Falls (Trans1) is 771 μM C, about nine times greater than that of the open ocean indicating that the Blood Falls subglacial environment is a source of DOC. The high DOC values (771 μM C) presumably result from ancient or contemporary *in situ* production of organic carbon, as there are no significant allochthonous sources in the Taylor Valley. Regardless of its origin, this DOC provides a vital substrate for heterotrophic bacteria, both within the subglacial environment and Lake Bonney.

Dissolved oxygen values were surprisingly high for water that has been potentially isolated from the atmosphere for possibly millions of years. Samples were collected in a dynamic zone of glacier melt (Trans3) and where the moat ice is actively thawing and refreezing (Trans4 and 5); these areas are in direct contact with the atmosphere, allowing for atmospheric exchange. The increase in DIN in Trans3-5 suggests an input of nitrogen, which may have come from microbial nitrogen fixation. Nitrogen fixation has been detected in microbial aggregates associated with the Lake Bonney ice cover (Pearl and Priscu, 1998). Additionally, specific nitrogen-fixing cyanobacteria (i.e. *Nostoc sp.*) and heterotrophic microorganisms were identified in ice-cover samples through nitrogenase gene (*nifH*) amplification (Olsen et al., 1998). The

low N:P ratio in the source waters of Blood Falls implies that any subglacial bacteria would not be phosphorus limited. Bacterial communities in all the lakes of the Taylor Valley have been shown to be P-deficient, particularly Lake Bonney (Priscu, 1995, Dore and Priscu, 2001), and bacterial P limitation is common in other lacustrine systems (Elser, 1995).

Biological analysis of Blood Falls discharge reveals the presence of a viable microbial assemblage. Growth on low nutrient, low salt agar (R2A) plates implies that some of the microorganisms are halotolerant (i.e. can tolerate with the hypersaline conditions in Blood Falls outflow), or that organisms present in glacial melt ice have been selectively enriched. In general, translucent and opaque colonies grew at room temperature incubations, while a variety of pigmented colonies were observed at lower (below 15 °C) incubation temperatures. It is not clear what relationship exists between the production of pigments and growth temperature, however similar results were observed by Skidmore et al (2000) when growing Artic subglacial sediment samples on R2A medium. A fraction of the isolates obtained from Blood Falls grew at both low (2 °C) and higher temperatures (≥ 22 °C), revealing the presence of psychrotolerant organisms capable of growth under increased temperatures. However, 85% fewer colonies appeared on agar plates incubated at room temperatures. The ability of isolates from Blood Falls to grow on marine agar shows that some portion of the microbial assemblage can grow under simulated marine conditions. An isolate from Blood Falls, capable of growth on marine agar, was phylogenetically related to *Schwanella frigidamarina* (99% similarity based on partial 16S rRNA data) (see Chapter 4). *S. frigidamarina* is a facultative iron-reducer that has been isolated from sea ice and

Antarctic lakes of marine salinity (Bowman, et al., 1997), adding a biological line of evidence to the supposition that the subglacial system beneath Taylor Glacier has a marine origin.

Bacterial activity. Incorporation of thymidine in Blood Falls outflow (average = 1.5×10^{-3} nM d^{-1}, SD = $\pm 1.36 \times 10^{-3}$) is low when compared to bacterial production in other lakes in the Taylor Valley, such as Lake Fryxell (0.45 nM d^{-1}) and the west lobe of Lake Bonney (0.05 nM d^{-1}) (Takacs and Priscu, 1998). Blood Falls bacterial production does however, fall within the range for samples from the Sargasso Sea or the Gulf Stream ($3.6\text{-}11 \times 10^{-3}$ nM d^{-1} and $2.4 - 13.2 \times 10^{-3}$ nM d^{-1}, respectively) (Rivkin and Anderson, 1997). Long doubling times such as those we computed for bacteria from Blood Falls (37-54 days) are not uncommon for microbes from cold environments. The doubling time for the organisms in Blood Falls based on thymidine incorporation is comparable to rates of permafrost bacteria (40 days) incubated at -15 °C (Rivkina et al., 2000). Bacterial activity in icy environments may ultimately be limited by the presence of liquid water. For example, in the permanent ice cover of Lake Bonney, liquid water only exists within the ice for 80-150 days during the austral summer which leads to doubling times that may exceed one year (Fritsen and Priscu, 1999). Still, heterotrophic, photosynthetic and diazotrophic microorganisms are metabolically active during this brief period (Priscu et al., 1998).

The fraction of the microbial assemblage capable of incorporating leucine were psychrotolerant, growing fastest at 30 °C (Figure 2.2), but still able to metabolize at near freezing temperatures. Consequently, these bacteria would never realize their maximum

growth potential *in situ*. The energy of activation and Q_{10} are similar to many other bacterial assemblages ranging from psychrophilic to thermophilic (Priscu and Goldman, 1984, Priscu et al., 1989 and Thamdrup et al., 1998), indicating common thermodynamic responses for the various enzyme systems.

Microbes capable of obtaining energy from chemical oxidation-reduction reactions might also live in the Blood Falls environment. Skidmore et al., (2000) reported the presence of nitrate and sulfate reduction, as well as methanogenic activity in subglacial basal ice and associated melt water. In addition to the visibly high levels of iron in Blood Falls, sulfur compounds are also present. For example, dimethylsulfide (DMS), which originates from microbial oxidation of dimethylsulfoniopropionate (DMSP), a major metabolite of marine algae, has been detected in Blood Falls outflow and in the west lobe of Lake Bonney (Priscu, unpublished data, Lee et al., 2004). Preliminary analysis of DNA extracted from Blood Falls subglacial flow waters detected the presence of 16S rDNA sequences whose nearest relative (98% sequence similarity) is *Thiomicrospira*, a sulfur oxidizing bacterium (see Chapter 4). Moreover, clones representing *Thiomicrospira* were the most dominant members found in the clone library.

Two broad ecological classes of sulfur-oxidizing bacteria exist, those that grow at acidic pH and those that grow at near neutral pH (Madigan et al., 2000). *Thiomicrospira* is a representative of the genera that operate at neutral pH and its presence is consistent with *in situ* pH and sulfate data from Blood Falls. Incubation experiments conducted at 4 °C by Sharp et al., (1999) showed production of sulfate in arctic subglacial slurries after one week. Microbially mediated sulfur oxidation may be an important subglacial

biological process occurring under microaerophilic conditions underneath the glacier and at the glacier surface interface.

Importance of Blood Falls to Lake Bonney Geochemistry and Microbiology. The west lobe of Lake Bonney is highly stratified with respect to salts and nutrients (Figure 2.3). Keys (1979) estimated that Blood Falls discharges an average of 2000 $m^3 y^{-1}$ of saline water (episodic discharges reach 6000 $m^3 y^{-1}$). The density of Blood Falls discharge (1100 kg m^{-3}) is also identical to that of the deep waters of the west lobe of Lake Bonney (Spigel and Priscu, 1996), indicating that Blood Falls discharge would sink beneath the lake's chemocline at 14 m. Lyons et al., (1998) concluded that Blood Falls contributed up to 95% of the total chloride flux to the west lobe of Lake Bonney in the 1994-1995 flow season. This contribution of Blood Falls to the deep waters of Lake Bonney is further corroborated by similarities in bacterial size (biovolume) between Blood Falls and the deep waters of the west lobe. Image analysis of bacteria in Blood Falls of December 2000 samples revealed small cells (range of length, width and volume = 0.59-0.91 μm, 0.44-0.45 μm, and 0.11-0.16 μm^3). These cells resemble those found below 22 m in the west lobe; cells in the deep water of other lakes in the Taylor Valley have bacteria that are about 40 % larger by volume (Takacs, 1999). Eh values in the surface waters of WLB are near 750 mV and drop to 240 mV below the chemocline (Lee et al., 2004), which is the same Eh value recorded from Blood Falls outflow (average = 246 mV). Evidently, the bottom waters of the west lobe of Lake Bonney (below 14 m) are strongly influenced by the Blood Falls outflow (Figure 2.3).

The biological connection between Blood Falls and WLB is not as straightforward. Ward et al. (2003) found bacterial incorporation of thymidine was stimulated when growth conditions were diluted compared to natural Lake Bonney bottom water, suggesting that *in situ* organisms may be stressed by the high salt, high iron conditions below the chemocline. However, after six days of incubation, thymidine incorporation activity did not increase further in the diluted samples while growth continued to increase in the undiluted samples. The authors conclude that substrate limitation prevented sustained growth in the diluted samples. Perhaps the organisms at depth in Lake Bonney are adapted to sustained, slow growth in their natural environment. Despite these strong similarities, the pH in the bottom waters of the west lobe of Lake Bonney is acidic (pH = 6) whereas pH measured at Blood Falls in 2002 was basic (pH ~9). This pH difference could have a significant effect on the microbial populations in Blood Falls as they enter the waters below the chemocline of West Lake Bonney and warrants further study.

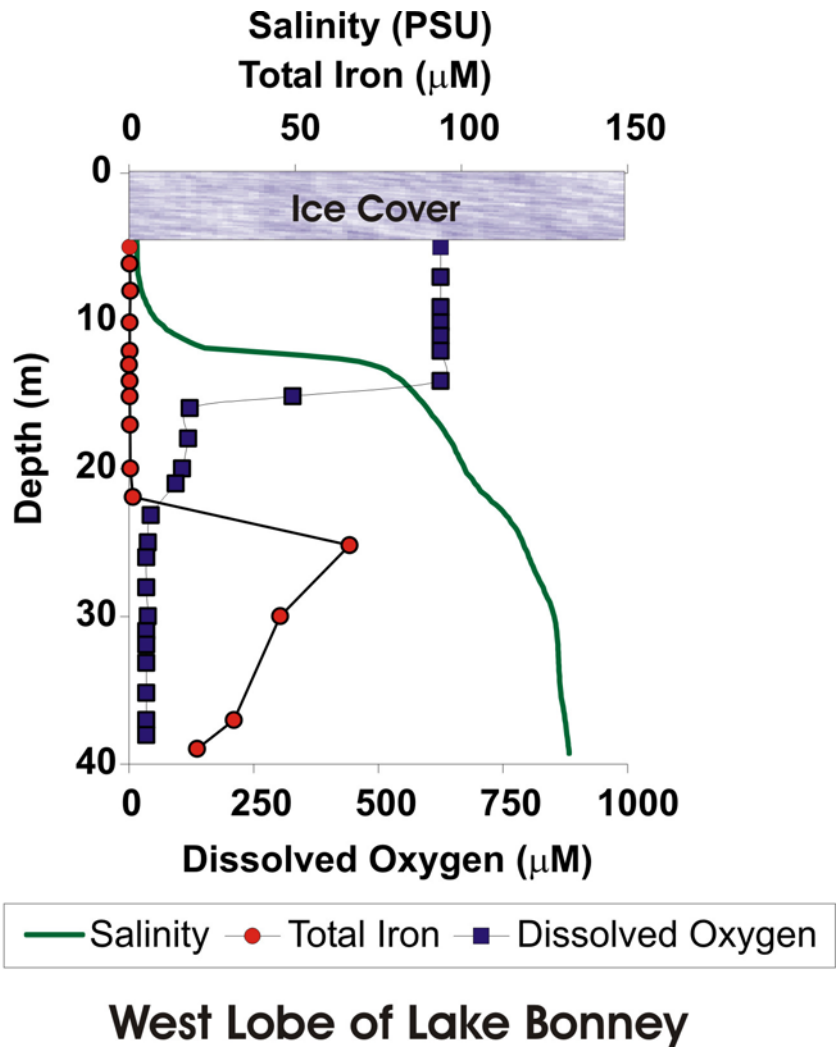

Figure 2.3. Geochemical profile for west lobe Lake Bonney. The lake is covered by 4.5 m of permanent ice. The solid line represents salinity in practical salinity units (PSU) the open circles represent total iron (μM) and the open squares show dissolved oxygen (μM).

Contribution to Subglacial Weathering. Subglacial flow influences glacier stability, sediment erosion, transport and deposition (Benn and Evans, 1998). Glacier bed movement also acts to grind and pulverize subglacial rocks, making available more surface sites for chemically and microbially mediated weathering reactions. In a polar desert such as the McMurdo Dry Valleys, subglacial processes would be slow, as most glaciers have frozen beds (cold-based glaciers) (Benn and Evans, 1998). However,

subglacial waters might influence the geochemical processes in the basal ice of the Taylor Glacier snout. A mass balance study of Taylor Glacier by Robinson (1984) revealed that as much as 50% of the lower ablation area of the Taylor Glacier basal ice may be melting and can be expected to behave as temperate ice. Robinson further reported that this area of pressure melting probably occurs as the glacier passes over extensive salt deposits, which have the effect of depressing the freezing-point of the basal ice, thereby extending basal melt even further. Recent ice penetrating radar data and subsequent modeling indicate the presence of water ponding or saturated sediment beneath the tongue of the Taylor Glacier, between 3-6 km up from the glacier snout (Hubbard et al., 2004). Temperature models deduce that this water is ~-7º C, implying this water must be hypersaline (Hubbard et al., 2004). Whether this subglacial water is chemically related to Blood Falls is unknown, but it would explain the episodically high values of dissolved ions in samples collected from Blood Falls outflow.

Lithium and silicon are two elements typically used to gauge water-rock interactions (Lyons and Welch, 1997, Pugh et al., 2003). Pugh et al., (2003) report an average of 242 μM Si in Blood Falls outflow during the 1999-2000 season. Lithium concentrations ranging between 47.1 – 91.1 μM reported by Lyons and Welch (1997) were the highest of all samples analyzed from the McMurdo Dry Valleys lakes and streams during the 1995-1996 season. Sr isotopic measurements of Blood Falls discharge also suggest a significant weathering component, as the $^{87}Sr/^{86}Sr$ values for Blood Falls (0.71146) are more radiogenic than seawater (Lyons, et al. 2002). In addition, the suspended load measurements from streams exiting from Blood Falls yield values from 25-925 mg L^{-1} (mean = 481) in 1998. Although suspended load measurements currently

only exist from this one year, the data indicate very high sediment yields related to the Blood Falls discharge.

Subglacial waters are expected to have a higher solute content, owing to prolonged contact with fresh bedrock and fine rock particles (Benn and Evans, 1998). The glacier-brine-bedrock interactions over extended time (possibly millions of years) have led to the unusual geochemical characteristics of Blood Falls; the ability of microorganisms to participate in subglacial weathering reactions suggests that biology may also play a role in the current composition of the outflow. Metabolically active heterotrophs like the organisms described here would produce CO_2 and organic acids that would enhance subglacial weathering.

Conclusions

Blood Falls provides a source of viable microbes, new nutrients, inorganic and organic carbon, and energetic mineral weathering products to the Lake Bonney system. The microbes from Blood Falls are metabolically active as demonstrated by distinct assays for heterotrophic activity. The source of carbon utilized by these organisms could be a legacy of photosynthesis by marine phytoplankton, from before Blood Falls was covered by the Taylor Glacier and cut off from sunlight. The presence of chemolithoautrophs capable of growth on sulfur compounds, as indicated by 16S rDNA results, implies that chemical energy, not solar energy, may be driving this system today. Reduced iron and ammonia are other energy sources that might be thermodynamically available to chemoautotrophs operating subglacially.

In the absence of drilling through the Taylor Glacier, the geomicrobiological environment of the subglacial habitat can be inferred by studying the chemistry and microbiology of the discharge and the deep waters of the west lobe of Lake Bonney. The discharge channels themselves are transition zones from subglacial to lake system and provide a glimpse of the subglacial environment, but not the true *in situ* conditions. Only through samples taken from the base of the Taylor Glacier at the source of Blood Falls will the true geomicrobiology of this subglacial system be determined. Blood Falls discharge has a major geochemical and biological influence on Lake Bonney. However, it remains unclear whether microbes from Blood Falls are able to survive and grow once transported to Lake Bonney. The chemistry of Blood Falls is discussed in greater detail in Chapter 3, and the microbial diversity of the outflow at Blood Falls is explored in Chapter 4. The effect of this outflow on the west lobe of Lake Bonney is revisited in Chapter 5.

CHAPTER 3

BIOGEOCHEMICAL EVOLUTION OF AN ANCIENT SUBGLACIAL OUTFLOW

Abstract

Taylor Glacier, Antarctica, overlies an ancient marine deposit forming what appears to be a saline subglacial lake or brine system. The source of this brine is believed to date to the Pliocene Epoch when the dry valleys were fjordlands. Marine waters remained in the upper valley following the recession of the Ross Sea and the salts are thought to have concentrated via evaporation and cryoconcentration. It is unclear the extent to which the waters below the Taylor Glacier were cryogenically concentrated and it is unknown what triggers the release of subglacial outflow to the surface at the snout of the glacier at a site called Blood Falls. Long-term monitoring of Blood Falls flow has revealed a distinct geochemical signature associated with subglacial release events. These release events discharge large amounts of subglacial water at Blood Falls and are herein referred to as ancient outflow events. Residual ancient outflow from past events is diluted with contemporary glacial melt during years where discharge is low or non-existent. The flow at Blood Falls during ancient release events is anoxic, hypersaline (~1375 mM Cl^-) high in sulfate (50 mM SO_4^{2-}) and total iron (3.8 mM) and slightly acidic (pH = 6.0-6.2). This outflow is also high in dissolved organic carbon (>400 μM C) and inorganic carbon (~50 mM C), and contains metabolically active microbial cells (10^5

cells ml^{-1}). Collectively these biogeochemical data provide insight into the structure of this subglacial ecosystem.

Introduction

The chemistry of subglacial outflow is often used by glaciologists to model the structure of the subglacial drainage system (Tranter et al., 1998). Interpreting the subglacial environment based on water chemistry assumes that the subglacial water is a product of its source (i.e. snow, glacial melt) and the subglacial reservoir through which it has traveled (Tranter et al., 1998). Conclusions based on chemistry alone can be complicated by chemical legacy of past events, bedrock lithology, and the geographical location of the glacier. Despite these potential complications, chemical analysis of the outflow can provide considerable information on subglacial geomicrobiology.

Meltwater from glacial ice is typically undersaturated with respect to ions (Tranter et al., 1998). In temperate glaciers, the undersaturated surface melt from the glacier travels to the glacier bed through conduits in the ice where it can then interact with rock surfaces. Consequently, high rates of chemical erosion occur in temperate and subpolar glacierized catchments from rock water interactions in the glacial bed (Benn and Evans, 1998). Since subglacial drainages are thought to be absent in the cold-based glaciers typical of high latitudes, the potential for weathering reactions is assumed to be minimal (Hodgkins, et al., 1998). In theory, run-off from cold-based or polar glaciers would consist primarily of supraglacial and englacial meltwaters which have dilute water chemistries; and the glacier ice and snow in the McMurdo Dry Valleys do show low values for all chemical constituents (Lyons et al., 1998).

Robinson (1984) hypothesized that subglacial brine sealed below the Taylor Glacier tongue causes the ablation zone to function more like a polythermal glacier. Hubbard et al. (2004) using ice penetrating radar detected areas of subglacial ponding approximately 3-6 km up-glacier of the terminus. At this distance, the glacier overrides an iron rich marble metasediment (Keys, personal communication). The subglacial "ponding" zone was measured to be 400-1000 m across and corresponds with a depression that is 80 m below sea level (Hubbard et al., 2004). The temperature in this region was modeled to be -7º C (Hubbard et al., 2004). The radar data is not explicit and the high reflectance described as "subglacial ponding" could be saturated soils, slush ice or a liquid water body (Hubbard et al., 2004). A temperature of -7º C suggests a hypersaline brine, which is presumably the source waters of the Blood Falls outflow.

Microorganisms can contribute to subglacial water chemistry (Sharp et al., 1999, Skidmore et al., 2000, Tranter et al., 2005), and water chemistry can implicate specific metabolic activities providing information on the actual subglacial environment. Tranter et al. (2005) outlined a series of weathering reactions likely to occur in the subglacial setting. For example, iron and sulfur oxidation can occur under oxic conditions according to equation 1.

$$4FeS_{2(aq)} + 16CaCO_{3(s)} + 15O_{2(g)} + 14H_2O_{(aq)} \leftrightarrow 16Ca_2{}^{+}{}_{(aq)} + 16HCO_3{}^{-}{}_{(aq)} + 8SO_4{}^{2-}{}_{(aq)} + 4Fe(OH)_{3(s)} \quad (1)$$

Where the subscripts refer to: aqueous (aq), solid (s) and gaseous (g). This pyrite oxidation and carbonate dissolution reaction produces HCO_3^- and SO_4^{2-} in a 1:1 ratio (Wadham et al., 2004). When heterotrophic microorganisms are present, organic matter

can also be oxidized generating CO_2 in excess of the 1:1 ratio from equation 1. Iron, sulfur, and organic matter oxidation consume oxygen and drive subglacial waters to anoxia (Tranter et al., 2005). In an anaerobic setting, electron acceptors other than O_2 become thermodynamically favorable; organic matter can be oxidized to CO_2 using Fe^{3+}, Mn^{4+}, and oxidized forms of nitrogen and sulfur as the electron acceptor. In highly reducing subglacial environments methanogenesis may occur, converting CO_2 to CH_4 (Skidmore et al., 2000 and Tranter et al., 2005). These aerobic and anaerobic reactions have been documented in arctic subglacial samples (Bottrell and Tranter, 2002, Wadham et al., 2002a, 2002b, 2004).

The chemistry of Blood Falls was first sampled by Hamilton et al. in 1958 and again by Black et al. in 1962 (Hamilton et al., 1962 and Black et al., 1965). However, in these studies, the researchers sampled the salt precipitates, or the "salt cone" that formed around the terminus of the Taylor Glacier, they did not sample true, unaltered outflow. Keys (1979) collected the first detailed chemical data in 1978 on what he described as a "discharge of saline icing at Blood Falls." Since the inception of the McMurdo LTER in 1992, flow from Blood Falls has been sampled routinely, providing over a decade of chemical data (data available at http://huey.colorado.edu/LTER/). It is clear from these geochemical data that the source water of Blood Falls is marine in origin (Lyons et al., 1998, 1999) and that the release of ancient subglacial fluid is episodic in nature resulting in complex and dynamic chemistry of waters sampled at Blood Falls (Lyons et al., 2005). In this chapter I present geochemical data that differentiates ancient outflow represented by subglacial Taylor brine, from diluted brine precipitates and contemporary glacial melt

flow. This paper builds on the LTER long-term data set with two additional years of data and new biogeochemical parameters.

Material and Methods

Blood Falls is a dynamic outflow feature at the terminus of the Taylor Glacier. Samples have been collected annually during routine LTER stream sampling (details of sample collections from 1993-2003 are available in Lyons, et al., 2005 and online at http://www.homepage.montana.edu/~lkbonney/). Samples from 2003 were collected via peristaltic pump methods (Appendix A. provides a detailed description of sample collection methods). Samples from 2004 were collected directly from the outflow into sterile vessels. The 1978 ion chemistry data were obtained from the published work of Keys (1979).

Geochemistry

Geochemical analysis was conducted as described in Chapter 3. Details on these procedures are available in the MCM LTER Limnological Methods Manual (Priscu and Wolf, 2000, available online at http://www.homepage.montana.edu/~lkbonney/). Briefly, pH was measured with a calibrated Beckman Φ12 pH meter. REDOX potential (Eh) was measured on samples collected in sealed scintillation vials. Samples were kept chilled (~ 4º C) until analysis (within 5 days). Eh was measured using a platinum silver/silver chloride combination electrode attached to a Beckman Φ12 meter. The electrode was standardized with a Zobell solution of Fe (III)/Fe (II) to 320 mV. Dissolved oxygen (DO) was determined by Winkler titration (Standard Methods, 1992). Dissolved

inorganic carbon (DIC) was measured by infrared gas analysis on acid sparged samples. Dissolved organic carbon (DOC) was measured on filtered (25 mm Whatman GF/F) acidified samples with a Shimadzu 5000 TOC analyzer. Major ions were measured according to the protocol of Welch et al. (1996). Total iron was measured using the ferrozine assay (Fulton et al., 2004).

Microbial Cell Counts

Outflow samples (10 ml) were collected into sterile 20 ml scintillation vials and preserved with filtered sterilized (0.2 μm) 37% formaldehyde (formalin) buffered with saturated sodium borate. Samples of outflow (3 ml) were stained with SYBR gold nucleic acid stain 25X (Molecular Probes) for 15 minutes as described by Lisle and Priscu (2004) then collected onto a 25mm 0.2 μm black polycarbonate filter (Poretics) supported by a 0.45 μm membrane filter. Filters were mounted on glass microscope slides with several drops of anti-fade solution (0.1% p-phenylenediamine in a 1:1 solution of phosphate-buffered saline and glycerol). Live cells were differentiated from dead cells based on the integrity of the plasma membrane using the Bac*Light* LIVE/DEAD® Bacterial Viability Kit (Molecular Probes, Inc.). Samples for viability were prepared in the same manner as samples for total cell counts. Cells were visualized and counted using a Nikon Optiphot epifluorescent microscope, equipped with a DM510 filter cube (Nikon) at a magnification of 1000X.

Incorporation Experiments

Blood Falls outflow was collected in December, 2004 directly into sterile 30 ml serum vials filled to overflowing and crimp sealed on site. Serum vials were kept chilled during transport and immediately processed (~ 4 h following sample collection). Samples were prepared in replicate (4 live, 3 killed controls) as described below for anaerobic light and dark incubations and unaltered light and dark incubations. 14 of 28 serum vials were flushed with N_2 gas to displace oxygen. The nitrogen gas was first passed through a 0.2 μm filter and each vial was flushed for approximately 3 min. The remaining 14 serum vials were not altered. Six gassed vials and six unaltered vials were killed using 1 ml of 0.2 μm filtered sodium borate buffered formalin to serve as controls. 1.9×10^6 DPM ml^{-1} of ^{14}C-labeled bicarbonate was added to each sample (28 total) with a sterile syringe. Seven gassed samples (four live, three killed) and seven unaltered samples (four live, three killed) were incubated in the dark at 2-4º C for one week. The other 14 bottles (four live gassed, three gassed killed and four unaltered lives and three unaltered killed) were incubated at 2-4º C with light (Photosynthetically active radiation at 400-700 nm (PAR) ~ 90 μmol photon m^{-2} s^{-1}). Incubations were terminated by filtering onto a precombusted 25 mm Whatman GF/F filter. Filters were placed into clean scintillation vials, acidified with 0.5 ml of 3N HCl, and placed on a heat block (60º C) until dried. Cytoscint scintillation cocktail (10 ml) was added to each sample and the radioactivity determined with a Beckman LS6000 scintillation counter. Bicarbonate incorporation rates were calculated from activity in live treatments minus killed treatments as outlined in equation 2.

$$\mu g\ C\ L^{-1}\ d^{-1} = \frac{(DPM_{LIVE} - DPM_{KILLED}) \times DIC\ (\mu g\ C\ L^{-1}) \times 1.06}{DPM_{ADDED} \times time\ (d)} \qquad (2)$$

Where DIC = dissolved inorganic carbon in the sample and 1.06 = the isotope discrimination factor (Lizotte et al., 1996). Samples for ^{3}H-thymidine incorporation were collected as described above for the bicarbonate incorporation studies except that 20 ml serum vials were used. Only dark, unaltered incubations were tested for thymidine incorporation. Samples in serum vials were inoculated with 20 nM (8 µl) ^{3}H-thymidine stock (4.3 x 10^{5} DPM ml^{-1}). Serum vials were incubated at 2-4º C in the dark for three days. Treatments were transferred to sterile 60 ml Nalgene containers at the end of the incubation and 20 ml ice cold 10% TCA solution was added to terminate the reaction and to precipitate nucleotides. Thymidine incorporation rates per day were calculated from the activity in live over killed treatments (equation 3).

$$nM\ thymidine\ d^{-1} = \frac{(DPM_{LIVE} - DPM_{KILLED}) \times (nM\ thymidine\ added)}{DPM_{ADDED} \times time\ (d)} \qquad (3)$$

Results

Geochemical Data

Sulfate (SO_4^{2-}) and chloride (Cl^-) concentrations measured in Blood Falls outflow revealed peak concentrations that are indicative of ancient subglacial outflow (Figure 3.1).

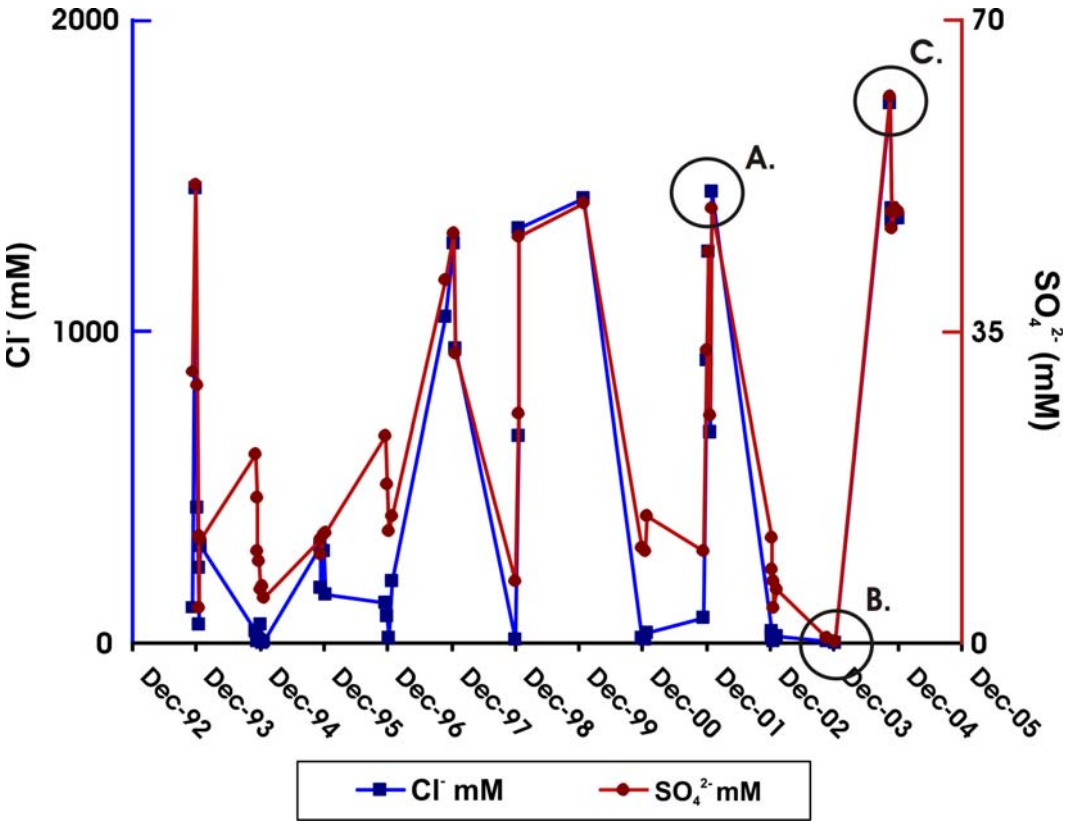

Figure 3.1. Concentrations of Cl^- and SO_4^{2-} plotted against time. Peaks of high Cl^- and SO_4^{2-} are indicative of ancient outflow events. Circled points represent samples discussed in greater detail in this dissertation. "A." represents the time when samples were collected for construction of a bacterial clone library (Chapter 4). "B." represents a year when ancient flow did not occur and biogeochemical data from these samples are compared in detail with "C," a representative ancient flow year for which extensive biogeochemical data exists (this chapter and Chapter 5).

The samples collected during these peaks had average concentrations of SO_4^{2-} = 48.3 mM (SD = +/- 5.0) and Cl^- = 1371 (SD = +/- 159) and SO_4^{2-}:Cl^- molar ratios consistently near 0.035. Based on SO_4^{2-} and Cl^- concentrations that correspond with these concentrations, ancient subglacial outflow events occurred during the following seasons of LTER monitoring: 1993-94, 1997-98, 1998-99, 1999-00, 2001-02 and 2004-05. Published data indicate that 1978 was an ancient outflow event (Keys, 1979). The molar ratios (SO_4^{2-}:Cl^-) covaried over the sampling period however, correlation values differed between ancient outflow events and contemporary melt events. Ancient outflow events (samples from the years listed above) exhibit a stronger correlation between SO_4^{2-} and Cl^- concentrations ($r=0.943$; $p<0.001$, $df=11$) than samples collected during non-flow events ($r=0.749$, $p<0.001$, $df=34$).

Geochemical parameters collected during the ancient outflow event of 2004 were consistent in samples collected in November through December (Table 4.1). The first sample collected (4 Nov 2004) was an exception and was more concentrated in dissolved ion chemistry and may represent a 'first flush' of concentrated brine. pH values near 6.2 and high DIC (~50μM) are indicative of ancient outflow, however these parameters are more susceptible to probe and/or collection bias and do not behave as conservatively as Cl^- (i.e., chloride is not controlled by biochemical or geochemical processes in aquatic systems, Lyons et al., 1999). Total iron in the sample collected on 13 November 2004 was 3.8 mM with 97% as Fe^{2+}. Dissolved oxygen (DO) measurements were taken during November and December of 2004. Samples collected in November (data not shown) were analyzed after 1-2 weeks resulting in possibly inaccurate DO measurements as oxygen can permeate the scintillation vial caps. Samples collected on 23 December were

processed immediately; and DO in samples collected directly from the glacier contained no detectable oxygen. Oxygen increased as the outflow moved down the terminal moraine; 0.6 mg O_2 L^{-1} on the slope and 8.8 mg O_2 L^{-1} at the base of the moraine before entering West Lake Bonney moat (Figure 3.2). There is insufficient data from past flow years to conclude whether the subglacial Taylor brine is consistently anoxic and Eh measurements (~ 100 mV) suggest that the brine may not be highly reducing. Therefore, SO_4^{2-} and Cl^- concentrations provide the best metric of flow conditions at Blood Falls.

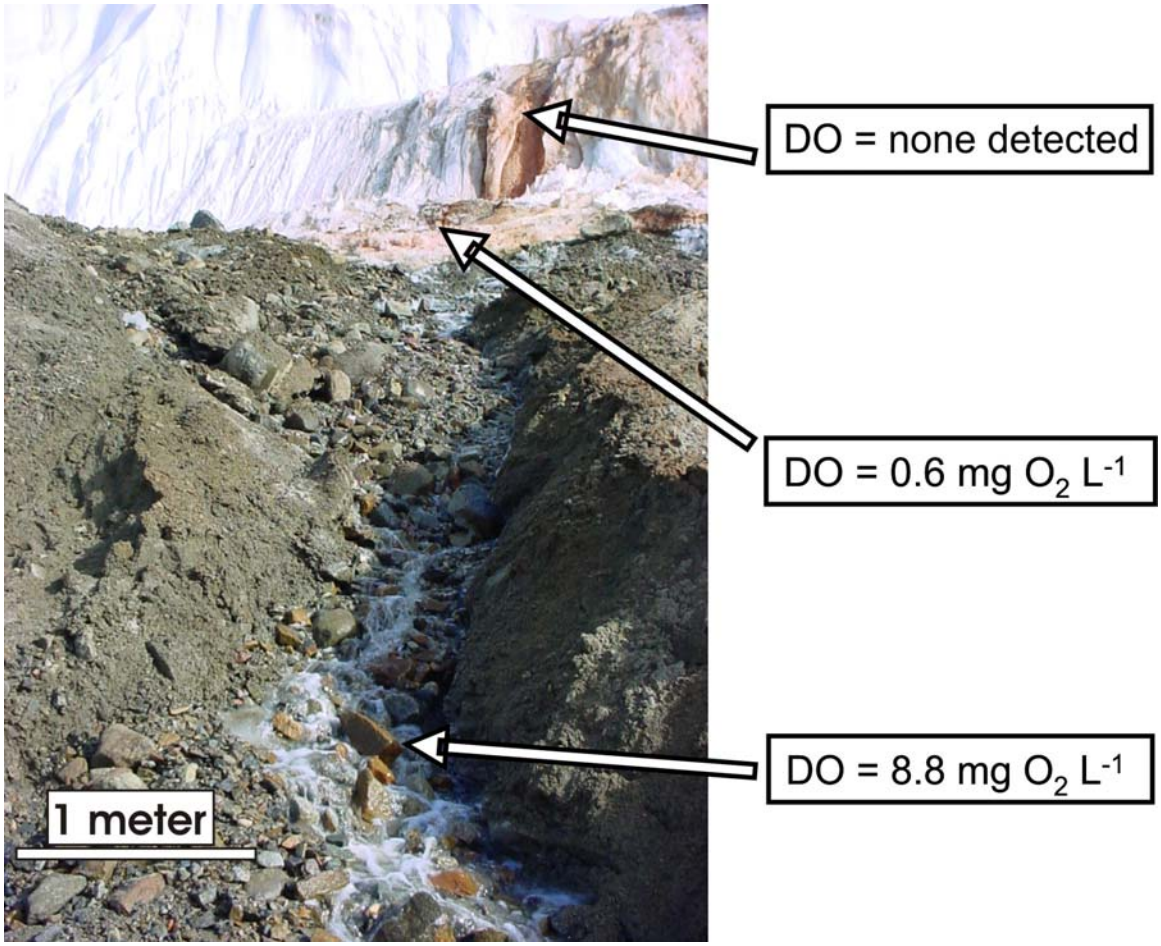

Figure 3.2. Spatial trends in dissolved oxygen (DO) of samples collected 23 Dec 2004.

Table 3.1. Summary of biogeochemical data from predicted ancient outflow events. †= data from Keys, 1979, exact date of sample collection not published, **= no oxygen measurements were made, but outflow was reported as clear at collection and accumulated iron oxides upon exposure to oxygen. na = not available

Collection Date	***Temp (° C)***	***pH***	***DOC (µM)***	***DIC (mM)***	***DO (mM)***	***Cells ($1x10^5$)***	***% live***	***Cl⁻ (mM)***	***SO_4^{2-} (mM)***	***SO_4:Cl***
11 Nov 04	-6.0	6.6	385	48	na	na	69	1730	61.4	0.035
13 Nov 04	-5.0	6.2	416	66	na	1.6	72	1395	48.4	0.035
14 Nov 04	-6.0	6.2	410	55	na	0.8	na	1356	46.6	0.034
3 Dec 04	-5.0	6.1	427	51	na	0.8	74	1362	48.9	0.036
23 Dec 04	-4.0	6.1	452	na	0.0	1.0	69	1364	48.4	0.035
2004 average	**-5.2**	**6.2**	**418**	**55**		**1.0**	**71**	**1441**	**50.7**	**0.035**
1978†	-5.1	6.2	Na	na	na**	na	na	1404	52	0.037
20 Jan 93	-5.3	6.5	203	na	na	na	na	1459	51	0.035
23 Nov 97	na	na	488	na	na	na	na	1045	41	0.039
2 Jan 98	na	na	432	na	na	na	na	1279	46	0.036
17 Jan 99	-4.0	6.4	Na	na	na	na	na	1329	46	0.034
18 Jan 00	-4.8	6.3	450	na	na	na	na	1424	49	0.035
4 Jan 02	-3.8	Na	Na	na	na	na	na	1254	44	0.035
24 Jan 02	-4.7	6.0	Na	na	na	na	na	1450	49	0.034

Biogeochemistry

Ancient outflow events (2004 samples) have different biological characteristics than contemporary melt events (2003 samples) (Table 3.2). Designation of these two samples as flow from ancient outflow and non-ancient flow contemporary melt was based on chemical analysis (i.e. SO_4^{2-} and Cl^- concentrations), but the biology is distinct as well. Bacterial densities averaged 1 x 10^5 cells ml^{-1} (SD = ±0.30 x 10^5) during ancient outflow events with approximately 70% of the cells present having intact cell membranes, cell densities were lower in 2003 (3 x 10^4 cells ml^{-1}, SD = ±0.69 x 10^5). Total iron, dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) concentrations were greater in ancient outflow when compared to contemporary melt (ancient outflow = 3772 μM Fe, 418 μM C as DOC and 55 mM C as DIC; contemporary melt: 22 μM Fe, 82 μM C as DOC and 0.46 mM C as DIC). Ancient outflow had a slightly acidic pH (6.20), whereas contemporary melt was basic (8.9). The effect of salt precipitation and loss of CO_2 upon equilibration with the atmosphere are likely factors in the drastic pH shift. pH measurements for ancient outflow (pH=6.2) are consistent with the pH below the chemocline of the west lobe of Lake Bonney; pH measurements presented in Chapter 2 were samples collected during a contemporary melt event.

Table 3.2. Comparison of biogeochemical parameters between a contemporary melt event (2003) and an ancient flow event (2004).

	Sample collection date:	
Parameter	**2003**	**2004**
pH	8.9	6.2
DOC (µM C)	81.5	418
Cell density (cells ml^{-1})	3.0×10^4	1.0×10^5
DIC (mM C)	0.46	55
DO (mM O_2)	0.3	0.0
Total Fe (µM)	22	3772
SO_4:Cl	0.383	0.035
	Contemporary melt event	***Ancient outflow event***

Productivity Measurements

Carbon fixation and thymidine incorporation rates were determined in samples from the outflow brine by measuring the incorporation of radiolabeled substrate into particulate material in live treatments relative to killed negative controls. Carbon fixation and thymidine incorporation were measurable but low in samples of ancient outflow (Figure 3.3). There was considerable error associated with all measurements, owing to the low rates. Live treatments were higher than kill treatments in the anaerobic, dark incubations and statistically significant to the $p = 0.050$ level (df=6). Bicarbonate incorporation in the anaerobic, dark incubations was 1.2 nmol C L^{-1} d^{-1} (SD = ± 0.7, p=0.058, df=2). Average label incorporated into particulate material was higher in the light incubations live treatments (samples not sparged) than the kills, however not statistically significant ($p = 0.295$). Bicarbonate incorporation in samples incubated under lighted conditions (samples not sparged) was 0.51 nmol C L^{-1} d^{-1} (SD = ± 0.46,

p=0.295, df=2). The remaining treatments (light treatment sparged and dark treatment not sparged) did not show average activity in live treatments that were higher than the kill treatments. Thymidine incorporation also occurred at low rates, 2.1 x 10^{-4} nM thymidine d^{-1-} (SD = ± 0.45).

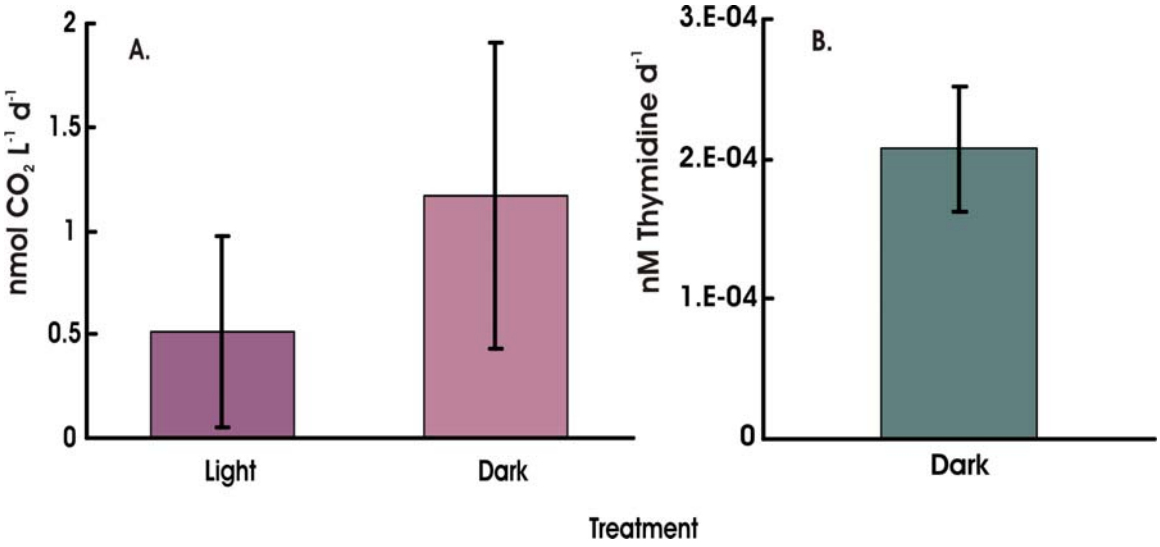

Figure 3.3. Metabolic activity measurements in samples of ancient outflow. Bicarbonate incorporation, light and dark treatments (A) and thymidine incorporation in the dark (B).

Discussion

Outflow Controls on Ancient Subglacial Taylor Brine

Ancient outflow at Blood Falls does not occur on a regular basis, but when outflow does occur, salts from the highly concentrated brine precipitate at the surface. These salts can be diluted during times of high melt or during the following season with glacier melt, yielding disparate chemistries at Blood Falls. Therefore, the flow at Blood Falls can represent several conditions: (1) ancient subglacial outflow, (2) a mixture of

subglacial brine and glacial melt or (3) a mixture of salt precipitates from previous ancient events diluted with contemporary glacial melt.

Ancient Outflow. Concentrations of SO_4^{2-} and Cl^- are highest during ancient outflow events, with a consistent molar ratio of SO_4^{2-}:Cl^- near 0.035. This ratio falls below the seawater line (Figure 3.4) indicating that ancient outflow is depleted in SO_4^{2-} relative to seawater. In artic subglacial environments, sulfate losses have been attributed to sulfate reducing bacteria (Wadham et al., 2004).

Subglacial Brine and Contemporary Melt Mixture. Samples with characteristic molar ratios of ancient outflow (SO_4^{2-}:Cl^- ~ 0.035), but with low concentrations of SO_4^{2-} and Cl^- imply that ancient flow is being diluted with glacial melt. A clear example occurred during 2001-2002, a year of above average summer temperatures (Foreman et al., 2004) during which there was also an ancient flow event at Blood Falls as indicated by SO_4^{2-} and Cl^- concentrations (Figure 3.1). Ancient outflow had begun by the collection of the 22 December 2001 sample (Figure 3.5). Samples collected on 24 January 2002 are 50% lower in SO_4^{2-} and Cl^- concentrations (arrow in figure 3.4 and 3.5) compared to other ancient outflow samples, yet, the SO_4^{2-}:Cl^- ratio (Figure 3.5 C) remained at 0.036 consistently since the start of ancient outflow. This decrease in Cl^- and SO_4^{2-} concentrations correspond with a period of above average PAR and above average temperatures (Figure 3.5 A) implying that the concentration of ions are being diluted by glacier melt.

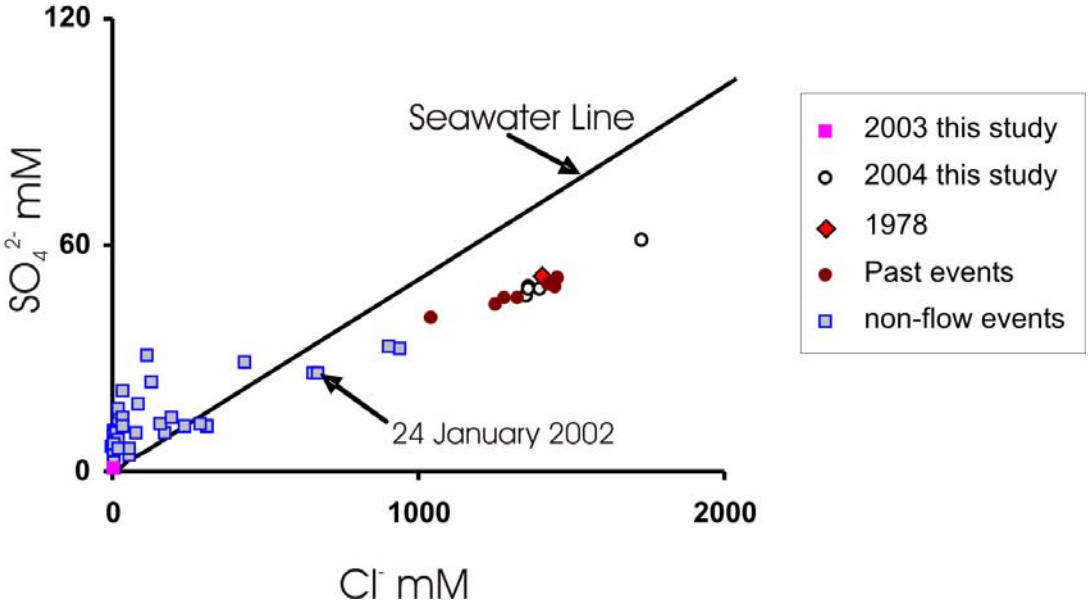

Figure 3.4 SO_4^{2-} concentrations plotted against Cl^- concentrations in samples of flow collected at Blood Falls. Ancient flow events from 2004 (hollow circles) and past events in the geochemical record (solid circles and diamond) fall below the seawater ratio. Data from Lyons et al. (2005), Keys (1979) and this study.

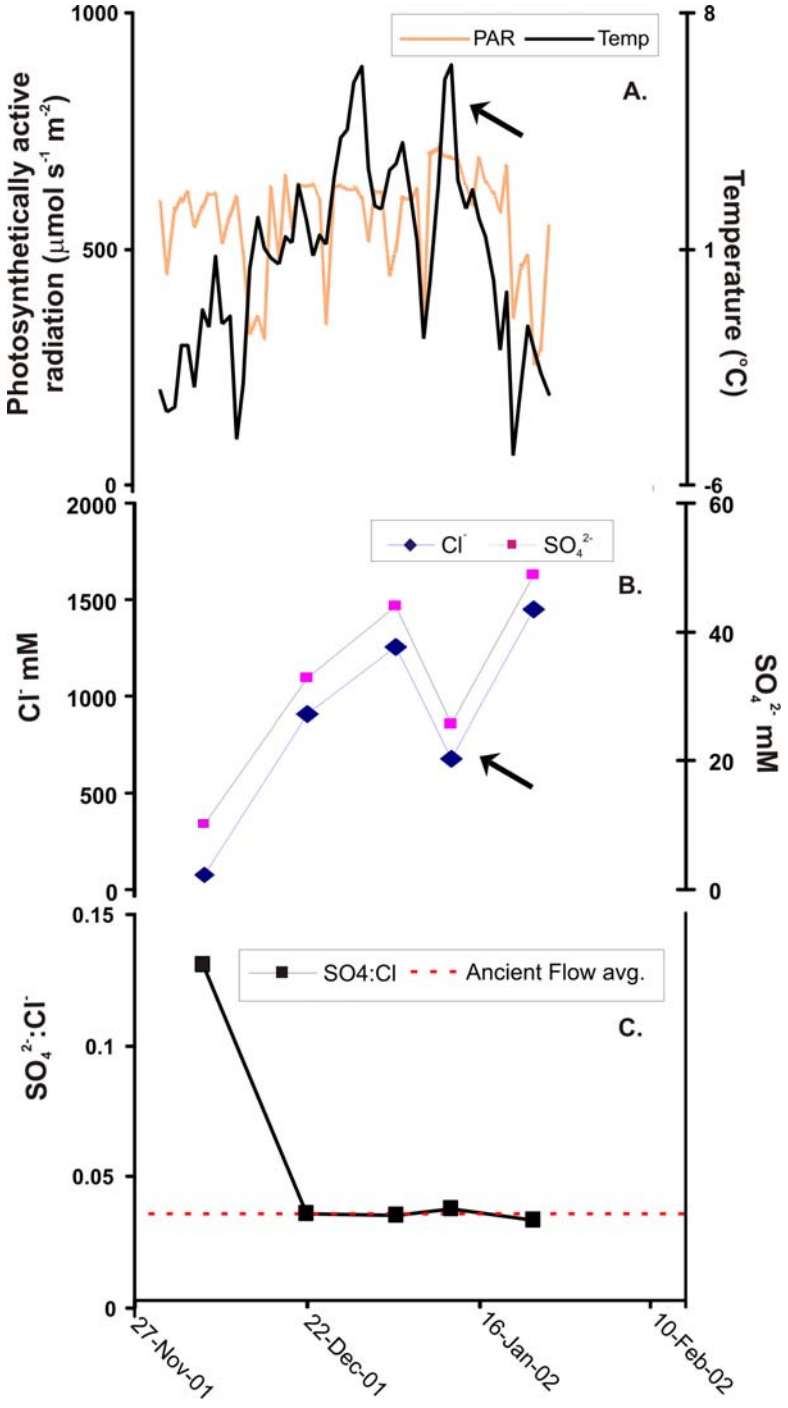

Figure 3.5. Time series for 2001-02 outflow samples. Panel A graphs average daily temperature and PAR collected from the nearby Bonney Snow Fence Meteorological station; the arrow in panel A points to an above average period of solar radiation and temperatures. Panel B shows a change in concentration (see arrow in B) of Cl^- and SO_4^{2-} in mid-January 2002, while the molar ratios (C) remain the same and correspond with the ratio of ancient flow Cl^-:SO_4^{2-} (0.035)

Salt Precipitates Diluted with Contemporary Melt. Black and Bowser (1968) documented the change or "aging" of salt precipitates following a 1962 flow event. The authors found that the salt precipitates were originally dominated by NaCl and in subsequent years, Na_2SO_4 became the dominate salt; and noted that the relative abundance of the two salts was reversed. The chemical distinctions between ancient outflow and contemporary melt was also observed by Lyons et al. (2005). Lyons et al. (2005), concluded that when concentrations of Cl^- were less than 500 mM, SO_4^{2-} was in excess of the seawater ratio; and conversely, the concentration of SO_4^{2-} was depleted to that of the seawater ratio when Cl^- concentrations are greater than 500 mM (Figure 3.4). It is clear that ancient flow events fall below the seawater ratio line, or are depleted in SO_4^{2-} relative to the expected concentration for water that was strictly concentrated from seawater without alteration. This "aging" of salts at Blood Falls likely occurs for a variety of reasons discussed below. A salt cone of precipitates remains at the glacier surface following an ancient discharge event. This salt deposit is selectively evaporated and cryogenically separated; the altered salts are then diluted with glacial melt as the austral summer progresses (Black et al., 1965). These processes cause fractional crystallization and changes in the salt composition, resulting in disparate concentrations of NaCl and Na_2SO_4 measured in contemporary melt years.

The total bulk composition of ancient discharge was not characterized here rather SO_4^{2-} and Cl^- concentrations were described as geochemical "markers" for ancient outflow. Other chemical constituents of outflow are likely related to ancient subglacial release events. For example, Si concentrations were discussed in Chapter 2 as a proxy for water-rock interactions as Si inputs to stream-water can only originate from chemical

weathering (Schlesinger, 1997). In the McMurdo Dry Valleys, Si concentrations in stream flow typically increase with stream length (Lyons et al., 1998). Blood Falls is the shortest "stream" in the Taylor Valley (estimated at ~100 m) yet Si concentrations in Bloodfalls flow during 1999-2000 (242 μM Si), a season with ancient outflow, were higher than all other sampled McMurdo Dry Valley streams (Pugh et al. 2003). Si concentrations the following season (2000-2001) measured at Blood Falls were 16 μM, which was at the lower end of the range of Si concentrations (range = 4 to 134) for the dry valley streams that year. This delineation of ancient flow events can help resolve disparate chemistries in the long term dataset from Blood Falls outflow.

The Biological Component of Ancient Outflow

Cells abundances in the ancient outflow samples from 2004 (1×10^5 cells ml^{-1}) were an order of magnitude higher than those reported for 2002 samples (1×10^4 cells ml^{-1}) or 2003 samples (3×10^4 cells ml^{-1}), both of which were contemporary melt years. Viability staining indicated that ~ 70% of the cells in the ancient outflow are alive, yet productivity measurements, using labeled bicarbonate and thymidine, were low. Different techniques to measure microbial metabolic activity might help resolve this disagreement. Thymidine incorporation rates measured in ancient outflow from 2004 (2.1×10^{-4} nM thymidine d^{-1}) were comparable to previously reported samples collected from Blood Falls in 2000 (0.7×10^{-3} nM thymidine d^{-1}, SD = ±0.45) another year of ancient outflow (Chapter 2, Mikucki et al., 2004). Bicarbonate incorporation rates were also low, but measurable (1.2 nmol C L^{-1} d^{-1}) in ancient outflow samples incubated under near *in situ* conditions. Blood Falls CO_2-fixation activity was orders of magnitude less

than the oxygenic photosynthetic primary production in the west lobe of Lake Bonney. For example, at 14 m depth (which corresponds to the deep chlorophyll maximum) primary production was ~ 15 μg C L^{-1} d^{-1} (Takacs et al., 2001). The low rates of CO_2-fixation in Blood Falls were statistically similar to rates reported for a subglacial volcanic lake (3.3 nmol C L^{-1} d^{-1}, p= 0.114) (Gaidos et al., 2004). Chemoautotrophic energy production in subglacial microbial assemblages is a significant finding as it implies the ability of subglacial ecosystems to function autonomously.

Conceptual Subglacial Models

Two models of Taylor Glacier's subglacial structure are proposed, based on past research and the biogeochemical data presented here. Outflow during 2004, a year of ancient outflow was anoxic, and polythermal glaciers in the Artic have been shown to store subglacial water through the winter providing time for microbial populations to drive the aerobic glacial melt water to anoxia (Bottrell and Tranter, 2002). In the Artic, where the subglacial hydrology is conducive to water storage through the winter and subsequent release in the spring, the first flush of outflow is anaerobic, while subglacial outflow collected later in the season is oxic (Tranter et al., 1997, Bottrell and Tranter, 2002). Outflow at Blood Falls was anaerobic for nearly two months of discharge during 2004. Discharge volumes were estimated to be as large as 6,000 m^3 during subglacial release events (Black et al., 1965, Keys, 1979). Therefore, the structure below the Taylor Glacier must be significantly large enough to generate these volumes of anaerobic outflow.

Model 1: The Subglacial Lake. In model 1 (Figure 3.6) remnant seawater is sealed below the Taylor Glacier and is considered a third lobe to Lake Bonney (Lyons et al., 2005). Seawater that was cryogenically concentrated and subsequently overridden by the Taylor Glacier would be contained in the 80 m depression 4-6 km upglacier. Based on the geochemistry presented here, this lake would be largely anaerobic. The surface layer of the lake would receive oxygen from the overlying glacial ice creating an oxic-anoxic interface for microorganisms to take advantage of the opposing chemical gradients of nutrient rich anaerobic brine and molecular oxygen from the dissolved glacier ice. Lake water brine would either travel through a discrete subglacial conduit the ~3-5 km to the glacier snout at Blood Falls, or physical shifts in the glacier may trigger the release of the subglacial lake water flushing aerobic surface waters first, with the remaining outflow consisting of deeper anaerobic lake water. In this model, both aerobic and anaerobic organisms would be expected to exist in the subglacial lake, but anaerobic lifestyles would dominate the community structure.

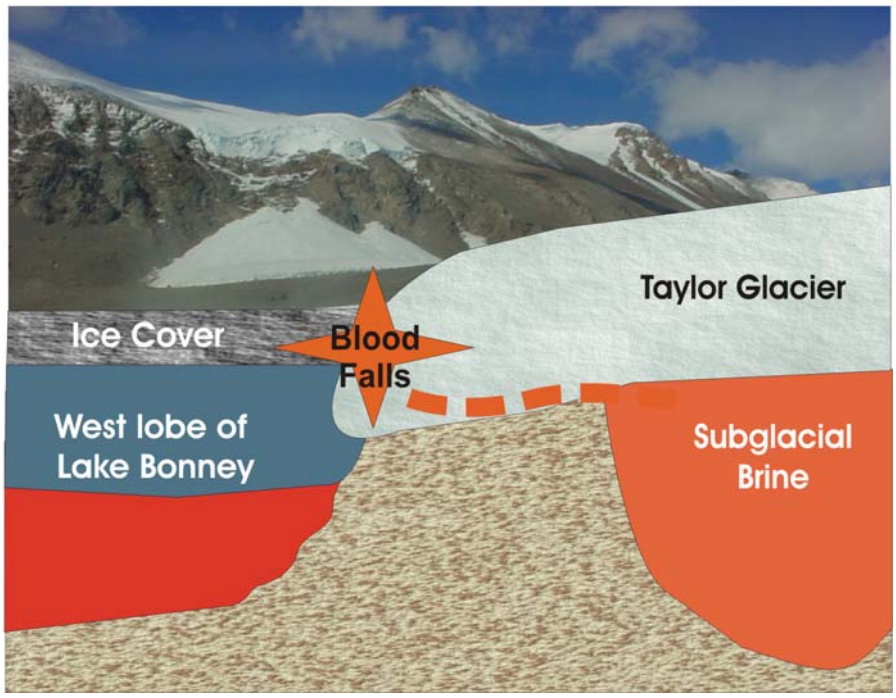

Figure 3.6. Model 1: the subglacial lake. Remnant seawater is sealed below the glacier and is released at Blood Falls through a discrete subglacial conduit. *Features not drawn to scale.*

Model 2: Distributed Subglacial Brine. Model 2 is adapted from a model first proposed by Keys (1980). In this model, the large depression detected by Hubbard et al. (2004) below the glacier contains a salt deposit precipitated from the ancient marine water (Figure 3.6). When these salts come in contact with the glacier bed, the basal ice is dissolved creating a brine that is distributed throughout the basal area below the glacier tongue. When enough volume is generated, pressure and glacier bed geomorphology cause the brine to converge near the glacier snout and eventually be released to the surface at Blood Falls. In this model, the outflow would be anaerobic given enough time distributed along the glacier bed for microorganisms to consume oxygen and drive the brine to anoxic. However, there would still be a supply of oxygen from the overlying

glacier ice above the putative lake and through any conduit or channels that the subglacial brine must travel before being released at Blood Falls. If the brine is distributed along the majority of the basal ice from the terminus to the brine source (~ 4-6 km upglacier) there would be significant oxic-anoxic interface.

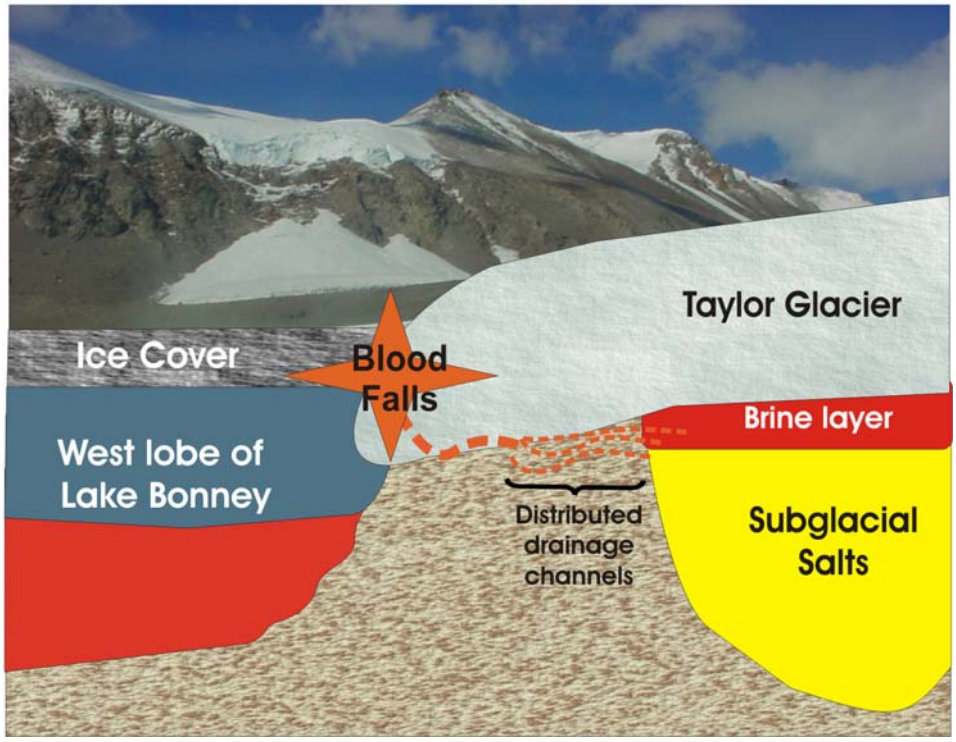

Figure 3.7. Model 2: a distributed subglacial brine. The brine layer forms when ancient salts are in contact with the overlying glacier ice, brine is then distributed along the basal layer of the glacier towards the tongue until its release at Blood Falls. *Features not drawn to scale.*

Conclusions

The chemistry of outflow waters below the Taylor Glacier collected at Blood Falls suggests a unique subglacial ecosystem with the potential for both aerobic and anaerobic metabolic and chemical processes. Significantly, this system has the biological potential for chemoautotrophy, a vital component for the maintenance of a permanently

cold and dark subglacial microbial community. Analysis of flow collected at Blood Falls reveals that outflow of ancient marine brine trapped below the Taylor Glacier has distinctive geochemical parameters. The unique chemistry of Blood Falls flow during ancient outflow years differs from contemporary melt events, biological parameters are distinct during ancient outflow as well. In the next chapter the biological community structure of ancient outflow is explored providing more detailed constraints on the subglacial habitat.

CHAPTER 4

BACTERIAL DIVERSITY ASSOCIATED WITH BLOOD FALLS, A SUBGLACIAL OUTFLOW FROM THE TAYLOR GLACIER, ANTARCTICA

Abstract

Blood Falls is the surface manifestation of subglacial brine that originates below the Taylor Glacier, in the McMurdo Dry Valleys, Antarctica. The geochemistry of Blood Falls shows that this brine is of marine origin. Molecular and culture analyses revealed a phylogenetically diverse microbial assemblage in Blood Falls, consisting of 16 unique phylotypes, identified by cloning, and seven unique phylotypes from culturing. The bacterial 16S rDNA clone library was dominated by a phylotype that had 99% sequence identity with *Thiomicrospira arctica* (46% of library), a psychrophilic marine autotrophic sulfur-oxidizer. The remainder of the library contained phylotypes from the *β*-, *δ*-, and γ-*Proteobacteria* and *Cytophaga-Flavobacteria-Bacteroides* divisions and included clones whose closest cultured relatives are from permanently cold marine environments, many of which are known to metabolize iron and sulfur compounds. Five isolates, including *Marinobacter sp.*, *Psychrobacter sp.*, *Janthiobacterium sp.* and two *Flavobacteria sp.*, had greater than 97% 16S rDNA sequence similarity to Blood Falls clones. These findings are consistent with the high iron and sulfate concentrations detected in Blood Falls, which are likely due to the interactions of the subglacial brine with the underlying iron-rich bedrock. My results imply that the brine below the Taylor Glacier hosts a

functional ecosystem with microorganisms capable of metabolizing in the absence of sunlight utilizing chemical energy present in reduced iron and sulfur compounds.

Introduction

The Taylor Glacier is an outlet glacier that drains the Polar Plateau in southern Victoria Land. The red discharge at the terminus of Taylor Glacier in the McMurdo Dry Valleys of Antarctica is commonly referred to as Blood Falls and results from reduced subglacial brine that episodically flows out from below the glacier; once exposed to the atmosphere, the brine is precipitated amassing a blood red salt cone at the northern end of the glacier terminus (Black et al., 1965). Ice penetrating radar data indicate the presence of water or slush below the glacier that corresponds to an 80 m depression in the bedrock topology (Hubbard et al., 2004). This depression is below sea level and forms what is believed to be a third lobe of Lake Bonney (Lyons et al., 2005). The ionic composition and ^{36}Cl values from Blood Falls are consistent with marine waters that have experienced cyrogenic concentration (Lyons et al., 1998, Lyons et al., 2005). The subglacial brine is thought to originate from the last incursion of marine water into the McMurdo Dry Valley network during the Pliocene Epoch (~5Mya) when the dry valleys were fjordlands (Elston and Bressler, 1981). Although geochemical studies have been conducted on the subglacial outflow since the early 1960s (Angino et al., 1964, Keys, 1979, Lyons et al., 2005), this study is the first to investigate the microbial diversity associated with Blood Falls.

Relatively few studies exist on the microbial diversity associated with subglacial environments because the subglacial setting was previously thought to be devoid of

biologically mediated processes (Raiswell, 1984) and subglacial samples are difficult to collect. Microscopic observations of dividing microbial cells and *in situ* sulfate production in subglacial outflow from an Arctic glacier provided the first evidence of active subglacial microbial communities (Sharp et al., 1999). Since Sharp and colleagues first presented data for an active subglacial microbial assemblage in the Canadian Arctic, there have been several reports of microbial cultivation and activity from subglacial sediments and waters (e.g., Skidmore et al., 2000, Bottrell and Tranter, 2002, Gaidos et al., 2004, Foght et al., 2004, Mikucki et al., 2004, Wadham et al., 2004).

The presence of microbial metabolism below glaciers has important implications for the processing of glacially "covered" carbon materials, such as tundra grasses for example, during periods of glaciation. Ice-core CO_2 records show a strong feedback between glacial, oceanic, atmospheric, terrestrial and biological carbon cycling during glacial and interglacial periods (Benn and Evans, 1998, Falkwoski et al; 2000). Subglacial microbial metabolism may have an important role in the cause and effect relationships of glaciation and atmospheric CO_2 dynamics.

The episodic release of subglacial brine at Blood Falls allows for the description of this subglacial environment in the absence of direct samples and has been used as a proxy to describe the ecological conditions that exists below the Taylor Glacier. This chapter describes the bacterial diversity of Blood Falls and the potential links between the diversity of these organisms and the geochemical conditions of the *in situ* environment.

Material and Methods

Site Description

The McMurdo Dry Valleys form the largest ice-free region in Antarctica (~ 4,000 km^2) and is characterized by strong winds, low precipitation (<6 mm water equivalents) and an average annual air temperature near -20°C (Priscu, 1998). This polar desert contains a mosaic of permanently ice-covered lakes, ephemeral streams, arid soils, exposed bedrock and both alpine and outlet glaciers. Dry polar desert conditions are believed to have persisted in the region for the past 15 million years (Sugden et al., 1992 and Summerfield et al., 1999). Several authors have hypothesized that marine waters remained in a basin below sea level in the upper valley and were subsequently covered by the advancing Taylor Glacier (Angino et al., 1965, Keys, 1979, Robinson, 1984, Lyons et al., 2005). Consequently, the Taylor Glacier, owing to the presence of subglacial brine near the terminus (Robinson, 1984), behaves more like a temperate or polythermal glacier. Liquid brine exists below the glacier, approximately 4 km up glacier from its terminus (Hubbard et al., 2004), which episodically drains at Blood Falls, the end of a conduit where subglacial brine emerges to meet the atmosphere.

The geochemistry of Blood Falls varies with the rate of subglacial discharge with low discharge waters being diluted with contemporary glacial surface melt. Chloride and sulfate concentrations are always highest during periods of active subglacial outflow (as described in Chapter 4). Ancient outflow is further characterized as slightly acidic (pH~6.2-6.5) with low dissolved oxygen concentrations. Dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) are high (~ 50 mM C and 400 μM C,

respectively). Additional information on the geochemistry of Blood Falls was discussed in Chapter 3 and can be found on the MCM LTER webpage (http://huey.colorado.edu/LTER/) and Lyons et al. (2005).

Culture work

Samples were collected for culture work during the austral summers (October – January) between 2001 and 2004 (Table 4.1). Outflow was collected directly into 74 mL serum vials by submerging the vials in the flow and crimp sealing them with a butyl rubber stopper with no head space. Serum vials were kept in the dark below 4 °C until return to McMurdo Station for incubation in selective media (approx 5-10 days). Media included R2A agar (Difco), Marine agar (Bacto) and thiosulfate-oxidizing agar (15%). Media are described in detail in Appendix C. Outflow sample (100 µl) was removed from the sealed serum vials with a 1 ml syringe, inoculated onto agar plates (15 g L^{-1}), spread with a sterile cotton swab and incubated at 2-4º C until colonies appeared (about 3-4 weeks). Enrichments for autotrophic microbes were done in selective medium using the Hungate method (Hungate, 1969). Enrichments for iron-reduction were prepared by inoculating 0.5 ml of outflow collected as described above, into 4.5 ml mineral media that was prepared under CO_2 gas and supplemented with Fe-OOH sludge (2 ml L^{-1}), (Lovley and Phillips, 1986) and pressurized with H_2 gas (30 psi), to a 0.7 M NaCl final concentration. Samples were serially diluted three times to a 10^{-3} extinction.

DNA Extraction

Samples for environmental DNA extraction were collected directly from Blood Falls outflow during the 1999-00 austral summer (Table 4.1). Samples (1L) were kept chilled (below 4 °C) and filtered immediately upon returning to the field camp (~ 4 h). Cells were collected onto a 90 mm membrane filter, placed in a sterile plastic bag; heat sealed and stored at -20° C until extraction. Filters were cut into pieces using flame sterilized scissors and tweezers and then processed according to the manufacturer's protocol (Mo Bio Laboratories, Inc. UltraClean Soil DNA Isolation Kit). The alternative protocol for maximum yields was followed. DNA extraction on a single isolated colony from each pure culture used the same methodology described above.

Table 4.1. Chloride concentration and type of flow at Blood Falls for each sampling season in this study. *indicates a year in which ancient outflow that had diluted chloride concentrations because of an unusually high glacial melt period (Chapter3).

Sample	**Field season**	**Average Cl^- (mM)**	**Average SO_4^{2-}:Cl^-**	**Blood Falls flow type**
Clone library	1999-2000	1420	0.035	Ancient outflow
Culture	2001-2002	1070*	0.035	Ancient outflow
Culture	2002-2003	22	0.448	Contemporary melt
Culture	2003-2004	2	0.206	Contemporary melt
Culture	2004-2005	1430	0.035	Ancient outflow

16S rDNA Clone Library Construction

The microbial 16S rDNA genes were amplified from extracted DNA using oligonucleotide primers 4F and 9F with 1492R (Table 4.2). Primers were purchased from Macromolecular Resources (Colorado State University). The 50-µl PCR reaction

contained 5 µl DNA template, dNTPs nucleotide mix (Fisher Scientific, 200µM final concentration), bovine serum albumin (BSA) (0.5 µg/ µl), 0.5 µM final concentration of each primer, 10x Taq Buffer (1.5 mM Mg^{2+}), 5x TaqMaster (a proprietary PCR enchancer, Eppendorf), and 1.25 units of *Taq* DNA polymerase (Eppendorf MasterTaq Kit). The reaction mixture was PCR amplified in an Eppendorf Mastercycler Gradient Thermal Cycler under conditions that included 15 sec of denaturation at 95º C, 45 sec of annealing at 55º C, and 1.5 min of primer extension at 72º C. These steps were repeated for 30 cycles. PCR products were purified using the QIAquick PCR purification kit (Qiagen) to remove primers, nucleotides, polymerases and salts. Purified PCR products were cloned using the pGEM-T Easy Vector System cloning kit with the pGEM-T Easy vector (Promega) according to the manufacturer's protocol.

Table 4.2. Oligonucleotide primers used for PCR amplification and sequencing reactions of 16S rDNA from Blood Falls genomic DNA, clones and isolates.

Primer use(s) and name	Direction	5'→ 3' Sequence
PCR and sequencing		
4F	Forward	TCCGGTTGATCCTGCCRG
9F	Forward	GAGTTTGATCTGGCTCAG
1492R	Reverse	GGTTACCTTGTTACGACTT
PCR, TGGE and sequencing		
341F-GC	Forward	CGCCCGCCGCGCGCGGCGGGCG GGGCGGGGGCACGGGGGGCCTA CGGGAGGCAGCAG
534R	Reverse	ATTACCGCGGCTGCTGG
Sequencing		
27F	Forward	AGAGTTTGATCCTGGCTCAG
515F	Forward	GTGCCAGCMGCCGCGGTAA
926R	Reverse	ACCGCTTGTGCGGGCCC
1068F	Forward	GCATGGCYGYCGTCAG
1391R	Reverse	GACGGGCGGTGTGTRCA

Sequencing of Clones and Pure Cultures

The clone library constructed from an environmental sample was screened by restriction fragment length polymorphism analysis (RFLP) with restriction enzymes Hin PI and MspI (New England Biolabs). Each 10 µl RFLP reaction contained 2µl of supplied enzyme buffer (New England Biolabs) with 2 units of each enzyme, and 8.7 µl of PCR product amplified directly from clonal colonies as described above, incubated at 37º C for 2 hours. Plasmid DNA was prepared for sequencing from clones using the QIAprep Miniprep kit (Qiagen) according the manufacturer's protocol. PCR product from pure cultures was prepared for sequencing using ethanol precipitation (Shapiro, 1981). Plates (96-well) containing DNA and primers were sent to Translational Genomics Research Institute (www.tgen.org) for sequencing. TGen uses Applied Biosystems (www.appliedbiosystems.com) automated sequencers.

Temperature Gradient Gel Electrophoresis (TGGE)

TGGE was used to test the effect of broth enrichment cultures on the growth of distinct microbial phylotypes. DNA was extracted from enrichment culture (1 ml) for each dilution and was amplified using PCR. Fragments (~ 221 bp) of the 16S rRNA genes were amplified from the genomic DNA using a set of bacterial primers (341F and 534R, Table 4.2), the forward primer contained a GC tail clamp (Muyzer et al., 1993). PCR conditions were as described above. PCR products (15 µl) were run on a temperature gradient gel (8% gel, run at 60 volts with a temperature gradient of 58-72 °C with a 0.8 °C ramp rate) using a DCode Universal Mutation Detection System (Biorad); protocol adapted from Muyzer et al.,1993). In the 10^{-3} dilution sample, two bands (one

dominant or bright band and one faint band) were observed in the gel. The dominant band was excised with a flame sterilized razor and the DNA was extracted from the acrylamide gel using the EZNA poly gel DNA extraction kit per manufacturer's instructions (Omega Bio Tek). Extracted DNA was reamplified and the phylotype was sequenced using the reverse primer 534R.

Phylogenetic Analysis

Nucleotide sequences were assembled in BioEdit (Hall, 1999). Any ambiguous or incorrectly aligned positions were checked and aligned manually on the basis of conserved primary sequence and secondary structures. Sequences were aligned using the ClustalX function in BioEdit, then further aligned manually to related sequences obtained from GenBank using BLAST (Version 2.0; National Center for Biotechnology Information; ncbi.nlm.nih.gov/BLAST/, Altshul et al., 1990). All assembled sequences were checked for chimeras using the Ribosomal Database Project II Chimera Check program (Cole al., 2003) and the Bellerophon Sever run with using two different correction models (Kimura and Jukes-Cantor) and with no corrections (Huber et al., 2004). Aligned sequences were imported into the Mega 2.1 program (Kumar et al., 2001) or BioEdit for phylogenetic analysis. A neighbor-joining phylogenetic tree was constructed using a Kimura 2-parameter model for estimating evolutionary distance between sequences. A maximum likelihood phylogentic tree was estimated from the nucleotide sequences using fastDNAml (version 3.3, Felsenstein, 1981, Olsen et al., 1994).

Estimations of Richness and Diversity

The richness and diversity of 16S rRNA genes were estimated from the clone library data. Numbers and proportional abundances of unique phylotypes were calculated based on the occurrence of unique RFLP patterns. Two or more representatives for each unique RFLP pattern were selected for sequence analysis. Sequence results were than used to confirm RFLP pattern assignments as unique phylotype. Unique phylotypes or operational taxonomic units (OTUs) were defined as having a final 16S rDNA sequence with greater than 3% difference in 16S rDNA sequence when compared to the other sequences in the clone library. Similarity between related sequences was determined using the software program DNADist (Version 3.5c, Felsenstein, 1993). Relative abundances of these unique phylotypes were determined based on matching RFLP patterns to the sequenced clone(s). Species richness (*S*) and species abundance (*N*) were determined from clone library data. These measurement (*S* and *N*) were used for subsequent diversity calculations including, Margalef's index (D_{mg}), Berger-Parker dominance (1/d), Simpson's index (1/D) the Shannon-diversity (*H'*) and Shannon evenness. A log series alpha value (α) was calculated for the clone library data and used to predict expected log species abundance values for each OTU (Magurran, 1988).

Results

Blood Falls is a dynamic feature that is subject to sample bias depending upon the date, time of day, and specific area of sample collection. The clone library constructed from a single sample collected during the 1999-2000 austral summer, during a season of ancient subglacial outflow, revealed a diverse microbial assemblage associated with

Blood Falls outflow. The clone library was dominated by phylotypes closely related to an obligate chemooautotroph and contained a variety of phylotypes related to organisms known to metabolize sulfur and iron compounds. This diversity was further detailed with isolates enriched over four austral summer seasons (2001-02 through 2004-05) and under variable flow conditions at Blood Falls (Table 4.1). The most abundant heterotrophic bacteria in aquatic environments are usually members of the *Proteobacteria* and *Cytophaga-Flavobacterium-Bacteroides* (CFB) kingdoms (Kirchman, 2002). These two groups dominated both the clone library and cultures from Blood Falls. Neighbor-joining and maximum-likelihood phylogenetic analysis of the 16S rDNA sequences yielded similar tree topologies, the neighbor-joining trees are presented (Figures 4.2 and 4.3). Five isolates were obtained that had greater than 97% sequence similarity (based on 1400 bp) to library clones. No *Archaeal* DNA was amplified from Blood Falls.

Clone Library

Clones (81 total) were screened by RFLP analysis and approximately 40 were selected for complete sequencing. Unique phylotypes were distinguished based on a conservative OTU definition (97% and greater sequence similarity was considered the same phylotype). The Blood Falls clone library contained 16 distinct phylotypes and was dominated by phylotypes of the *Proteobacteria* kingdom (67 clones or 83%) (Figure 4.1 A). Of the total *Proteobacteria*, the γ-, δ- and β- subdivisions were represented by 64%, 15% and 4% respectively. The *Cytophaga-Flavobacterium-Bacteroides* kingdom contained 16% of the total clones. A sequence was distantly related to other uncultured clones in the *Green Non-sulfur* (GNS) division, including: an "uncultured *Chloroflexi*"

from pasture soil (~ 29%; 100 bp) and to OPB 11 and OPB 12 (~ 34%; 1000 bp) from Obsidian Pool in Yellowstone (Hugenholtz et al., 1998).

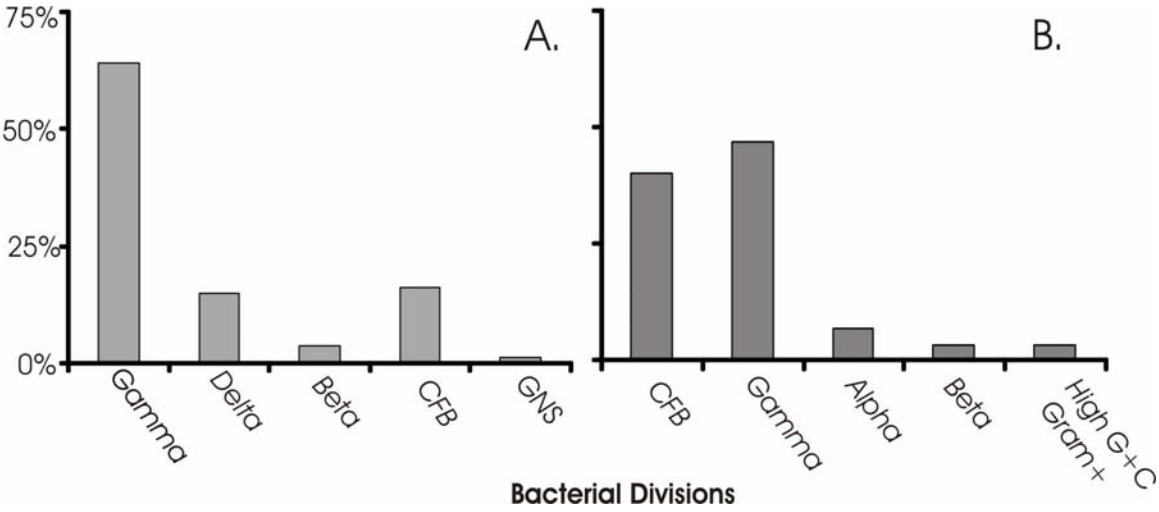

Figure 4.1. Distribution of represented bacterial kingdoms found in Blood Falls. Percent distribution in the clone library (A). Clones that were not sequenced were identified based on RFLP pattern (n=81). Percent distribution of bacterial divisions (B) in cultured isolates (n=30).

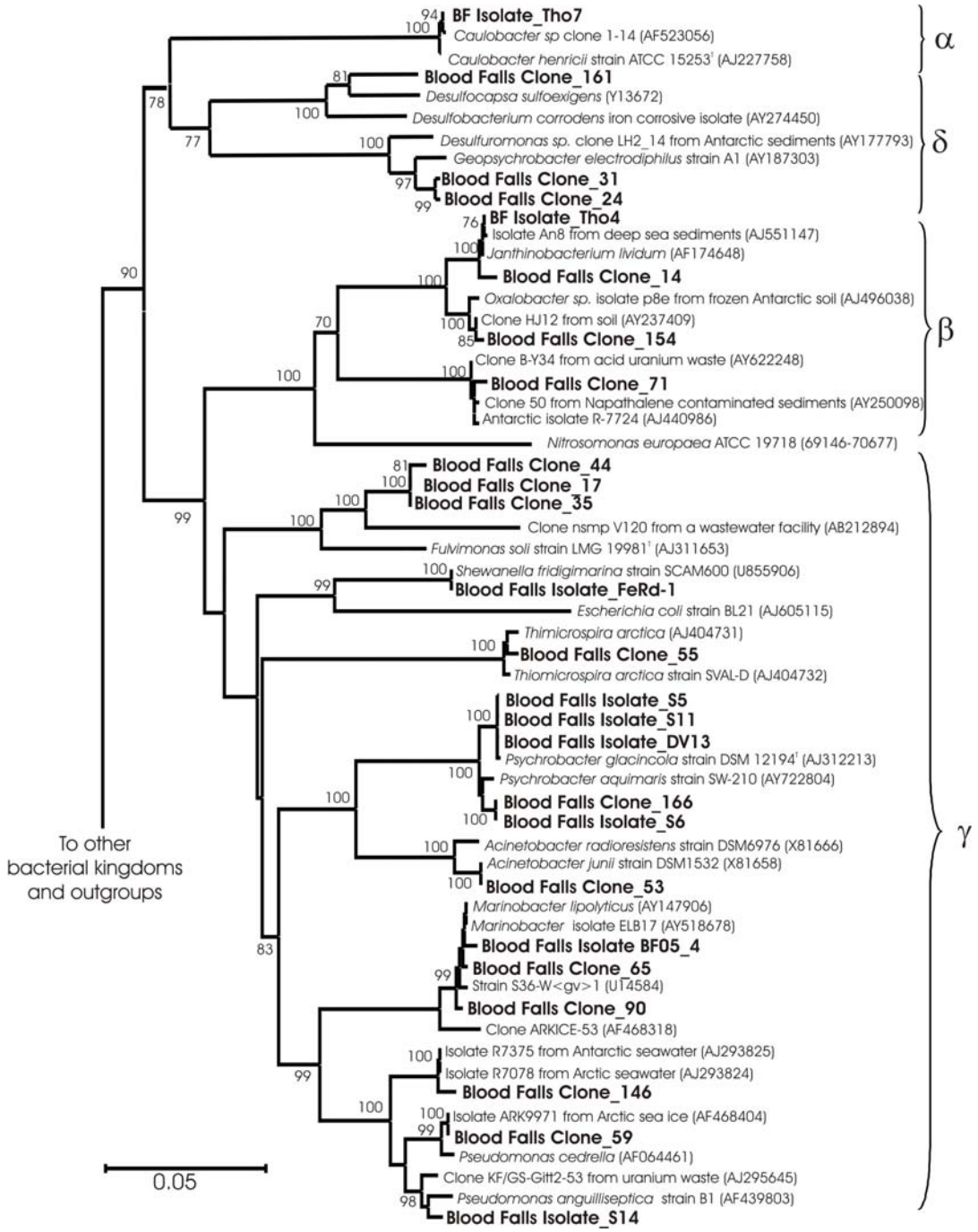

Figure 4.2. Neighbor-joining phylogenetic tree of *Proteobacteria* 16S rDNA sequences constructed with a Kimura 2-parameter model. Selected sequences from the major bacterial lineages were used to the root the tree. Results of bootstrap analysis with 100 replications are noted. The scale bar represents 0.05 nucleotide substitutions per sequence position. Isolates and clones from Blood Falls are in **bold**. GenBank accession numbers are listed in parentheses.

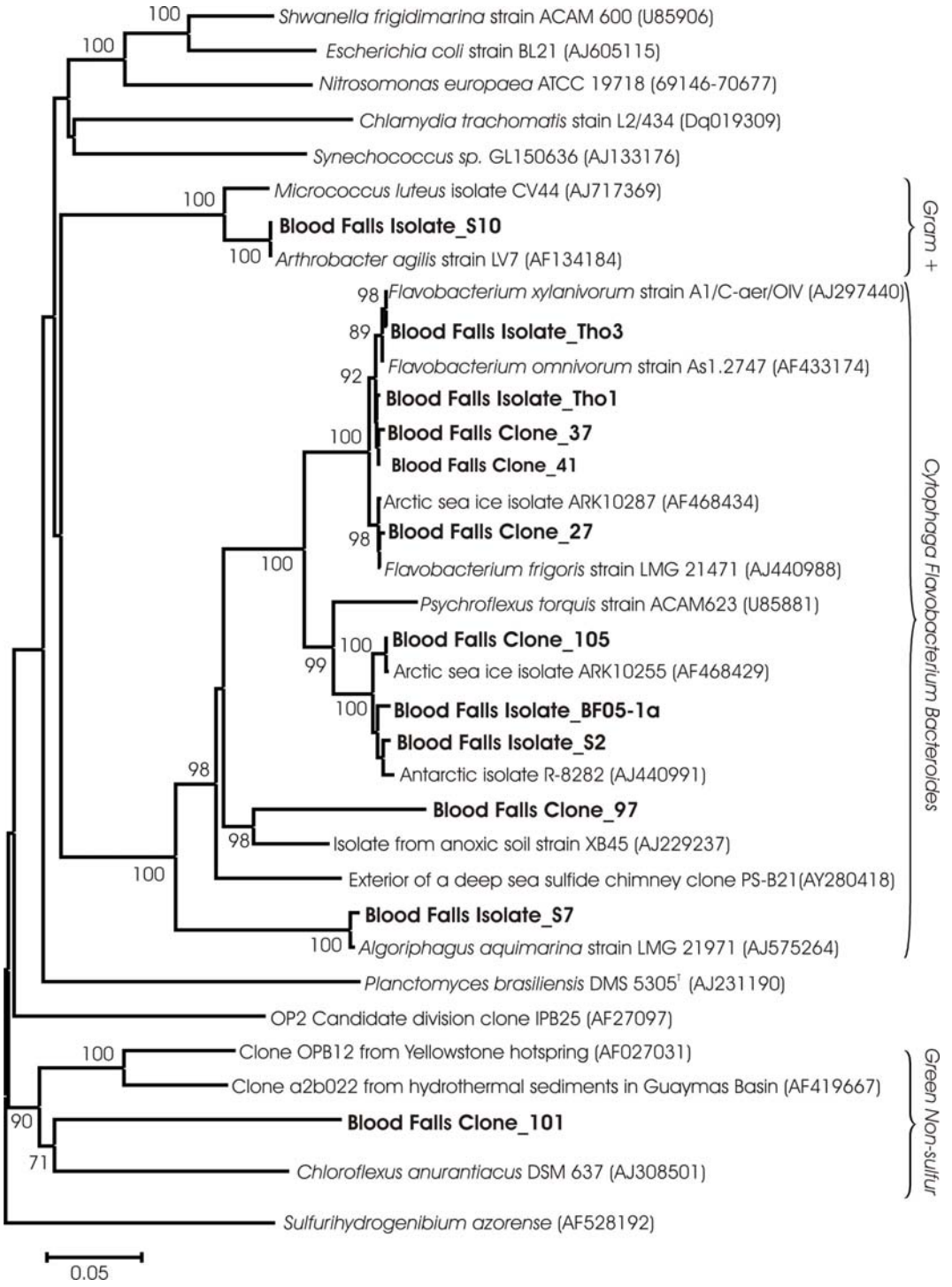

Figure 4.3. Neighbor-joining phylogenetic tree of 16S rDNA sequences from selected isolates and clones constructed with a Kimura 2-parameter model. Results of bootstrap analysis with 100 replications are noted. The scale bar represents 0.05 nucleotide substitutions per sequence position. Isolates and clones from Blood Falls are in **bold**. GenBank accession numbers are listed in parentheses.

Diversity

Microbial diversity data from the Blood Falls clone library was analyzed by constructing a species distribution curve (i.e., a relative abundance curve) (Figure 4.4). Based on the species distribution curve for Blood Falls, there are a few abundant clones (*Thiomicrospira sp.*, *Geopsychrobacter sp.* and *CFB* members), but most sequences were rare generating a long right handed tail. The log abundance plot (Figure 4.4 A) reveals that the data fit a log series distribution. The log series α, was calculated from the clone library data and superimposed on the species distribution curve (Figure 4.4 B) and describes the species abundance found in the Blood Falls clone library (P > 95% based on goodness of fit where χ^2=6.14 with 15 degrees of freedom (Magurran, 1988).

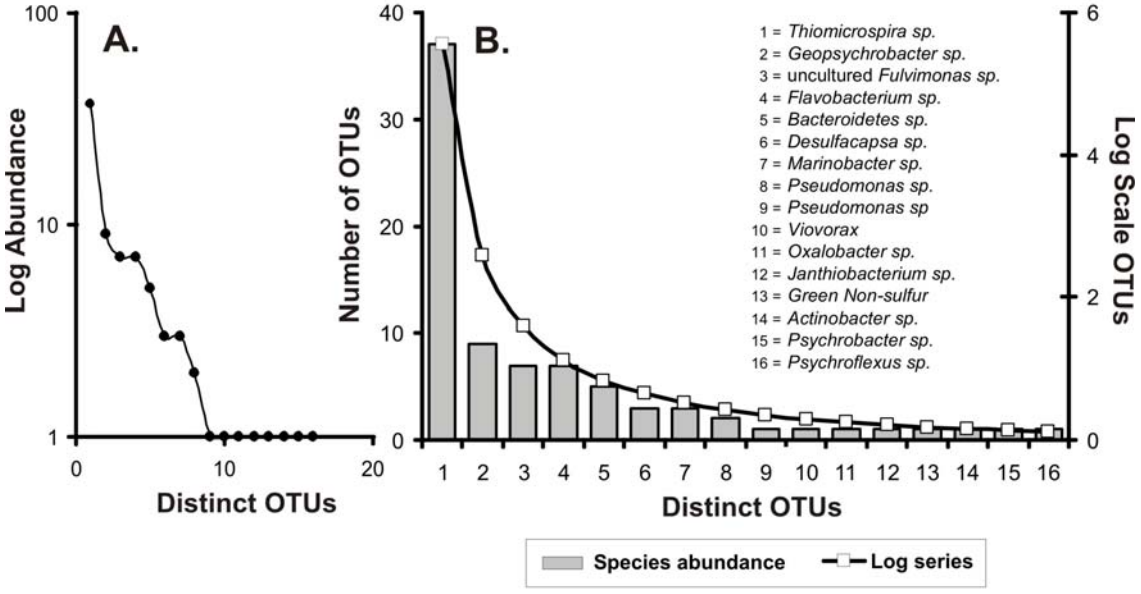

Figure 4.4. Species distribution curves based on bacterial 16S rDNA clone library data from an environmental sample of Blood Falls outflow. OTUs were determined based on RFLP pattern and confirmed with sequence analysis. Log abundance (A), and the log series curve fitted to the species distribution curve (B).

The Margalef index (D_{mg}), which provides a measure of richness, was 3.41 for Blood Falls (Table 4.3). The Berger-Parker (1/d) is a measure of dominance that expresses the proportional importance of the most abundant species (Magurran, 1988) and was 2.19 for Blood Falls. Shannon diversity (H') which takes both evenness and species abundance into account was 1.75 for the Blood Falls clone library. Values for Shannon diversity typically range between 1.5 and 3.5 and rarely surpass 4.5 (Magurran, 1988, Margalef, 1972). Shannon evenness, calculated from H' was 0.63 for Blood Falls. The Simpson's index (1/D) is a dominance measure since it is weighted towards the abundance of the most common species rather than providing an estimate of species richness (Magurran, 1988) and for Blood Falls equals 4.26. The Chao1 uses the number of singletons (OTUs that appear once) and doubletons (OTUs that appear twice) to estimate diversity found in a given sample. Chao1 estimates were calculated using the web interface provided by Kemp and Aller (2004, www.aslo.org/lomethods/free/2004/0114a.html). Observed/predicted values of Chao1 indicated that only 53% of the total diversity in Blood Falls was sampled implying that the measurements presented here may be conservative estimates of the bacterial diversity present in Blood Falls.

Table 4.3. Diversity estimates for Blood Falls based on clone library data constructed from environmental samples of outflow.

Diversity:	
Species richness (*S*)	16
Individuals (*N*)	81
Margalef's index (D_{mg})	3.41
Berger-Parker Dominance (1/d)	2.19
Shannon diversity index (H')	1.75
Shannon evenness	0.63
Simpson's (1/D)	4.26
Log series index (α)	5.97
Predicted S_{Chao1}	30.25
Observed/predicted S_{Chao1}	0.59

Cultured Isolates

The majority of bacterial isolates obtained from Blood Falls environmental samples during the 2001-2005 austral summers, were members of the *γ-Proteobacteria* and *CFB* (Figure 4.1 B and Table 4.4) with *Flavobacterium sp.* (10 isolates) *Psychrobacter sp.* (9 isolates) and *Marinobacter sp.* (3 isolates) being the most commonly isolated organisms. Culturing also revealed several organisms not detected in the clone library including an *Arthrobacter sp.* that was closely related to an isolate from sea ice (Junge et al., 1998) and an *α-Proteobacteria* that clustered with the *Caulobacter sp.* (Abraham et al., 1999).

Table 4.4. Isolates obtained from Blood Falls over the course of four austral summers. The sample source indicates the character of Blood Falls at the time of sample collection, enrichment media are described in Appendix C.

Year	# of isolates	Closest Genus	Bacterial Division	Sample Source	Enrichment Media
November 2001 (ancient flow year)	1	*Flavobacteria sp.*	CFB	Slush ice	R2A
	1	*Actinobacter sp.*	Gamma	Sediment and ice	R2A
	1	*Psychrobacter*	Gamma	Slush ice	R2A
November 2002 (contemporary melt year)	1	*Schewanella*	Gamma	Outflow	Fe-reducing
	3	*Flavobacterium sp.*	CFB	Outflow	Marine
	1	*Bacteriodes sp.*	CFB	Outflow/slush ice	Marine
	8	*Psychrobacter sp.*	Gamma	Outflow/slush ice	Marine
	1	*Actinobacter*	Gamma	Outflow	Marine
	1	*Pseudomonas*	Gamma	Outflow	Marine
	1	*Arthrobacter sp.*	Gram +	Outflow	Marine
November 2003 (contemporary melt year)	2	*Flavobacterium sp.*	CFB	Outflow	Thiosulfate-Ox
	1	*Caulobacter sp.*	Alpha	Outflow	Thiosulfate-Ox
	1	*Janthiobacter sp.*	Beta	Outflow	Thiosulfate-Ox
November 2001 (ancient flow year)	4	*Flavobacteria sp.*	CFB	Outflow	Marine
	3	*Marinobacter sp.*	Gamma	Outflow	Marine

Autotrophic enrichments yielded no strict chemolithotrophs, however the ferrihydrite in the iron-reducing enrichment media was reduced after approximately 5 months of incubation. The iron-reducing dilution series was tested for enrichment of a single phylotype using TGGE analysis. Two distinct bands were observed and the DNA sequence of these bands showed high sequence similarity to *Shewanella frigidimarina* (strain ACAM 591). *S. frigidimarina* is reported to be a chemorganotrophic marine organism (Bowman et al., 1997). 100 ul aliquots of the enrichment culture were transferred to marine agar and incubated at 10º C, colonies formed within 3 days. Phylogenetic analysis of the isolate (named BF_FeRd-1) confirmed a close relation to *S. frigidimarina* (99% sequence similarity of 1400 bp). It is important to note that in this study, BF_Rd-1 was originally enriched in media containing CO_2 as its sole carbon source, with hydrogen and Fe (III) as the only available electron donor and acceptor, respectively. Bowman et al. (1997) did not report autotrophic growth of strain ACAM 591, and it is possible that organic carbon substrates present in the inoculum were not eliminated with the dilution transfers. Alternatively, the *Shewanella* isolate (BF_Rd-1) may be able to fix CO_2.

Discussion

Microbial Diversity Measurements

Microbial Diversity in Blood Falls Outflow. Although caution is required when using diversity measurements on small data sets (Atlas, 1984, Wintzingerode et al., 1997), they can provide useful comparisons of the environmental diversity despite the

inherent biases of clone library construction, particularly when a number of indices are used in concert. The bacterial 16S rDNA Shannon diversity value calculated for Blood Falls (H' = 1.75) is comparable to measurements from other icy and glaciated environments (Table 4.5) but significantly lower than the diversity present in temperate arid soils (H' ~ 6.8, Dunbar et al., 1999). Blood Falls had twice the diversity found in Antarctic pack ice (H'=0.84-0.81) and was ~ 60% greater than Artic pack ice diversity (H'=1.06-1.09), despite the similarity in phylotype composition found in these locations, similar species detected in both Blood Falls and polar pack-ice included: *Shewanella fridigimarina*, *Psychrobacter sp., Marinobacter sp., Pseudomonas sp.,* and *Acinetobacter sp.* (Brinkmeyer et al., 2003). The forefields of two receding glaciers were sampled by Sigler and Zeyer (2002), at the glacier terminus and at a distance of 150 m from the terminus. The Shannon diversity decreased for these two glaciers from 2.31 and 2.83 at the glacier termini to 1.97 and 2.25 at 150 m distance from the terminus. Both forefields showed higher diversity (~ 24 %, SD = ± 11%) than Blood Falls. The glacier forefields sampled in the Sigler and Zeyer study are located in Switzerland at 46 ºN latitude, whereas Blood Falls is located at 77 º S and it is a common paradigm in ecological studies that species diversity declines from the tropics to higher latitudes (Rosenzweig, 1995). Still, little data exist on the latitudinal variation in microbial diversity and this paradigm might not hold true when describing patterns of microbial diversity.

Simpson's dominance values (1/D) reported for the Swiss glaciers (range = 6.0-13.0) suggested the assemblage was dominated by a few phylotypes; a similar species distribution is implied from Blood Falls data (Blood Falls 1/D = 4.3). The arid soils had a more even species distribution (1/D = 0.97, Evenness = 0.97) than the samples from

Blood Falls. The diversity measures presented here show distinct differences in the structure of icy systems versus a temperate desert soil environment. Examining multiple measures of diversity including: type of organism present (composition), number of types (richness) and the relative abundance of types (structure), as demonstrated here, provide a more complete description of microbial diversity in a given system (Magurran, 1988).

Table 4.5. Shannon diversity (H'), Simpson's dominance (1/D) and Shannon eveneness values for Blood Falls and other ecological systems. Two glaciers were studied in the Sigler and Zeyer report (^{A}) = Damma Glacier (^{B}) = Rotfirn Glacier. nr = not reported

Habitat sampled	**H[1]**	**1/D**	**Evenness**	**Latitude**	**Phylotype detection method**	**Reference**
Blood Falls	1.75	4.3	0.63	77 ºS	RFLP and cloning	This study
Antarctic pack ice	0.81-0.83	nr	nr	70 ºS	RFLP and cloning	Brinkmeyer et al., 2003
Arctic pack ice	1.06	nr	nr	75 ºN		
	1.09	nr	nr	80 ºN		
Glacier terminus	2.31^{A}, 1.97^{B}	13^{A}, 7.4^{B}	nr	46 ºN	DGGE	Sigler and Zeyer, 2002
150 m from glacier	2.83^{A}, 2.25^{B}	8.6^{A}, 6.0^{B}	nr			
Arizona arid soils	6.6-7.1	1.0	0.97	36 ºN	RFLP and cloning	Dunbar et al., 1999

The use of 16S rDNA clone analysis for determining microbial diversity includes bias from DNA extraction, PCR, cloning and gene copy number (Farrelly et al., 1995). Given these potential problems, the frequency of the 16S rRNA gene is not necessarily a reflection of the *in situ* microbial community structure (Wintzingerode et al., 1997). Although sequenced microbial genomes of Blood Falls phylotypes currently do not exist, several close relatives have been analyzed and their 16S rDNA operon number(s) are

listed in Table 4.6. (img.jgi.doe.gov and Klappenbach et al., 2001). *Pseudomonas sp.* and *Acinetobacter sp.* for example, have high rRNA gene copy numbers but were not abundant clones retrieved from the Blood Falls library. There appears to be no correlation with the16S rDNA gene copy number and the frequency of phylotypes detected ($r = -0.235$ $p = 0.419$) implying that copy number is not a factor in the frequency of clones from Blood Falls. Despite the biases, diversity descriptions based on 16S rDNA genes provide a useful means of comparison with other systems and this approach has been used more recently in microbial diversity studies (Moyer et al., 1994, Dunbar et al., 1999, Inagaki et al., 2003.)

Table 4.6. Number of *r*RNA operons in phylogenetic groups related to the clones obtained from the Blood Falls outflow clone library. Taxon ID listed for data obtained from DOE-JGI. rrndb = rRNA Operon Copy Number Database (rrndb.cme.msu).

Organism	Taxon ID	# 16S rRNA operons	Data Source
Geobacter metallireducens	269799	1	DOE-JGI
Geobacter sulfurreducens PCA	243231	2	DOE-JGI
Psychrobacter cryopegella 497		1	rrnadb
Psychrobacter arcticum 273-4	259536	4	DOE-JGI
Marinobacter articus T2		2	rrnadb
Acinetobacter sp. ADP1	62977	7	DOE-JGI
Thiomicrospira denitrificans	39766	4	DOE-JGI
Thimicrospira crunogena	39765	3	DOE-JGI
Desulfuromonas acetoxidans 891		2	rrnadb
Pseudomonas sp.	208964	6	DOE-JGI
Pseudomonas sp.	205922	6	DOE-JGI
Pseudomonas sp.	223283	5	DOE-JGI
Pseudomonas sp.	205918	5	DOE-JGI
Pseudomonas sp.	160488	7	DOE-JGI

Microbial Diversity in Subglacial Environments. It follows that genetic diversity might be quite distinct between other subglacial systems and the Taylor subglacial

system, given the marine origin of Blood Falls. The *in situ* geochemistry and preglaciation history of each glacier bed must play a role in the extant microbial diversity present. Blood Falls has an ancient marine origin and is therefore geochemically similar to marine environments (Lyons et al., 2005), and the majority of the phylotypes described from Blood Falls samples were related to marine organisms (Ravenschlag, et al. 1999, Bowman et al., 2000, Bowman and McCaig, 2003) supporting the contention that the preglaciation environment determines the subglacial microbial community.

Available energy sources must also influence the metabolic phylotypes present in the subglacial community. Similar energy sources found in Blood Falls (i.e. iron and sulfur minerals from bedrock), would be available in other subglacial environments and it follows that similar metabolic phylotypes would also be detected. This notion is supported by reports of sulfur oxidation and iron- and sulfur-reduction activity below other cold-based or polythermal glaciers such as the well studied Haut Glacier and Finsterwalderbreen in Svalbard (Sharp et al., 1999, Wadham et al., 2004, Tranter et al., 2005).

Phylogenetic diversity reported for subglacial systems from temperate latitudes differs considerably from that of Blood Falls. This difference is likely due to the physical hydrology of the glacier, as well as the pre-glacial ecosystem. Foght et al. (2004), using cultivation, examined the diversity of basal sediments from the Franz Josef and Fox Glaciers in New Zealand and showed that the majority of isolates detected were members of the *β-Proteobacteria.* The two New Zealand glaciers studied are wet-based glaciers with melt water communication between glacier ice and the basal environment. The Taylor Glacier is polythermal and supraglacial water would not be expected to reach the

glacier bed (Fountain, 1999). A molecular survey using sequencing of DGGE fragments from samples collected from a subglacial volcanic crater lake in Iceland also revealed a system dominated by members of the *β-Proteobacteria* (Gaidos et al., 2004). Bacterial diversity associated with sediment-laden basal ice from the Greenland Ice Sheet Project (GISP2) was distinct from Blood Falls diversity as well, where *α-Proteobacteria* and Gram-positive bacteria were the most abundant phylotypes cultivated (Sheriden et al, 2003). Glacier hydrology, in addition to the preglaciation ecosystem and available microbial energy sources, is a third factor that affects subglacial microbial diversity.

Collectively, the data described in the preceding sections indicate that the environmental factors creating the subglacial environment (preglacial ecosystem, glacier bed substratum, and glacier hydrology) all influence the structure of subglacial microbial diversity.

The Ecological Habitat Below the Taylor Glacier

The species abundance data for Blood Falls fits a log series distribution (Figure 4.3) and describes a habitat where the biological assemblage is composed of a small number of abundant species and a larger proportion of rare species that are present in relatively low number. The log series species distribution occurs where one or only a few factors control the ecology of the community (for example, the availability of water or sunlight). The Taylor Glacier subglacial microbial diversity is controlled by low temperatures and accessible mineral energy sources and oxygen. The ability to gain energy efficiently at low temperatures from iron and sulfur minerals or available organic carbon would be a physiological advantage below the Taylor Glacier and in other

subglacial environments. All clones in the Blood Falls library were related to uncultured clones or isolates obtained from permanently cold environments such as cold marine sediments, Antarctic and Arctic coastal sediments, sea ice or polar lakes as determined by BLAST search analyses. In support of the Blood Falls diversity data fitting a log series distribution, phylotypes related to organisms from cold marine environments that metabolize iron and sulfur compounds were the most abundant species in the Blood Falls log series. The proposed microbial metabolic niches below the Taylor Glacier are discussed in detail below and highlighted in Table 4.7.

Sulfur Oxidation and Autotrophy. The Blood Falls clone library was dominated by sequences closely related (99%) to an obligate chemolithoautotrophic sulfur-oxidizing bacterium, *Thiomicrospira arctica*, that was originally isolated from Arctic marine sediments (Knittel et al., 2004). *T. arctica* is a true psychrophile and grows optimally with 250 mM Na^+ concentrations; but growth was demonstrated at concentrations as high as 1240 mM. These values are consistent with Na^+ concentrations in Blood Falls (~ 1175 mM during primitive flow). The genus *Thiomicrospira* is an important group in hydrothermal vent communities and is widely distributed in marine environments suggesting this genus may play an important role in global carbon and sulfur cycles (Brinkhoff and Muyzer, 1997). *CFB*-type isolates obtained on thiosulfate-oxidizer agar were related (95.1% sequence similarity) to deep sea isolates that are capable of oxidizing thiosulfate (Teske et al., 2000). This suggests that other phylotypes in Blood Falls may be involved in sulfur oxidation.

There is growing evidence that biological sulfur oxidation is an important subglacial process. Significant biological sulfate production has been measured in subglacial sediment and water samples by incubation experiments (Sharp et al., 1999) and stable isotopic measurements (Bottrell and Tranter, 2002). Bottrell and Tranter found that biological sulfur oxidation occurred under partially anoxic conditions with Fe (III) derived from Fe-OH minerals as the oxidant. My phylogenetic data support these previous studies and supports the notion that Fe and S have an important role in the metabolism of the Taylor Glacier subglacial system.

The majority of clones in the Blood Falls library (46%) were related to an obligate chemoautotroph, implying the capacity for subglacial chemosynthetic primary production. This notion is further supported by the measurable CO_2-fixation that occurred in the dark in Blood Falls ancient outflow samples (Chapter 3). The rates calculated for Blood Falls (1.2 nmol C L^{-1} d^{-1}) were statistically similar ($p= 0.114$) to rates measured in samples from a subglacial volcanic lake (3.3 nmol C L^{-1} d^{-1}) (Gaidos et al., 2004). Together these results imply *in situ* primary production by chemoautotrophs can provide new organic carbon to the subglacial environment below the Taylor Glacier, in the total absence of sunlight, and at permanently low temperatures.

Table 4.7. Description of nearest cultivated relatives to selected Blood Falls clones and isolates obtained from ancient subglacial outflow samples during December 1999 and December 2004.

Putative Ecological Role	Blood Falls Clone or Isolate	Closest cultivated relative	% similarity	% of library	Comments	Reference
Primary producer	C55	*Thiomicrospira arctica*	98.8	45.7	Chemoautotrophic sulfur oxidizer, psychrophilic arctic marine sediments	Knittle, 2004
Sulfur and Iron reducers	C24, C31	*Geopsychrobacter electrodiphilus*	98.0	11.1	Anaerobic, psychrotolerant, from marine sediments: chemoorganotroph using Fe(III), S^o, Mn(IV) or AQDS as e^- acceptors	Holmes et al., 2004
	C161	*Desulfocapsa sulfoexigens*	92.7	3.7	Anaerobic, psychrotolerant, from marine sediments: disproportionates elemental sulfur, sulfite and thiosulfate using Fe(III) as a scavenging agent	Finster et al.,1998
Heterotrophs	C65, C90, *Isolate* BF05_4a	*Marinobacter lipolyticus*	96.1	3.7	Moderate halophile with lipolytic activity	Martin et al., 2003
	C154	Antarctic soil isolate	98.3	1.2	Protease producing isolate	Wery et al., 2003
	C105	*Psychroflexus tropicus*	95.5	1.2	Hypersaline lake isolate	Donachie et al., 2004
	Isolate BF05-1_2	Abyssal strain AII3 *Cytophaga-Flavobacterium*	95.1	na	Thiosulfate oxidizing strain isolated from marine sediments from 4,500 meters depth	Teske et al., 2000

Sulfur and Iron Reduction. Clones belonging to the *δ-Proteobacterium* lineage comprised 15% of the library, of which 11% clustered with members of the sulfur- and iron-reducing *Geobacteraceae* group. The closest sequence match (98% identity) for these clones was *Geopsychrobacter electrodiphilus*, a psychrotolerant bacterium capable of growth using Fe(III), Mn(IV) and elemental sulfur as electron acceptors (Holmes et al., 2004). The remaining 4% of the *δ-Proteobacterium* clones were more distantly related to their nearest Blast relatives. These clones had 93.2% similarity to an isolate (strain STP 23) from anaerobic lake sediments that can disproportionate sulfite, ferment lactose and can use sulfite and thiosulfate as electron donors and acceptors (Sass et al., 1998) and 92.2% similarity to *Desulfocapsa sulfoexigens*, an anaerobe isolated from marine surface sediments that disproportionates elemental sulfur and thiosulfate (Finster et al., 1998). My findings are also consistent with geochemical studies from other subglacial environments. Sulfate reduction was measured in the subglacial environment of two Arctic glaciers by sulfate loss in incubations of basal ice from the John Evans Glacier in Canada (Skidmore et al., 2000) and using stable isotope analyses of subglacial outflow from Finsterwalderbreen in Svalbard (Wadham et al., 2004). Sulfate reduction could operate under the Taylor Glacier as a complement to the sulfur oxidation described above since it could provide substrate for sulfur reducing lifestyles through the oxidation of iron-sulfur minerals.

Organic Matter Degradation. The remaining clones and isolates were related to organisms that require organic carbon for growth, including the chemoorganotrophs described above. Isolates in this study were not tested for their carbon utilization

abilities, but the existing literature on the nearest cultivated organisms to the clones and isolates from Blood Falls implies the ability to degrade an array of carbon compounds. The *Cytophaga-Flavobacterium-Bacteroides* group (*CFBs*) was well represented (16%) in my clone library and its members were readily cultivated from Blood Falls samples (40% of isolates). These results are consistent with findings for other aquatic marine environments where *CFBs* are described as organotrophs, proficient in the degradation of high molecular weight organic matter such as cellulose and chitin (Kirchman, 2002). It should be noted that most cultivated *Cytophaga* and *Flavobacteria* are aerobic, and all known members of the *Bacteroides* are anaerobes (Kirchman, 2002). A *β-Proteobacterium* (clone 154) was closely related (98.3%) to an Antarctic soil isolate that demonstrated a variety of extracellular enzymatic activities, including chitinase activity (Wery et al., 2003). The majority of *CFB* isolates (10) and clones (7) from Blood Falls clustered with the *Flavobacteria*, the remaining *CFB* clones (5) and isolates (1) clustered with the *Bacteroides*. A *Marinobacter sp.*, which was both cloned and cultivated from Blood Falls outflow, had 96.1% sequence similarity to a cultivated *Marinobacter,* (*M. lipolyticus*), an organism with lipolytic activity. The above discussion suggests that heterotrophs below the Taylor Glacier might be capable of degrading a broad range of organic substrates including recalcitrant and high molecular weight compounds.

Conclusions

What type of characteristics would be expected for microbes inhabiting the subglacial environment below the Taylor Glacier? Based on geochemical and physical observations one might expect organisms that have adapted to the permanent darkness

and cold and that are tolerant of elevated salinities. Effective metabolic regimes would include those that could harvest energy from the bedrock or from legacy marine organics. Given the length of time that this marine system has been isolated from phototrophic production (~2 Mya) the ability to degrade and consume increasingly recalcitrant organic carbon would also be a beneficial trait.

The function of microorganisms cannot be accurately assessed by 16S rDNA sequences alone and caution must be used when interpreting ecosystem function from sequence similarity data. However, these results are consistent with the scenario proposed by Tranter and colleagues (2002, 2005), who predicted that subglacial microbially mediated chemical weathering reactions would consume oxygen via sulfide oxidation and organic matter combustion driving the system towards anoxia, and increasing CO_2 concentrations. Once conditions were driven to anoxia, sulfides and organic matter would be oxidized using Fe (III) or sulfate as the electron acceptor. If brine was distributed throughout the basal layer of the Taylor Glacier, and in contact with the glacial basal ice, as described in the conceptual subglacial model (#2, Chapter 3), this brine layer would be thin enough to become anoxic and support the anaerobes described here while maintaining oxygen for the aerobes where the brine is in contact with the overlying glacier ice. The phylotypes I describe from Blood Falls support the metabolic scenario described by Tranter et al. (2002, 2004) and add to it the element of chemoautotrophy, indicating that subglacial systems can be sustained independent of new carbon fluxes by *in situ* CO_2 fixation.

CHAPTER 5

THE ECOLOGICAL IMPACT OF SUBGLACIAL OUTFLOW ON A PERMANENTLY ICE-COVERED, PROGLACIAL LAKE

Abstract

Subglacial outflow from the Taylor Glacier, Antarctica, discharges saline water that advects below the west lobe of Lake Bonney (WLB) chemocline (~ 22m). As the subglacial outflow discharges from the glacier, it mixes with moat water. The immediate flux of saline (conductivity ~ 105 mS cm^{-1}), and iron rich (~ 3.5 mM), subglacial brine into the WLB moat is a disturbance event that reduces microbial activity. As the salts from Blood Falls outflow were diluted with fresh glacial melt, microbial growth in the moat area was enhanced, for example agar plate recovery was 0% in samples of moat water collected during a subglacial discharge event versus ~20%-60% recovery in samples of moat water collected when outflow was diluted. These results, together with other biogeochemical parameters, imply that saline subglacial brine is initially toxic, but once diluted becomes a source of nutrients for microbial growth allowing organisms to recolonize the moat area and take advantage of the reduced nutrients available from the outflow. The pulse of nutrients makes the moat zone around Blood Falls a "hot spot" for microbial activity in what would otherwise be a region of low productivity. The geochemistry of the Taylor Glacier subglacial outflow is similar to the water below the WLB chemocline (>~18m). Genotypically, however, the subglacial outflow is quite

distinct and, despite the geochemical similarities in the two waters, microbial diversity does not appear to be transported from the subglacial outflow to the lake.

Introduction

Subglacial outflow from the Taylor Glacier enters the west lobe of Lake Bonney (WLB) via Blood Falls, a saline seep located at the snout of the glacier. The release of subglacial brine at the surface of Taylor Glacier is episodic. True subglacial brine is characterized by high chloride and sulfate concentrations (> 1400 mM and 50 mM respectively) and is referred to as ancient outflow. Many salts from the ancient subglacial brine precipitate upon contact with the atmosphere at the surface of the Taylor Glacier. These salts are diluted by contemporary glacier melt in the absence of continuous ancient subglacial outflow, before the brine enters WLB.

Santa Fe Creek, a stream fed primarily by surface (supraglacial) melt from the Taylor Glacier, enters the moat of WLB ~50 m north of Blood Falls where it converges with Blood Falls, creating a zone of dilution. I term the confluence of these features the "Blood Falls moat system" in this chapter to distinguish it from the rest of the WLB moat.

The Blood Falls moat system is a front ("meeting of waters"), or an ecotone, between subglacial waters, fresh glacial melt and lake water. The chemistry of these waters may act to maintain this front as a sharp transition between moat and lake due to their densities. Santa Fe stream and surface waters of WLB have been considered as "fresh" (Cl^- range = 1.4 - 10 mM, Spigel and Priscu, 1996, Lyons et al., 2005), compared to subglacial outflow at Blood Falls (1440 mM) (Keys, 1979, Lyons et al., 2005, Chapter

3). The Blood Falls moat system is conceptually similar to an estuary, where freshwater rivers meet the sea, only in reverse. Estuarine waters are known to host a variety of biochemical reactions including, the dissolution of particulate substances, flocculation, chemical precipitation, biological assimilation, and absorption of chemicals on and into particles of clay, organic matter and silt (Day et al., 1989).

An ecological disturbance can be described as "a punctuated, episodic event that results in element redistribution within and between ecosystems, involving element transport between the biosphere, hydrosphere, lithosphere, and atmosphere" (Hungate et al., 2003). I contend that the episodic hypersaline outflow originating below the Taylor Glacier as it enters the fresh surface waters of WLB fits this definition of disturbance. An ecosystem can experience both positive and negative feedback responses from a disturbance event (Hungate et al., 2003). Biological and geochemical data presented in this chapter indicate that the Blood Falls moat system exhibits both positive and negative responses to the influx of ancient subglacial outflow.

Methods

Site Description

Lake Bonney occupies two steep-sided basins (the east and west lobe) in the Taylor Valley (Spigel and Priscu, 1996). The Taylor Glacier terminates at the west lobe of Lake Bonney and is in direct contact with the lake water to a depth of ~20 m. Typically, a moat forms between the glacier and the permanent ice cover of Lake Bonney between November - February (Figure 5.1). This moat forms over the submerged terminus of the Taylor Glacier and can range in depth from 3-5 m. The bottom of this

moat is glacier ice, not lacustrine sediments (personal observation). Subglacial release at Blood Falls flows over terminal moraine sediments and into the moat area. As the austral summer progress, Santa Fe Creek, a glacial fed stream of Taylor Glacier, converges with Blood Falls flow. The moat water is part of the shallow (~0.3-1 m deep) floodplain of the Santa Fe Creek at this point of convergences, and is no longer overlying the submerged glacier terminus. Shortly (~ 50 m), beyond this convergence, the moat transitions into the west lobe of Lake Bonney (WLB).

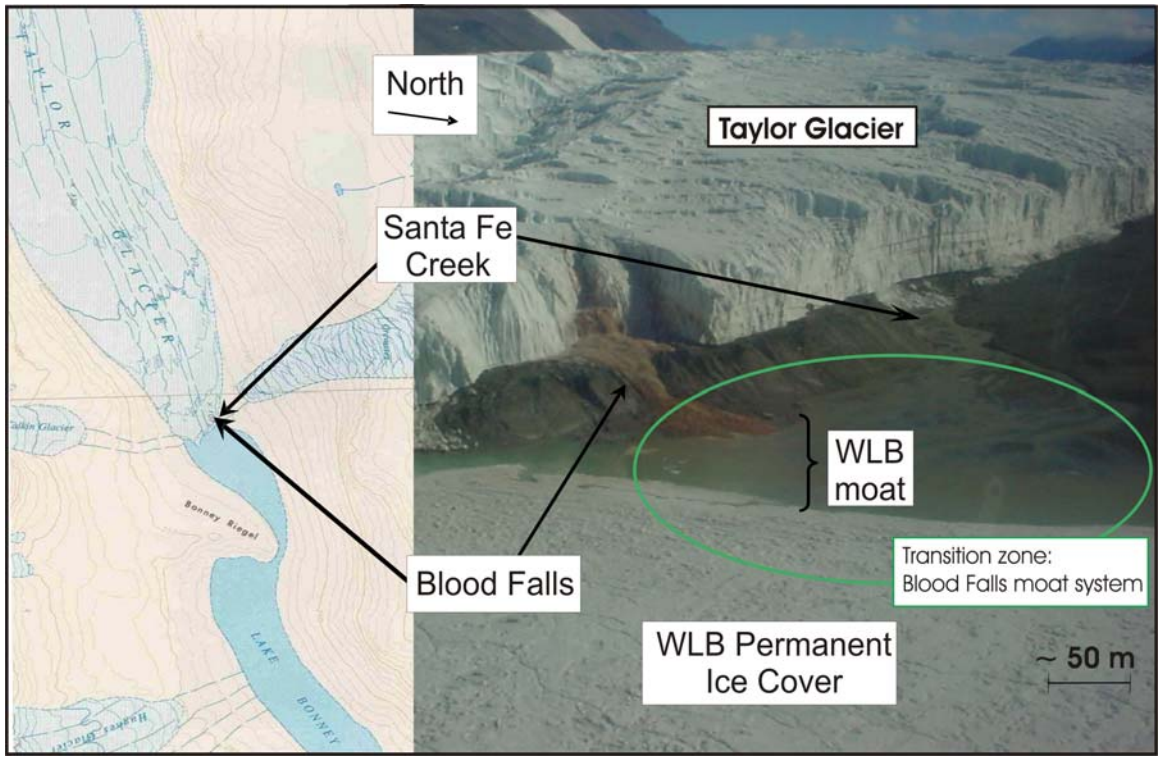

Figure 5.1. Location of Blood Falls relative to the west lobe of Lake Bonney (WLB). The Santa Fe Creek and Blood Falls flow converge in the WLB moat.

Glacier to Lake Transect

Samples were collected along a transect from Blood Falls into WLB. Water was collected for biogeochemical analyses along the transect during a season of high ancient subglacial flow (i.e. subglacial outflow as described in Chapter 4) and when subglacial outflow was low or nonexistent and Blood Falls flow was diluted with contemporary glacial melt (fresh water) during the austral summers (October – January) of 1999, 2003 and 2004. Four 10-inch holes were drilled through the ice cover at (~5, 80, 150 and 425 m from Blood Falls); the farthest site represented the LTER limnological sampling site (Spigel and Priscu, 1998), representing the deepest portion of the lake (~ 37 m). Holes were drilled on 15 November 2003 and water was collected on 16 November 2003 using a 2-L Niskin sampling bottle. The samples were designated BFT-X (where X is numbered 1-17, Figure 5.2). Samples were also collected on 20 December 2003 from and around the source of Blood Falls flow and designated BFS-X (Where X is numbered 1-10, Figure 5.3). Blood Falls moat system samples were collected during the 2004 season from holes drilled on 14 November 2004.

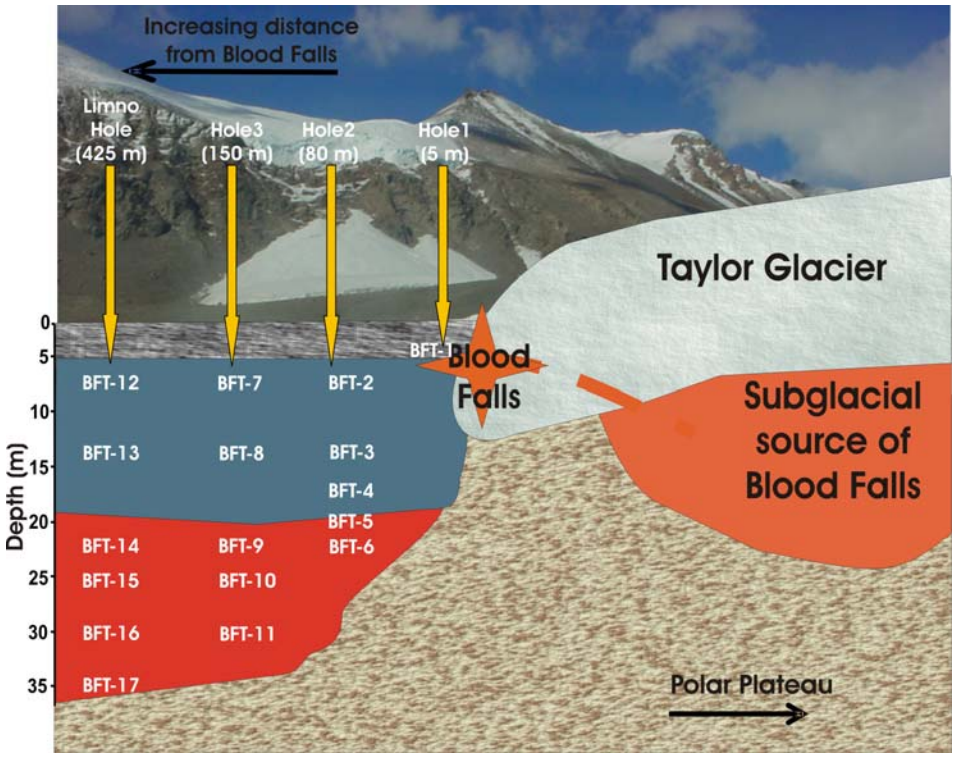

Figure 5.2. Sample origin for points along a transect from Blood Falls outflow to the limnological sampling hole. *Features not to scale.*

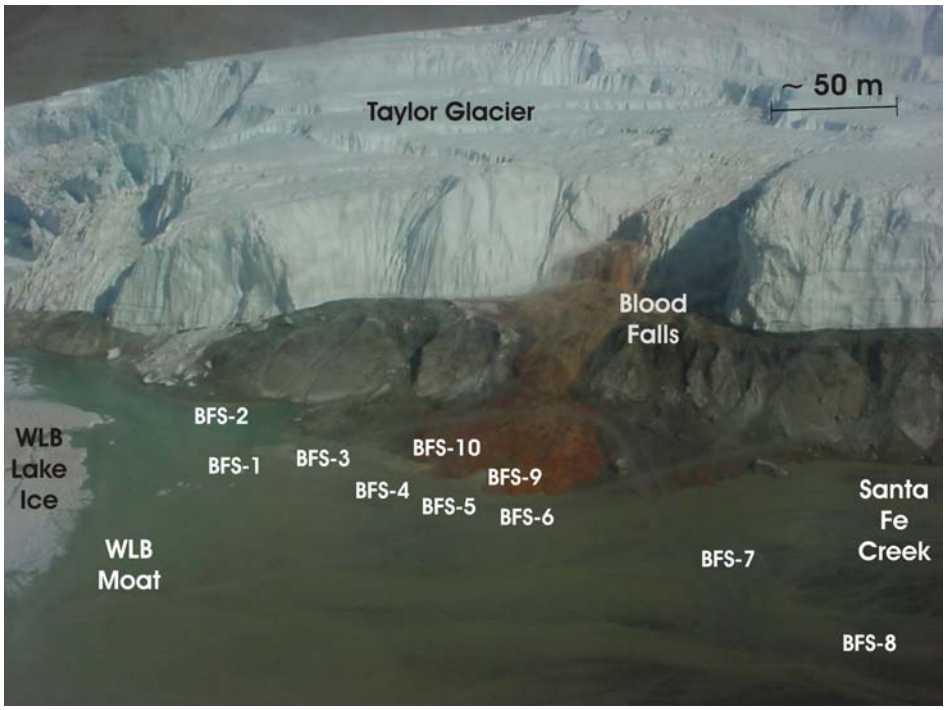

Figure 5.3. Sample location for points around the source of Blood Falls flow.

Geochemistry

Geochemical methods are available in the MCM LTER Limnological Methods Manual (Priscu and Wolf, 2000, available online at www.homepage.montana.edu/~lkbonney/ and were described previously (Chapter 3). In summary, pH was measured with a calibrated Beckman Φ12 pH meter. Dissolved oxygen (DO) was determined by Winkler titration (Standard Methods, 1992). Dissolved inorganic carbon (DIC) was measured by infrared gas analysis on acid sparged samples. Dissolved organic carbon (DOC) was measure on filtered (Whatman GF/F) and acidified samples with a Shimadzu 5000 TOC analyzer. Nitrate (NO_3^-) and nitrite (NO_2^-) concentrations in water samples were measured with a Lachat autoanalyzer according to Standard Methods (1992). Nitrite levels were determined by diazotization, with sulfanilamide, and then coupled with a diamine to produce a pink dye, analyzed spectrophometrically on a Lachat autoanalyzer. Nitrate was reduced to nitrite by passing the sample through a copperized cadmium column and analyzing for nitrite, as above, giving a nitrate plus nitrite concentration. The nitrate concentration was determined by subtracting the nitrite concentration from the total (nitrate + nitrite) concentration. Ammonium (NH_4^+) concentrations were measured separately by reaction with alkaline phenol followed by sodium hypochlorite, forming indophenol blue. Sodium nitroprusside was added to enhance sensitivity and the sample was analyzed spectrophometrically on a Lachat autoanalyzer. Dissolved inorganic nitrogen (DIN) was reported as the summation of nitrate, nitrite and ammonium ($DIN = NO_3^- + NO_2^- + NH_4^+$). Major ions were measured according to the protocol of Welch et al. (1996). Chlorophyll-*a* (CHL) was extracted into 90% acetone for 24 h at <0 °C in the dark from particles

collected on a Whatman GF/F filter, and the subsequent concentration was determined fluorometrically. Total iron was determined as described by Fulton et al. (2004), using the ferrozine assay. Geochemical data (including: DOC, DO, pH, total Fe and ions: Na^{+}, Cl^{-}, SO_4^{2-}, Ca^{2+}) were characterized using hierarchical cluster analysis with the Pearson's measure of correlation and a furthest neighbor linkage using the SPSS software package (Version 13.0, LEAD Technologies, Inc, 2004); data used for cluster analyses were transformed to range in value between 0 and 1.

Microbial Cell Counts

Outflow samples (10 ml) were collected into 20 ml scintillation vials and preserved with filtered sterilized (0.2 μm) 37% formaldehyde (formalin) buffered with sodium borate. Aliquots of outflow (3 ml) were stained with SYBR gold nucleic acid stain 25X (Molecular Probes, Inc.) for 15 minutes as described by Lisle and Priscu (2004), then concentrated onto a 25mm 0.2 μm black polycarbonate filter (Poretics) supported by a 0.45 μm membrane filter. Filters were mounted on glass microscope slides with several drops of anti-fade solution (0.1% p-phenylenediamine in a 1:1 solution of phosphate-buffered saline and glycerol). Cells were visualized and counted using a Nikon Optiphot epifluorescent microscope, equipped with a DM510 filter cube (Nikon) at a total magnification of 1000X.

Agar Plate Counts

Samples were collected for culture work during the austral summer between 2001 and 2004. Outflow was collected into serum vials (74 ml), by submerging the vials directly into the flow, and crimp sealed with butyl rubber stoppers leaving no head space.

Serum vials were kept dark and below 4° C until return to McMurdo for incubation in selective media (approx 5-10 days). Media included R2A agar (Difco) and Marine agar (Bacto). Sample (100 µl) was removed from the sealed serum vials with a 1 ml syringe and inoculated onto agar plates (15 g L^{-1}) or serially diluted into sterile phosphate buffered saline to a 10^{-4} dilution and then plated. Samples on plates were spread with a sterile cotton swab and incubated at 2-4° C until colonies appeared (about 3-4 weeks).

DNA Extraction

Samples for DNA extraction were collected directly from Blood Falls outflow during the 1999-2000 and 2003-04 austral summers. Samples collected during the 1999-2000 season were kept chilled (below 4° C), and filtered (1 L) within 4 hours, onto a 90 mm membrane filter, placed in a sterile plastic bag, heat sealed and stored at -20° C until extraction. Cells from 1 L of outflow and across the 17 transect points, collected during the 2003 season, were concentrated on 45 mm membrane filter, placed in a sterile cryovial, flash frozen in liquid nitrogen and stored at -80° C until extraction. Filters were cut into pieces using flame sterilized scissors and tweezers and then processed according to the manufactures protocol for the Mo Bio Laboratories, Inc. UltraClean Soil DNA Isolation Kit (Catalog # 12800-50). The alternative protocol for maximum yields was followed.

PCR Amplification of 16S rDNA

The microbial 16S rDNA genes were amplified from extracted DNA using a nested PCR approach. The oligonucleotide primers 27F and 1391R were first used to amplify larger fragments of 16S rDNA. The 50-µl PCR reaction contained 2 µl DNA template, dNTPs nucleotide mix (Fisher Scientific, 200µM final concentration), 0.5 µM final concentration of each primer MasterTaq Kit (Eppendorf) was used including; 10x Taq Buffer (1.5 mM Mg^{2+}), 5x TaqMaster (a proprietary PCR enhancer), and 1.25 units of *Taq* DNA polymerase (Eppendorf MasterTaq Kit). The reaction mixture was PCR amplified in an Eppendorf Mastercycler Gradient Thermal Cycler under conditions that included 15 sec of denaturation at 95° C, 15 sec of annealing at 60° C, and 15 sec of primer extension at 72° C. These steps were repeated for 20 cycles. 2 µl of PCR product was used as template for the subsequent nested reaction. Nested fragments (~ 221 bp) of the 16S rRNA genes were amplified from the PCR amplified DNA using a set of bacterial primers (341F and 534R) in which the forward primer contained a GC tail clamp (see table 5.1). The reaction mixture was PCR amplified under the same conditions as described above except that the steps were repeated for 25 cycles.

Table 5.1. Oligonucleotide primers used for PCR amplification of 16S rDNA from Blood Falls genomic DNA. R=A or G

Primer uses and name	***Direction***	***5′→ 3′ Sequence***
PCR		
27F	Forward	AGAGTTTGATCCTGGCTCAG
1391R	Reverse	GACGGGCGGTGTGTRCA
PCR and DGGE		
341GC	Forward	CGCCCGCCGCGCGCGGCGGGCG GGGCGGGGGCACGGGGGGCCTA CGGGAGGCAGCAG
534R	Reverse	ATTACCGCGGCTGCTGG

Temperature Gradient Gel Electrophoresis (TGGE)

TGGE techniques were applied in this study to examine the presence of bacterial phylotypes in PCR products across the transect from Blood Falls into the water column of Lake Bonney. PCR amplified products (15 μls) were separated on a temperature gradient gel (8% gel, run at 60 volts with a temperature gradient of 58-72 °C with a 0.8 °C ramp rate) using a DCode Universal Mutation Detection System (Biorad); protocol adapted from Muyzer et al., 1993).

Analyses of TGGE Data

TGGE gels were stained with SYBR gold and imaged with a Polaroid Photo Documentation camera (Fisherbrand); images were analyzed using Image J software (version 1.33u) which converts DNA bands into peaks. Presence and absence of peaks, as compared to a standard, were manually determined for each lane of the gradient gel. The total number of bands for each sample was counted for richness (R) values. The similarity of banding patterns (presence/absence of bands) was quantified between each

sample using the Jaccard index where the Jaccard index value is equal to 1 if the two populations are identical or zero if they are completely dissimilar (Magurran, 1988). Jaccard's similarity index values were used to construct a hierarchical cluster dendogram using furthest neighbor linkages with the SPSS statistical software package (Version 13.0, LEAD Technologies, Inc, 2004).

Substrate Incorporation Experiments

Outflow from Blood Falls was placed in 20 ml scintillation vials (10 ml outflow) for aerobic incubations and 20 ml serum vials (20 ml outflow) with no headspace, with sterile pipettes, crimp-sealed with butyl rubber stoppers for anaerobic incubations. Samples were prepared in replicate (4 live, 3 killed); formalin (0.2 μm filtered sodium borate buffered formalin) was added to killed treatments (5% final concentration). Samples in serum vials were inoculated with 20 nM ^{3}H-thymidine stock. Serum vials were incubated at 2-4º C, in the dark, for 14 hours, 20 hours, 3 days, 7 days, and 14 days. Treatments were transferred to sterile 60 ml Nalgene containers at the end of the incubation and 20 ml ice cold 10% TCA solution was added to terminate the reaction. Thymidine incorporation rates were calculated from the activity in live minus killed treatments (Takacs and Priscu, 1998).

2003 melt samples were incubated with the addition of Fe (II). $FeCl_2$ solution (5 mM) was prepared in a serum vial and made anaerobic by flushing the head space with (0.2 μm filtered) N_2 gas. $FeCl_2$ was transferred to outflow samples (1L) using a sterile syringe to 20mM (final concentration) and transferred to serum vials with sterile pipettes and crimp sealed. ^{3}H-thymidine (20 nM) was added to samples and the samples were

incubated at 2-4º C in the dark for 14 days. Reactions were terminated and rates calculated as described above.

Results

Geochemical Patterns

The reduced, nutrient rich outflow is oxygenated as it exits the subglacial environment at Blood Falls and spreads across the lake ice surface, mixing with the moat. Various minerals, such as mirabilite, halite and gypsum (Black et al., 1965, Black and Bowser, 1968, Lyons et al., 2005) precipitate at the surface and then dissolve in glacier melt or stream flow with from Santa Fe Creek, and finally enter the moat waters of WLB.

Geochemical data (dissolved oxygen, pH, dissolved organic carbon, total iron, ions (Na^+, Cl^-, SO_4^{2-}, Ca^{2+})) collected along the transect from Blood Falls into WLB (Table 5.2) were used for cluster analysis (See Appendix D for list of distance values). The resultant dendogram (Figure 5.4) shows that Blood Falls ancient outflow shares the highest similarities with WLB 25 m depth water, (Pearson's R between transect samples BF-2004 and BFT10 (Hole3, 25m) = 0.661 and between BF-2004 and BFT15 (Limno hole, 25m) = 0.748). There is some correlation between the outflow samples collected in 2004 and all the 2003 samples collected throughout the water below the chemocline (~18-35 m), although values were not as high as for the samples mentioned above, all were positive. Contrarily, correlation values between 2004 subglacial outflow (BF-2004) and the 2003 Blood Falls flow (BF-2003) were negative as were correlation values between BF-2004 and all 2003 hypolimnion samples.

Table 5.2. Summary of biogeochemical parameters collected along a transect from Blood Falls into the WLB water column (samples named BFT) and around the Blood Falls moat system (samples named BFS). ND = not detected, na = not available.

Sample	*pH*	*Cell density cells ml^{-1}*	*CFUs R2A*	*CFUs Mar*	*Fe µM*	*Chl*-a *µg L^{-1}*	*DIC mM C*	*DO µM O_2*	*DOC µM C*	*DIN µM*	*Na^+ mM*	*Cl^- mM*	*SO_4^{2-} mM*	*Ca^{2+} mM*	*Richness*
BFT-1	8.5	7.6E+05	1.4E+05	5.1E+05	14.5	43.20	0.6	524	210	36	6	6	0.06	0.7	21
BFT-2	na	4.2E+05	1.2E+03	1.2E+03	0.8	3.21	1.4	669	78	21	13	16	0.17	1.4	24
BFT-3	6.4	7.7E+04	7.2E+02	5.3E+03	0.3	7.63	34.1	315	381	94	427	659	6.86	21.9	20
BFT-4	6.2	2.1E+05	0.0E+00	2.8E+03	0.3	0.76	46.7	70	696	237	1048	1515	15.77	47.2	21
BFT-5	6.2	8.5E+04	0.0E+00	2.7E+03	0.4	0.92	52.8	89	720	240	1074	1538	16.00	47.7	18
BFT-6	6.0	1.3E+05	0.0E+00	4.7E+03	5.5	0.11	55.8	51	780	259	1271	1785	18.57	50.0	17
BFT-7	na	5.1E+05	3.0E+04	2.0E+02	8.2	3.40	30.9	556	77	17	15	17	0.18	1.4	23
BFT-8	6.5	1.0E+05	6.5E+02	3.8E+03	0.2	7.10	na	302	279	64	315	453	4.72	16.1	25
BFT-9	6.2	2.4E+05	0.0E+00	2.3E+03	0.4	0.35	45.9	83	744	257	1181	1640	17.06	49.8	17
BFT-10	6.0	1.2E+05	0.0E+00	0.0E+00	36.3	0.22	45.1	0	902	288	1434	2066	21.50	50.0	17
BFT-11	6.0	1.1E+05	0.0E+00	0.0E+00	17.2	0.06	6.2	0	1253	357	1568	2209	22.99	55.3	19
BFT-12	7.9	2.4E+05	8.0E+03	1.4E+03	4.7	2.14	1.2	719	82	16	16	19	0.19	1.6	23
BFT-13	7.2	7.9E+04	2.3E+03	2.4E+03	0.6	3.50	7.1	831	163	26	159	246	2.56	8.0	19
BFT-14	6.2	1.9E+05	0.0E+00	5.4E+02	0.5	0.18	46.2	89	757	257	1202	1620	16.86	50.5	16
BFT-15	5.9	4.2E+04	0.0E+00	1.2E+02	57.5	0.29	55.1	0	909	296	1452	2007	20.89	50.9	16
BFT-16	5.9	5.3E+04	0.0E+00	0.0E+00	23.7	0.15	52.8	0	1256	362	1634	2254	23.45	57.5	21
BFT-17	5.9	5.8E+04	0.0E+00	0.0E+00	16.2	0.43	52.2	0	1423	402	1614	2370	24.66	58.6	22
BFS-1	7.7	na	na	na	27.4	14.1	0.4	283	44	16	na	0.6	0.22	na	na
BFS-2	9.2	na	na	na	15.9	18.6	0.3	247	34	10	na	0.7	0.21	na	na
BFS-3	8.5	na	na	na	33.7	ND	0.4	299	31	19	na	0.6	0.21	na	na
BFS-4	8.4	na	na	na	34.9	ND	0.4	268	58	18	na	0.7	0.21	na	na
BFS-5	8.6	na	na	na	37.2	ND	0.5	279	42	19	na	0.6	0.20	na	na
BFS-6	8.2	na	na	na	36.2	6.2	0.5	279	44	19	na	0.7	0.22	na	na
BFS-7	8.5	na	na	na	38.1	6.0	0.4	298	51	19	na	0.7	0.22	na	na
BFS-8	8.4	na	na	na	43.2	5.0	na	287	36	20	na	0.6	0.20	na	na
BFS-9	9.1	na	na	na	78.6	ND	0.8	293	63	10	na	15.7	7.47	na	na
BFS-10	9.1	na	na	na	2.3	0.1	1.3	257	114	10	na	26.9	24.54	na	na

Blood Falls flow samples collected during 2003, a year of contemporary melt, are geochemically distinct from ancient outflow (Pearson's R between BF-2004 and 2003 = -0.528), and are most geochemically similar to surface water samples (6 m) across the lake (i.e. Pearson's R= 0.737 between BF-2003 and BFT-12 (Limno hole, 6 m). The cluster analysis also shows geochemical stability across the WLB water column; similar depths across the lake cluster together, this pattern was also found by Spigel and Priscu (1996, 1998) using extensive density measurements across both lobes of Lake Bonney.

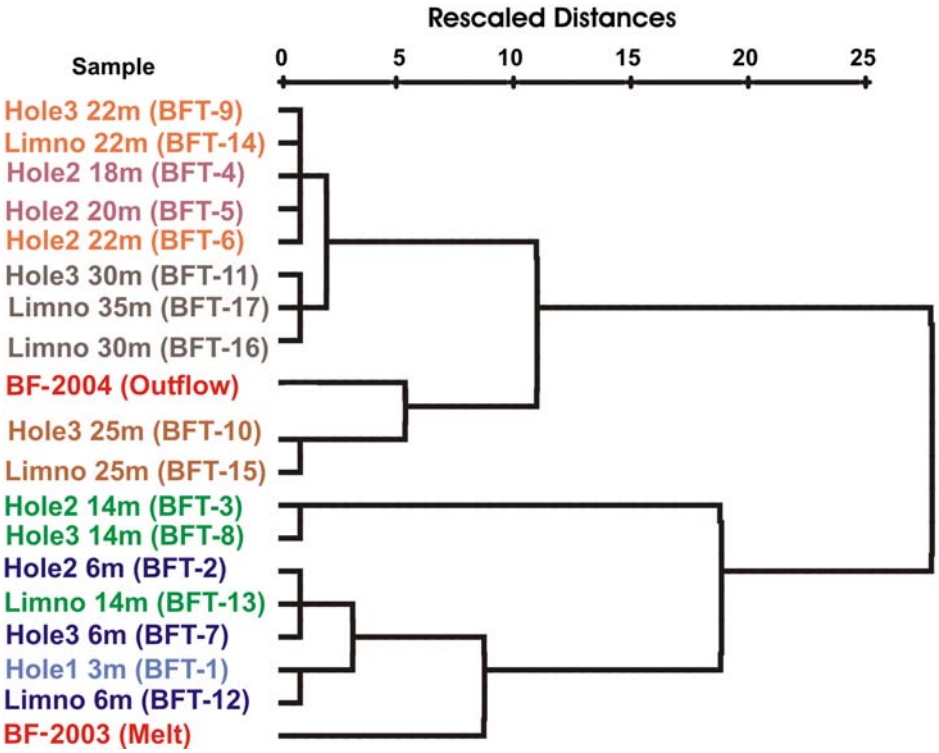

Figure 5.4. Pearson's correlation analysis of geochemical parameters (DOC, DO, pH, total Fe and ions: Na^{+}, Cl^{-}, SO_4^{2-}, Ca^{2+}). Data intervals were measured with the Pearson's correlation and clustered using the furthest neighbor method. Parameter values were standardized to range between 0 and 1.

Microbial Diversity Patterns

Chemical and temperature denaturing gradient gel electrophoresis methods (DGGE and TGGE respectively) are not without limitation. For example, distinct DNA fragments may co-migrate within the gel and fragments with only a few base pair differences may not be resolved (Muyzer and Smalla, 1998). However, DGGE and TGGE have been used successfully to profile microbial communities (reviewed in Muyzer and Smalla, 1998) including a study of bacterial community structure in Antarctic lakes (Pearce, 2000). TGGE analysis of samples collected along the transect from Blood Falls to the deep waters of west lobe Lake Bonney revealed that 16S rDNA diversity varied among samples (Figure 5.5).

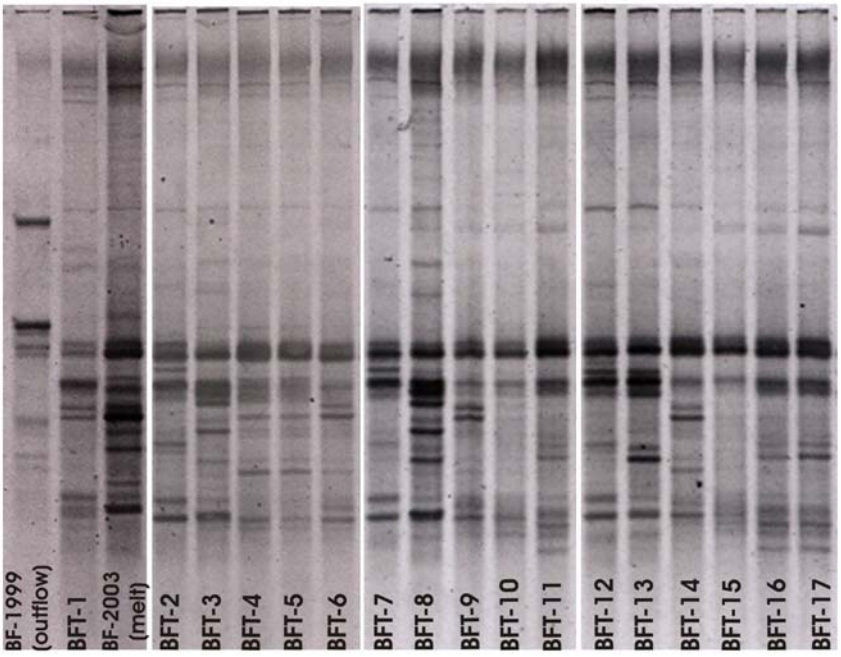

Figure 5.5. Temperature gradient gel electrophoresis gel showing banding patterns for each sample along the transect from Blood Falls to the limnological sampling hole (BFT-1 through BFT-17) and samples collected from outflow during an ancient outflow event and a contemporary melt year (1999 and 2003, respectively).

Cluster analyses of TGGE banding patterns (Figure 5.6) based on presence or absence of bands, using the Jaccard measure of similarity, shows that ancient outflow contains an assemblage of phylotypes distinct from all other samples. Ancient outflow samples are most similar to samples from BFT-5 (Hole2, 20m), Jaccard index =0.400 and BFT-6 (Hole2, 22m), Jaccard index = 0.307. On average, there are less genotypic similarities (significant genotypic differences at the $p<0.001$ level) between ancient outflow (BF-1999) and other WLB water column transect samples (BF-1999 Jaccard average similarity = 0.289), whereas 2003 contemporary melt samples have greater overall similarity with the WLB water column samples (Jaccard average similarity between BF-2003 and other transect samples = 0.407). The microbial diversity observed in the melt year outflow clustered most closely with moat samples from that same year BFT-1 (Hole1, 3 m), Jaccard index = 0.529, as well as other surface samples across the lake (6 m depths: BFT-2, BFT-7 and BFT-12). These data show that genotypically, ancient outflow samples are distinct from both contemporary melt samples and from the WLB water column.

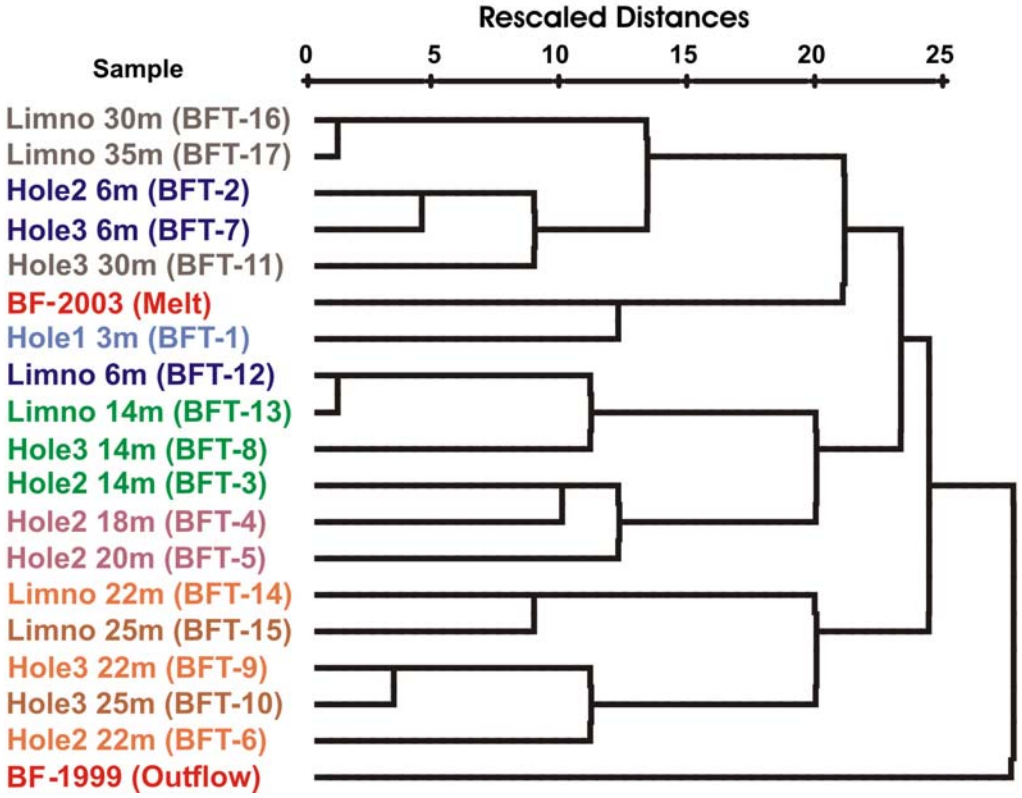

Figure 5.6. Hierarchical cluster analyses of TGGE banding patterns of outflow and WLB water column samples (based on presence/absence of bands). The furthest neighbor cluster method was used with the Jaccard measure for binary data.

Biological Implications

Biogeochemical data further support the notion that ancient outflow and contemporary melt flow samples collected from Blood Falls flow were distinct (Table 5.3). Ancient outflow events release high concentrations of DIN, DOC and DIC to the WLB moat. DIN in ancient outflow and the moat water (~ 95 μM) and was fully reduced (100% DIN=NH_4^+) in 2004. In contemporary melt years, moat DIN was 17 times lower and only 36% appeared as NH_4^+. DOC concentrations were high during ancient outflow events with 420 μM C and 454 μM C in the outflow and moat water, respectively, and

twice that measured in the contemporary melt moat (210 μM C). DOC concentrations in the moat from both years was twice that or greater than concentrations in 6 m (BFT-2, 7 and 12) water across WLB (79 μM C; SD= +/-2). DIC was almost an order of magnitude greater in ancient outflow and corresponding moat water samples (~55 μM C) than in contemporary melt moat samples (0.6 μM C).

Table 5.3. Biogeochemical parameters of ancient flow versus contemporary melt. Samples collected at the outflow sources and in the moat during an ancient flow event (2004) are shaded. nd = not detected.

Parameter	***Ancient Outflow (2004 averages)***	***Moat (ancient outflow event-2004)***	***Moat (contemporary melt event-2003)***
pH	6.2	6.3	~8.5
Bacterial density (cells mL^{-1})	1.0×10^5	2.7×10^4	7.6×10^5
% recovered (R2A/MAR)	0/0	0/0	20/67
DOC (μM C)	420	454	210
DIC (μM C)	55	56	0.6
DO (μM O_2)	0.0	210	525
Chl-*a* (μg/L)	0.23	0.32	43.2
DIN (μM)	94	95.5	5.4
%DIN as NH_4^+	100	100	36
Fe (μM)	3450	2323	14.5
SO_4^{2-} (mM)	50	52	0.06
Cl^- (mM)	1440	1459	6.0
SO_4:Cl	0.035	0.036	0.010

CHL concentration was low (0.32 μg L^{-1}) in the moat during ancient outflow (2004). In contrast, the CHL concentration in the contemporary melt moat (2003), was 43.2 μg L^{-1}, which is more than 7 times higher than the deep chlorophyll maximum of WLB at 13 m (~ 6 μg L^{-1}; Priscu, 1995). Primary production was not measured in the

moat, but the high concentrations of CHL imply that photosynthetic organisms are active and possibly more productive closer to Blood Falls (Figure 5.7).

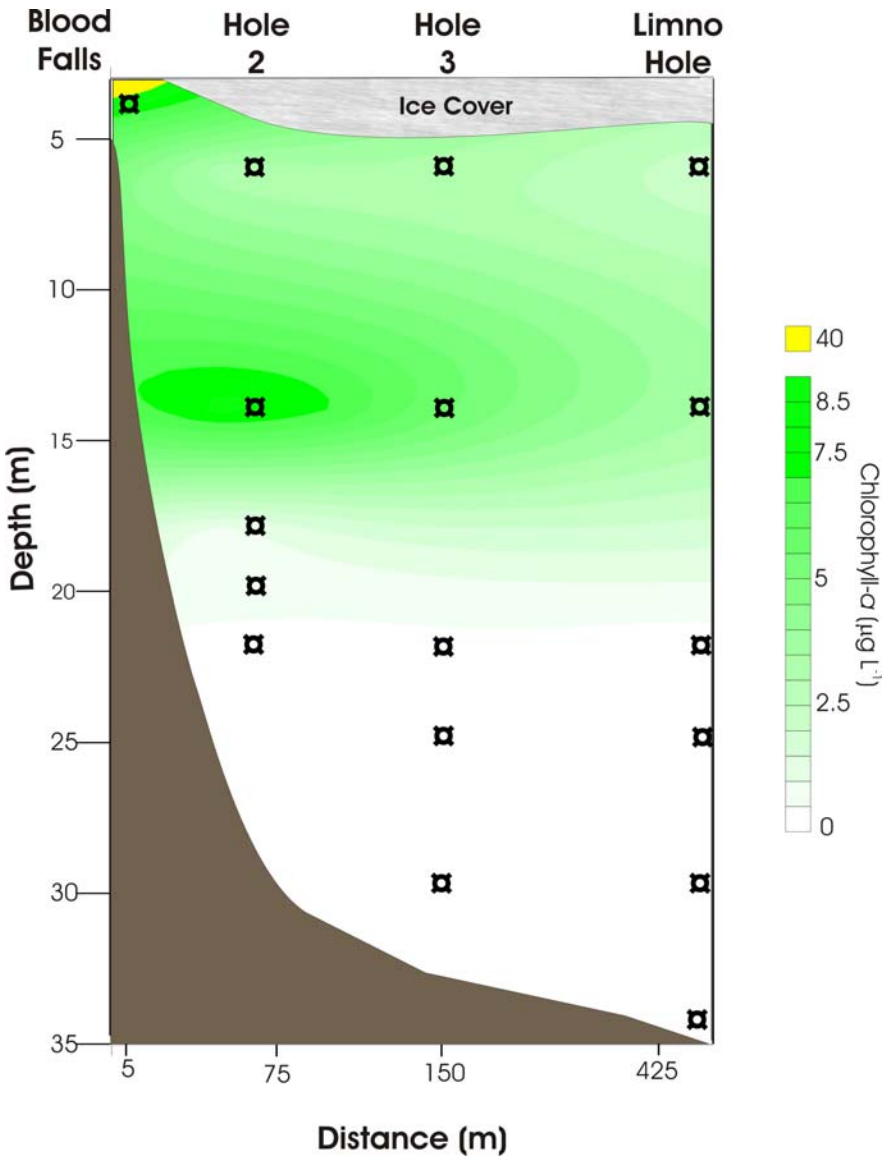

Figure 5.7. Contour plot of Chlorophyll-*a* concentrations (µg L^{-1}) in the west lobe of Lake Bonney. Data for the contour plot were interpolated by krigging the 17 transect points (BFT1-17). ¤ = actual transect data points used to create the map. Note that CHL for the sample point closest to Blood Falls (BFT1) is an order of magnitude greater and not on the scale. The ice thickness over Hole2 ~ 3.4 m, and ~4 m over the Limno hole.

Recovery of microorganisms in the Blood Falls moat system on agar plates (Table 5.3), was significantly higher during contemporary melt events (2003) than during ancient flow events (2004, no colonies recovered). Colony forming units (CFUs) on R2A plates were 1.4 x 10^5 CFUs ml^{-1} (20% recovery) and 5.1 x 10^5 CFUs ml^{-1} on marine agar plates (67% recovery). Water column samples that were incubated on marine agar during 2003 resulted in colony recovery from most depths along the water column transect (no colonies were recovered from 30 and 35 m samples on either agar) with an average of 5% recovery across the water column for all samples collected (Figure 5.8). Average recovery of colonies on R2A was lower (2% average recovery across the water column transect) and occurred only in samples of water above the chemocline. Samples collected from the outflow and the moat during ancient flow yielded no colonies after 5 months of incubation at 4º C. Eventually, five colonies appeared on agar plates inoculated with ancient outflow following >6 months incubation. These cells were most closely related to *Marinobacter sp.* and *Flavobacterium sp.* (as described in Chapter 4).

Bacterial production rates in moat samples (Table 5.4) from a contemporary melt year (2003) were statistically higher than samples of moat water from an ancient flow year (2004; $p<0.001$; $df = 6$). Contemporary melt samples amended with 5 mM Fe (II) showed dramatically reduced (90% less) thymidine incorporation rates (2.1 x 10^{-4} nM thymidine d^{-1}) compared to unamended treatments; rates were not statistically different from incorporation rates for ancient outflow samples the following year ($p=0.833$). Incorporation rates per cell (Table 5.5) in samples collected during contemporary melt were significantly higher ($p<0.001$) than the activity per cell of samples collected during ancient outflow events and samples amended with 5 mM Fe (II).

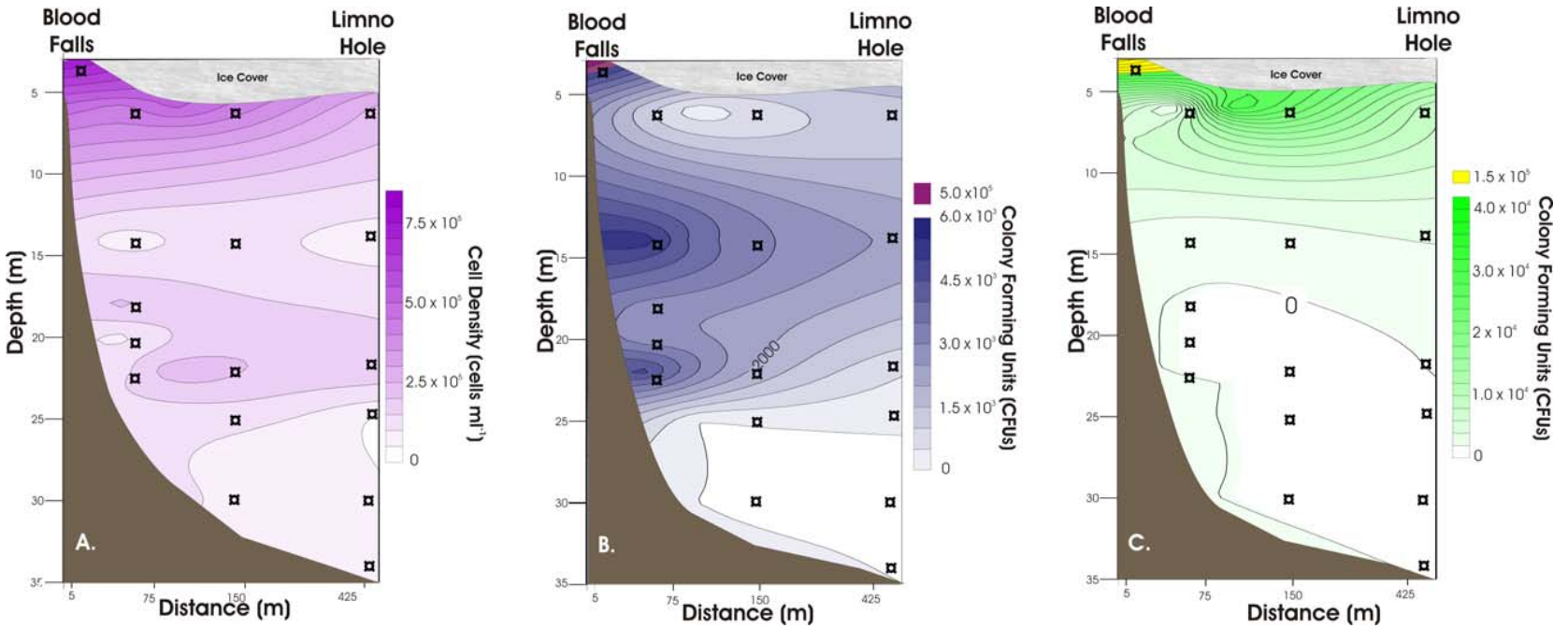

Figure 5.8. West lobe of Lake Bonney contour map of cell densities (A), colony forming units (CFUs) grown on marine agar (B), and CFUs grown on R2A agar (C). Data for contour maps were interpolated by krigging from the 17 transect points (BFT1-17). ¤ = actual transect data points used to grid the contour maps. Note that CFUs for the sample point closest to Blood Falls (BFT1) are exponentially larger (10^5) and not on the scale.

Table 5.4. Thymidine incorporation rates in Blood Falls flow samples collected during an ancient outflow event (2004) and during contemporary melt (2003). Note that sample treatment and incubation time vary among samples.

Sample Date:	***Inc. Time (h)***	***Mean nM Thymidine day^{-1}***	***St. Dev.***	***Treatments:***
13-Nov-04	20	2.8×10^{-4}	2.3×10^{-4}	Scint vials; aerobic
10-Nov-04	20	1.7×10^{-4}	2.6×10^{-4}	Scint vials; aerobic
6-Dec-04	72	2.3×10^{-4}	6.2×10^{-5}	Serum vials; anaerobic
2004 Ancient outflow (Average)		*2.3×10^{-4}*	*2.8×10^{-4}*	
21-Dec-03	144	5.5×10^{-2}	1.2×10^{-2}	Serum vials; anaerobic
21-Dec-03	14	1.5×10^{-2}	7.5×10^{-4}	Scint vials; aerobic-*Light*
21-Dec-03	14	1.1×10^{-2}	1.9×10^{-3}	Scint vials; aerobic-*Dark*
21-Dec-03	278	4.2×10^{-2}	1.6×10^{-2}	Serum vials; anaerobic
2003 Contemporary melt (Average)		*3.8×10^{-2}*	*1.4×10^{-2}*	
21-Dec-03	278	2.1×10^{-4}	6.3×10^{-5}	Serum vial; anaerobic + 5 mM Fe (II)

Table 5.5. Thymidine incorporation averages, cell density, and bacterial productivity per cell for Blood Falls flow samples collected during an ancient outflow event (2004) and during contemporary melt (2003) and contemporary melt samples amended with 5 mM Fe (II).

Sample	***nM Tdr d^{-1}***	***Cells ml^{-1}***	***nM Tdr d^{-1} cell^{-1}***
2003 average	3.8×10^{-2}	6×10^{4}	6.4×10^{-7}
2004 average	2.3×10^{-4}	1×10^{5}	2.3×10^{-9}
2003 samples with 5 mM Fe(II)	2.1×10^{-4}	6×10^{4}	3.5×10^{-9}

Discussion

Ancient Outflow as a Disturbance Event

Ancient outflow from the Taylor Glacier occurs episodically and acts as a disturbance event to the moat microbial community. Diluted outflow supplies nutrients for bacterial activity in the Blood Falls moat during melt years, while ancient outflow acts to disrupt the moat assemblage by inhibiting bacterial activity. Samples collected around Blood Falls during contemporary melt (BFS samples, Table 5.2) show that despite dilution of chloride and iron concentrations, DOC and other nutrients are still present in the diluted flow. These nutrients may act to enhance bacterial activity in the Blood Falls moat system. Bacterial activity in samples of Blood Falls flow during the 2003 melt year averaged ~3.8 x 10^{-2} nM thymidine d^{-1}, which is comparable to rates reported for WLB (0-0.05 nM thymidine d^{-1}, Takacs and Priscu, 1998), but orders of magnitude greater than activity in ancient outflow (average = 2.3 x 10^{-4} nM thymidine d^{-1}, Table 5.4). Contemporary melt samples amended with 5 mM Fe (II), which is similar to the total Fe concentrations measured in 2004 subglacial outflow (3.8 mM), showed 90% less activity than unamended samples. These data imply that the concentration of iron in subglacial influx may be inhibitory to the moat microbial assemblage. In addition to high concentrations of iron, ancient outflow would also deliver a hypersaline shock, producing an initial inhibitory disturbance event to the Blood Falls moat system. Ward et al. (2003) have suggested that bacterial productivity is limited in the depths below the chemocline (~18 m) in WLB due to high concentrations of salts and metals, supporting my results for the moat system.

The complete lack of recoverable organisms during ancient discharge is surprising, but clearly demonstrates a biological change in the Blood Falls moat system. The high recovery rates (20-67%) from melt year samples (2003) imply that the heterotrophic bacteria at the site of Blood Falls outflow were actively reproducing and prepared to take advantage of available nutrients at the time of plating. The dominant populations, following a disturbance event, tend to be generalists with enhanced physiological tolerance and substrate utilization capabilities (Atlas et al., 1991). Research indicates that the majority of bacterial populations grown on agar plates from environmental samples are *r*-selected populations with high growth rates, capable of growth at high nutrient concentrations (Atlas, 1984, Atlas and Bartha, 1998). The growth strategies utilized by microbes at the surface where outflow is released would be different from organisms living below the Taylor Glacier. In addition, organisms transported from the subglacial environment in discharge may be metabolically stressed by the change in geochemistry in the moat (i.e. increase in oxygen concentration and decrease in salinity).

Advective Outflow Into the West Lobe of Lake Bonney

The density of ancient subglacial discharge is ~1,100 kg m^{-3} (Keys, 1979) and would reach density equilibrium at ~ 22-25 m depth in the WLB water column (Spigel and Priscu, 1996). It is not surprising that the geochemistry in the bottom depths of WLB are similar to ancient outflow and the influx of dense sublglacial brine should deliver any cells present in the brine to these deep depths below the WLB chemocline. Preliminary culture work shows that some organisms my proliferate both below the Taylor Glacier and in the WLB water column, however, based on similarity measures of TGGE banding

pattern diversity, the majority of the microbial assemblage transported with the brine does not appear to be present in WLB.

Cell density, colony forming units (Figure 5.8) and CHL concentrations (Figure 5.7) are highest at the point where Blood Falls mixes with the WLB moat water. The contour plots also show what appears to be a higher concentration of these parameters in water column samples collected closer to Blood Falls. These data were not shown to be statistically significant however, this could be due to insufficient samples. Lawson et al. (2004), detected evidence for the lateral mixing of moat waters extending into the under-ice environment near large stream inputs using stable isotope analysis of organic matter, and although the extent of lateral mixing as a result of Blood Falls and Santa Fe Creek inputs are unknown, mixing surely occurs and may account for the higher concentrations of CHL, for example, closer to the Taylor Glacier.

Ancient outflow contains numerous cells (1×10^5 ml^{-1}), including metabolically active and cultivable cells (Chapter 3 and 4). It remains unclear whether these cells survive and grow in the WLB water column. A *Marinobacter* isolate (BF05-1a, described in Chapter 4) was most closely related to isolate ELB-17, previously identified throughout the water column of the east and west lobes of Lake Bonney (Ward and Priscu, 1997) and isolated from samples of 22 m WLB water during my 2003 transect study (Table 5.6). Isolate BF05-1a was obtained from samples collected directly at the source of primitive outflow, but it would be difficult to conclude that organisms in ancient outflow grow in Lake Bonney. If organisms like BF05-1a, are hardy enough to survive transport from the subglacial environment, they should be able to thrive in WLB, which has similar geochemical conditions. The low Jaccard index values of similarity

between ancient flow and all WLB water column samples (<0.400) implies that the majority of phylotypes are not found concomitantly in ancient subglacial outflow and WLB, despite the geochemical similarities between ancient outflow and WLB bottom waters.

Table 5.6. Checklist of phylotypes cultivated from WLB at 22 m depth and Blood Falls outflow compared to phylotypes detected by cloning. Blood Falls flow type refers to whether sample for cultivation was collected during an ancient outflow event or contemporary melt event. Clone library (Chapter 4) was constructed from samples collected during ancient outflow.

	Cultivated from:		***Blood Falls flow type:***	***Represented in BF Clone Library***
Organism	**WLB 22m**	**Blood Falls**		
Arthrobacter sp.	√	√	Melt	
Marinobacter sp.	√	√	Ancient	√
Flavobacterium sp.		√	Both	√
Psychrobacter sp.		√	Both	√
Janthiobacter sp.		√	Melt	√
Pseudomonas sp.		√	Melt	√
Caulobacter (alpha)		√	Melt	
Schwenella frigidimarina		√	Melt	

Conclusion

The discharge of ancient subglacial outflow from the Taylor Glacier is an episodic disturbance to the Blood Falls moat system. Ancient outflow contains high salt and iron concentrations that may be toxic to some microorganisms. The outflow also contains nutrients for bacterial growth that, once diluted, may stimulate growth in the Blood Falls moat system. Subglacial outflow is altered as it travels to the surface, through the moat and into the lake water. Despite this connectivity, the microbial diversity profiles for

each compartment are different (subglacial outflow, moat and lake). Geochemically, WLB bottom water is related to Taylor Glacier subglacial outflow (Figure 5.4), yet analyses of biological parameters suggest a disconnect exists between subglacial outflow and WLB bottom water. This "change" to the subglacial biota occurs in the Blood Falls moat system where water from Lake Bonney, Blood Falls and the Santa Fe Creek converge.

CHAPTER 6

MICROBIAL ECOLOGY STUDIES OF THE SUBGLACIAL ENVIRONMENT: CONCLUSIONS AND BROADER IMPLICATIONS

The dynamics of the Earth system are tightly linked with physical, chemical and biological processes (Knoll, 2003); the common currency of these biogeochemical linkages is the cycling of the elements (Schlessinger, 1997). The role of microorganisms in the cycling of elements is crucial for the maintenance of planetary habitability; since microbes are tightly involved in most of the biogeochemical transformations that secure the steady state of energy flow in the biosphere.

The McMurdo Dry Valleys of Antarctica represent the closest Earthly analog to Martian environments (Cameron et al., 1976, McKay et al., 2005). Subglacial environments functioning autonomously on Earth, for possibly millions of years, suggest that subglacial systems on Mars would be plausible sites for exobiological exploration. As early Mars lost its atmosphere and cooled, water at the surface would have experienced evaporation and cryogenic freezing (Burt and Knauth, 2003) similar to the brines of the dry valleys and brine below the Taylor Glacier. Subglacial environments, similar to the Taylor subglacial system described here, would provide stabile microbial refuge and protection from harsh Martian surface conditions.

Chemical analysis of subglacial outflows provides insight into the subglacial habitat (Tranter et al.,1998). Until recently, subglacial chemistry was thought to be

strictly abiotic reactions (Raiswell, 1984). Advances in cold-environment microbiology and subglacial geochemistry, has changed our view of the subglacial system (Nealson, 1999, Priscu and Christner, 2004, Tranter et al., 2005). We now know microbial metabolism is an important component of subglacial processes and that microbially transformed subglacial carbon is a significant component of the global carbon budget (Sharp et al., 1999).

Blood Falls is the subglacial outflow of the Taylor Glacier in the dry valleys of Antarctica. The discharge is episodic, leading to disparate chemistries of water collected at Blood Falls, where outflow meets the surface (Chapter 3). Chloride and sulfate concentrations, and specifically their molar ratios, are a conservative metric for distinguishing between ancient subglacial outflow events and contemporary glacial melt mixing with residual outflow (Chapter 3).

Geochemical analyses of outflow indicate that the source water of Blood Falls is ancient seawater (Lyons et al., 1998, 2005) that has been modified through freezing and evaporation. Chapters 2 and 3 presented data that imply microbial processes are an integral part of the geochemistry of the outflow. The physical hydrology of the Taylor Glacier and the geomorphology of the underlying bedrock create a situation where ancient marine brine is stored below the glacier having minimal communication with the surface, for extended periods of time, possibly millions of years. This brine, therefore, provides significant insight into stable, cold-based subglacial processes.

The microbial diversity of subglacial systems remains largely unexplored. Microbial diversity data presented in Chapter 4 indicates that the microbial assemblage below the Taylor Glacier is shaped by three main factors: (1) the legacy ecosystem prior

to glaciation (2) the glacier hydrology (i.e. the extent to which surface or supraglacial waters are in contact, if at all, with the subsurface glacial environment) and (3) the bedrock lithology.

The Taylor Glacier is a polar glacier, lacking crevasses and other direct connections from the surface to the bed (Fountain et al., 1999); hydrological processes in the ablation zone of the Taylor Glacier appears to be controlled by the presence of subglacial liquid brine (Robinson, 1984, Hubbard et al., 2004). Thus, the subglacial brine and the microbial assemblage living in the brine are isolated from the surface. The phylogenetic analysis of the Taylor subglacial outflow in this study revealed a microbial assemblage dominated by clones capable of utilizing iron and sulfur minerals for energy and growth. Such lithotrophic lifestyles match the physical constraints described for the Taylor Glacier subglacial system and may be common to other subglacial microbial communities. Evidence for chemoautrophic growth in the outflow was detected by measurements of CO_2 fixation as presented in Chapter 3. Samples from subglacial outflow collected at Blood Falls fixed CO_2 in dark incubation experiments at rates (1.2 nmol C L^{-1} d^{-1}) comparable to that reported for an Arctic subglacial volcanic lake (Gaidos et al., 2004). The most dominant clone in the Blood Falls 16S rDNA library was closely related to the 16S rDNA sequence of *Thiomicrospira arctica*, an obligate chemoautotroph (Chapter 4). In sum, these data support the notion that the subglacial environment below the Taylor Glacier can function autonomously using *in situ* chemosynthetic and heterotrophic activity to obtain carbon and energy sources in the absence of light.

The Taylor Glacier's subglacial outflow influences the proglacial ecology of Lake Bonney (Chapter 5). Outflow from the subglacial environment changes as it interacts

with the west lobe of Lake Bonney moat water before it eventually enters into the deep saline water of the lake proper. The microbial diversity in subglacial discharge is distinct from that of the deep waters of Lake Bonney despite geochemical similarities between the two waters suggesting subglacial outflow is altered as it flows from the glacier to the lake.

The research presented in this dissertation has demonstrated that Blood Falls outflow and the subglacial brine it represents is an important site for the study of subglacial ecology and the efflux of subglacial fluids on proglacial ecosystems. Opportunities for further study of the subglacial system below the Taylor Glacier are vast. Measurements that link *in situ* microbial activity with subglacial geochemistry, for example labeled substrate experiments, would further define the interactions between metabolic guilds of the subglacial ecosystem. It is difficult to infer metabolic function from phylogentetic data alone therefore it is important to obtain pure cultures of the autotrophic (i.e. *Thiomicrospira sp.*) and heterotrophic members (i.e. *Flavobacterium sp.*) of the subglacial assemblage to further study the metabolism and ecological interactions within the system.

In the absence of drilling through the Taylor Glacier, the geomicrobiological environment of the subglacial habitat was inferred by studying the geochemistry and microbiology of the subglacial discharge at Blood Falls. The discharge channels themselves are transition zones from the subglacial to the lake ecosystem and provide a glimpse of the subglacial environment. However, it is important to note that the data obtained from the study of Blood Falls outflow is not a true representation of the subglacial condition. Only samples taken from the base of the Taylor Glacier at the

source of Blood Falls will allow us to determine the true geomicrobiology of this subglacial ecosystem.

Obtaining subglacial samples, to study true *in situ* conditions, would require drilling through the Taylor Glacier. To ensure that the integrity of this closed system is maintained, meticulous aseptic drilling techniques would be essential. Future multidisciplinary investigators are obliged to consider the unique ecology of Blood Falls and the Taylor Glacier subglacial system and take precautions to protect it.

APPENDICES

APPENDIX A

COLLECTING AND PROCESSING SAMPLES OF ICE

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COLLECTING AND PROCESSING SAMPLES OF ICE

Introduction

A crucial step for any ecological study, but particularly in habitats with low biomass, is sample collection. Sampling procedures should be designed so that the integrity of the sample is not altered; and should reflect *in situ* conditions as much as possible (Atlas, 1992). For example, anoxic sediments should not be exposed to oxygen while processing if isolation of strict anaerobes is the desired goal; typically an anaerobic hood is used for deep sediment samples. Using the same logic, ice cores should be manipulated in cold rooms, reagents and tools used for cleaning should be pre-chilled and any melting of the sample should occur at temperatures just above freezing. Chemical or physical alteration of a sample during processing is one concern; microbiologists must also prevent the contamination of their samples with omnipresent, non-indigenous organisms.

Sample Collection - Working with Ice and Frozen Samples

Ice core contamination sources can occur from the drill or any tool used to collect the ice, drilling fluids, handling, storage, transport and/or final processing (Rogers et al., 2005). When ice forms, it naturally incurs inconsistency. Fluids which have not reached their eutectic temperatures, sediments and particles are excluded from the ice matrix as

sediment pockets or brine channels (Benn and Evans, 1998). Drilling equipment and drilling fluid could introduce contaminants into deeper portions of drilled ice cores through pockets and channels. Simply rinsing off the ice sample with sterile solutions and then melting a composite sample is not enough to ensure sample integrity. Rogers et al. (2004) suggest that for the effective decontamination of ice, one must institute proper sterilization methods and use controls that include the use of indicator organisms and nucleic acids as well as seek replication by independent investigators when possible.

This appendix describes sample collection and decontamination protocols developed for icy samples. Three sample manipulations are described: (1) the analysis of drilling fluid contamination in the glacier ice overriding Lake Vostok; and methods developed for the decontamination of deep ice cores (Christner et al., 2004), (2) samples collected from the ice-cover of Lake Fryxell, collected for the enrichment of naturally occurring heterotrophic microorganisms, and (3) sample collection strategies from Blood Falls.

Study Site Descriptions

Lake Vostok. Lake Vostok is located near the center of the East Antarctic Ice Sheet (78°27' S, 106°52' E), and lies beneath 4 km of accretion and meteoric glacier ice (circled in Figure A.1). Lake Vostok is 250 km long and more than 700 m deep and is the largest and most studied of all known Antarctic subglacial lakes (Priscu et al., 2003). Ice cores were collected through the drilling operations at the Russian Vostok Research Station situated directly over Lake Vostok. Samples for this study were made available

by the United Stations National Ice Core Laboratory (NICL) in Denver, CO. (http://nicl.usgs.gov)

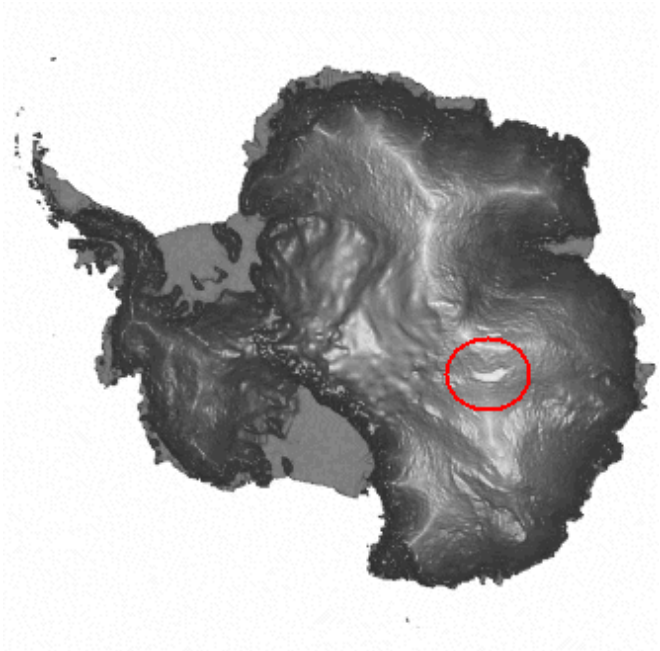

Figure A.1. Satellite image of Antarctica with the location of Lake Vostok, in East Antarctica circled. As the ice float over the lake, a flat spot is visible (www.earthinstitute.columbia.edu; Satellite map courtesy of NASA / U.S. Geological Survey).

Taylor Valley. The McMurdo Dry Valleys, located at 76º30'-78º30'S, 160-164ºE, form the largest ice-free region on the Antarctic continent. Taylor Valley is the site of the NSF-funded LTER. The dry valleys form the coldest, driest desert on Earth and contain a mosaic of permanently ice-covered lakes, ephemeral streams, arid soils, exposed bedrock and large polar glaciers (Priscu et al., 1998). Lake Fryxell (Figure A.2) is located in the eastern end of the Taylor Valley, and is one of the lakes monitored annually by the McMurdo LTER program. The lake ice cores discussed in this appendix were collected from Lake Fryxell's permanent ice cover.

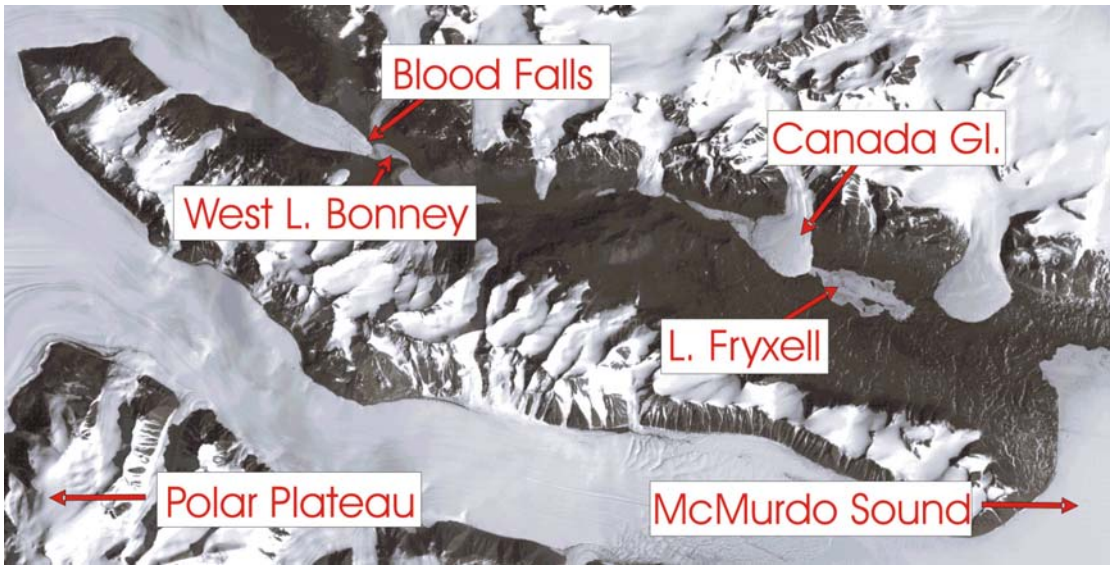

Figure A.2. Major features of the Taylor Valley. Lake Fryxell is closest to the McMurdo Sound which is the eastern most lake in the Taylor Valley. Taylor Glacier is an outlet glacier of the east Antarctic ice sheet as it enters the valley from the west.

Blood Falls. The Taylor Glacier, located at the western end of the Taylor Valley (Figure A.2) is an outlet glacier of the East Antarctic Ice Sheet and terminates into the west lobe of Lake Bonney (WLB) (Fountain et al., 1999). Taylor Glacier also provides the largest fraction (over 60%) of stream flow to Lake Bonney (Chinn, 1993). The Taylor Glacier has three major outflows into Lake Bonney: (1) Santa Fe stream in the north, (2) and Lyons stream in the south, both generated from supraglacial flow, and (3) Blood Falls, a distinct subglacial stream. Subglacial release from Taylor Glacier occurs predominantly at the northern end of the glacier terminus. The distinct red coloration of Blood Falls, resulting from iron oxides, provides a highly visible landscape feature.

Deep Ice Cores - Vostok Decontamination

Introduction

Preventing contamination is paramount when the environment of study is one of low biomass and one that has never before been sampled for the presence of microorganisms. Unequivocal claims for the presence of life in such environments require high levels of attention to decontamination protocols. Lake Vostok, a deep subglacial lake buried hundreds of meters below the surface and sealed from the atmosphere for millions of years is a classic case of biological controversy. The recovery of organisms from the deep subsurface is a good example of similar controversy that was overcome by sound decontamination protocols. The presence of a deep subsurface biosphere was originally a theory (Gould, 1992) until microbial evidence began to emerge (Sørensen et al., 2004). Convincing the inherently skeptical scientific community that life was prevalent in the subsurface in the absence of sunlight and that isolates did in fact come from hundreds of meters below the surface required robust controls.

Sediment cores from below the seafloor are obtained by drilling with seawater as the drilling fluid. Although sulfate is present at high concentrations in seawater (approximately 28 mM), it is absent in marine sediments more than a few meters below the surface due to the activity of sulfate-reducing bacteria (Claypool and Kvenvolden, 1983). Collected sediment cores are par cored with sterile tools under anaerobic conditions. Detection of sulfate in par-cored samples would indicate intrusion of the drilling fluid into the interior of the core. Injection of fluorescent beads (≈ 0.5-2 μm) or chemical tracers into the drilling fluid are other measures used for detecting

contamination (Smith et al., 2000). The presence of fluorescent beads or a non-indigenous chemical tracer would be easily detected if either migrated into interior samples.

Collecting deep (> 300 m depth) ice cores requires drilling fluid to prevent the lithostatic pressure from the surrounding ice sheet to cause plastic collapse of bore holes (Talalay and Gundestrup, 2002a, 2002b). Typically *n*-butyl acetate or kerosene with an added densifier is used as drilling fluid for these deep cores. Therefore, ice core samples collected from deeper depths, must be decontaminated as these drilling fluids are not sterile.

Background

A "wash and ablate" method was developed to decontaminate samples of the glacier ice overriding Lake Vostok (Fig. A.1), during the summer 2001. The outside of each core was rinsed with a sterile solution and ablated at room temperatures until approximately 50% of the ice core mass was removed. Samples of the rinsed and ablated core were processed differently for subsequent chemical and biological analyses. Chemistry samples were rinsed with twice filtered, autoclaved dionized water, then two thirds of the core's mass was ablated. Samples for biology were first rinsed with filtered 95% ethanol and then rinsed with twice filtered, autoclaved dionized water. Following processing, a final composite sample from a core retrieved from 763 m below the surface (V763), maintained a strong, distinct scent of kerosene. This observation prompted the investigation of kerosene penetration into the ice cores and the need to explore alternate methods of sample processing for biological studies.

Lake Vostok Glacial Ice Samples. The methods presented below are for cores collected from 179 m (179 m) and 2749 m (V2749) below the ice surface at Vostok Station. Core V179 was recovered from borehole BH5 with a dry drill where no drilling fluid was used. Core V2749 was collected from Vostok borehole 5G in which drilling fluid was used for collection at deeper depths (down to 3623 m). The drilling fluid used in the 5G borehole was commercial grade kerosene-based jet fuel (trade names: JetA, TC-1, JP-8), which has a complex combination of C9 to C16 hydrocarbons produced by the distillation of crude oil and includes other trace components typical of high end jet fuel such as toluene, ethylbenzene, and 1,2,4-trimethylbenzene. For cores drilled to depths greater than 300 m, the fuel mixture was supplemented with the halogenated solvent CFC-11 which acted as a densifier. Following the Montreal Protocol and subsequent ban on CFCs, the freon derivative Foran 141b was used as a densifier.

The contrasting drilling techniques used to recover core V179 (borehole BH5) and V2749 (borehole 5G) at Vostok station make them suitable samples to compare potential drilling fluid penetration into the core. The outer portion of the cores, (V179 and V2749), were reported to have up to 3 orders of magnitude higher bacterial density than the innermost sample (Christner et al., 2005). Clearly, decontamination protocols are an important aspect of microbiological investigations with ice cores, particularly when the actual *in situ* numbers of bacterial cells are low, between 10^2-10^4 cells ml^{-1} (Priscu et al., 1999 and Karl et al., 1999).

Methods

Ice core preparation. A 3 cm thick disc of each core was removed from cores V179 and V2749 with a band saw in a -10º C cold room. The outermost layer of ice (at least 50 mm of the core radius) was scraped with a 95% acetone-rinsed stainless steel microtome blade and ice scrapings at 2-5 mm intervals were collected and melted in glass conical bottles with Teflon-lined screw caps that were previously rinsed with acetone and baked at 100º C for 3-4 hours.

Kerosene detection. Detection and quantification of the kerosene signature was determined by gas chromatography in a HP 5890 GC column attached to a HP 5970 series Mass Selective detector. The organic fraction of melted samples was extracted using 1 part methylene chloride and 3 parts melted ice core shavings (melted in a gas tight container) in a glass conical vial with a Teflon lined screw cap. An internal standard of methyl stearate was included in the extraction process to monitor extraction efficiency. Samples were inverted and mixed for 10 min. After separation, the methylene chloride fraction was removed with a syringe. The aqueous phase was re-extracted to ensure the removal of all organics. A sample of Jet A kerosene was separated on a VG 70E GC column with a mass spectrometer detector to determine the hydrocarbon signature indicative of the Jet A mixture for comparison with signatures obtained from ice core samples.

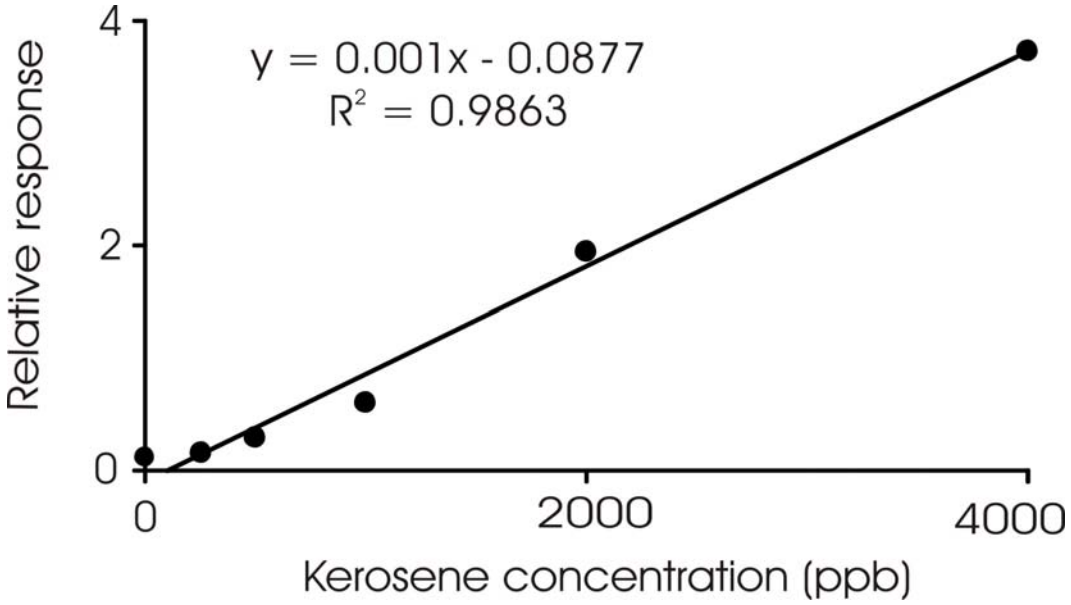

Figure A.3. Kerosene concentration standard curve based on relative response of increasing kerosene concentrations to the methyl stearate internal standard.

Hydrocarbons typically fragment into ions of masses 57, 71, 85, and smaller (masses are denoted by *m/z*, the ratio of the mass to the number of ion charges). The ion with a mass of 71 (ranging between 70.70 and 71.70) represented the specific marker compound for hydrocarbons and was scanned for by setting the mass spectrometer to run in the selective ion mode. HP Chem Station software (version B.02.05) was used to calculate peak heights of the mass 71 ion. The most abundant ion of methyl stearate has a mass of 74 (73.70 to 74.40) which was used to calculate the relative response. The total peak area of the hydrocarbon signature (mass 71) divided by the peak area of the internal standard (methyl stearate; mass 74) provided a relative response value. The relative response value was plotted against kerosene concentrations to generate a standard curve (Figure A.3). This standard curve was used for determining the concentration of kerosene in melted ice core samples. Although not representative, laboratory DI water

was used as a blank since Vostok glacial ice melt, free from any contaminants, was not available.

Results

Concentrations of kerosene were over 3000 ppb in the outermost layer of core V2749; the kerosene concentration was at the level of the DI water blank when approximately 5 mm of the ice core is scraped away (Figure A.4). The comparisons shown in Figure A.4 were based on the mass 71 ion extracted from the total GCMS spectrum. A more detailed examination of the spectral output provides more information on the actual carbon compounds associated with the ice (Figure A.5 B). Kerosene was clearly present in the outermost layer of core V2749 (Figure A.5 E), but at the level of the DI water blank with removal of 1 cm of the core exterior (Figure A.5 F). A comparison of the GCMS spectra from the outermost layer of core V179 (Figure A.5 C) reveal a signature that is not indicative of Jet A (Figure A.5 A); several peaks detected were almost out of the scan range implying that diesel or some other similar heavy distillate of crude oil might be present on the outside of the core. Despite differences in hydrocarbon composition, this signal is absent when the outermost layer of the ice core is scraped (Figure A.5 D).

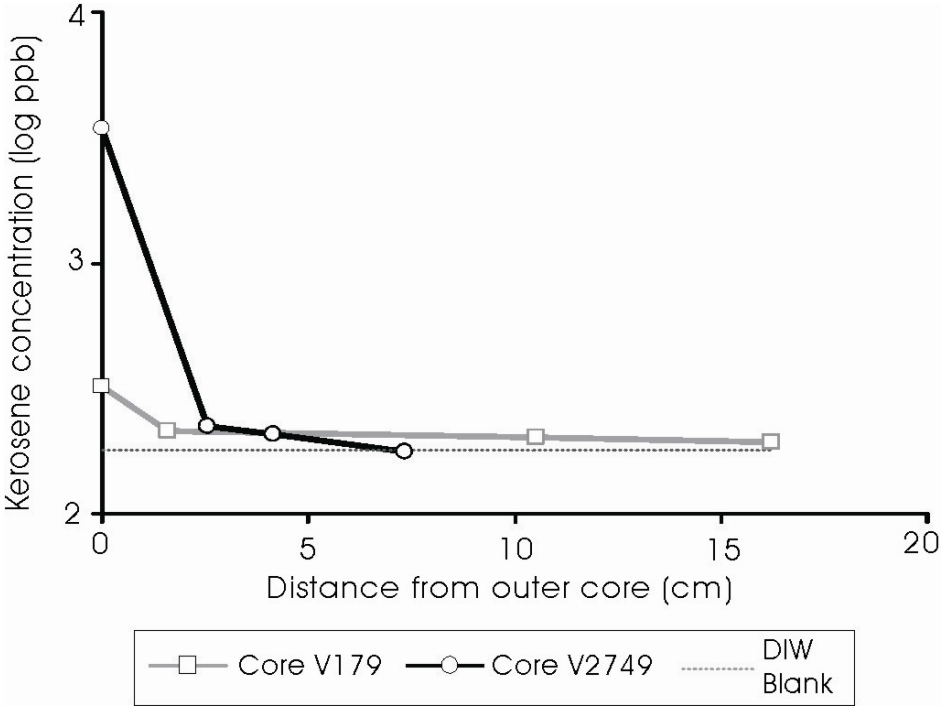

Figure A.4. Concentration of kerosene detected on the outermost scrapings of core V179 and core V2749.

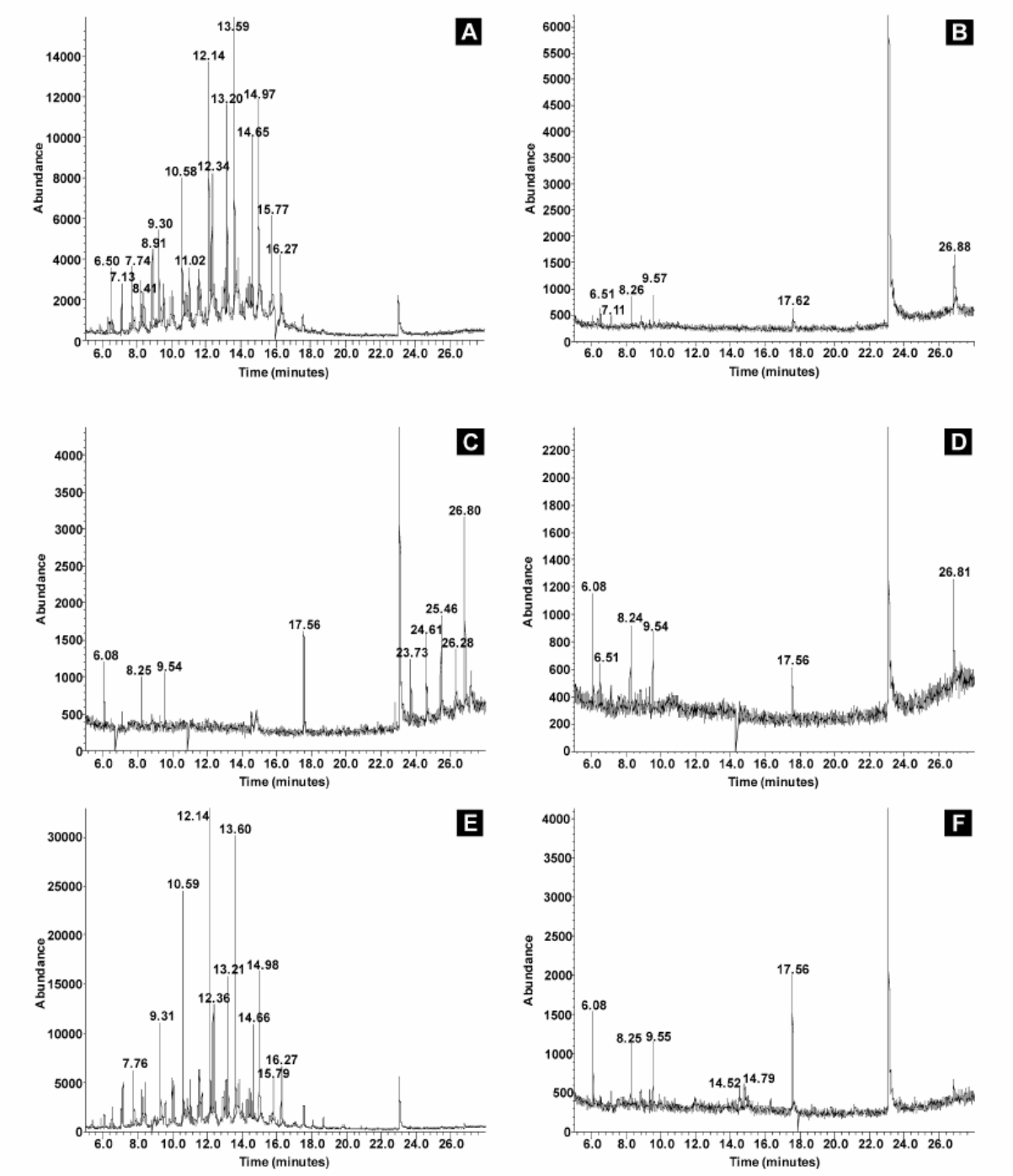

Figure A.5. Mass spectra obtained by gas chromatography of Jet A fuel or kerosene standard (A) DI water blank (B) the outermost 1.0 cm of core V179 (C) and an internal sample, > 1.5 cm (D), the outermost 1.27 cm of V2749 (E) and an internal sample, >1.5 cm (F).

Discussion and Conclusions

Deep ice cores retain the signature of the drilling fluid used for their collection and this drilling fluid can contaminate biological samples if proper decontamination procedures are not followed. These results indicate that rinsing and ablating is not sufficient for removing potential drilling fluid contamination. Even cores drilled without drilling fluid may retain residual hydrocarbons from handling and the use of diesel generators to operate drilling equipment. Concise recommendations for the standardization of ice core processing for biological studies were proposed by Rogers et al., (2005). The additional removal of the outer 1-2 cm of ice cores by physical scraping and the analysis of the extent of drilling fluid penetration should be included to this standard protocol as outlined by Christner et al. (2005).

Fryxell Lake Ice

Introduction

A Bell 212 helicopter crashed on the ice cover of Lake Fryxell on January 16, 2003 releasing approximately 200 gallons of hydrocarbon-based fluids. The fluids consisted primarily of a mix of JP8 and JP5 fuel with some hydraulic fluid (Alexander and Stockton, 2003). The ice-cover was isothermal at the time of the spill, and water and sediment filled cavities were present as described by Priscu et al. (1998) and Adams et al. (1998). There are concerns about the migration of these fluids within the ice cover and the subsequent contamination of the pristine Lake Fryxell below. This appendix presents data from a baseline study of the microorganisms present in uncontaminated lake ice.

Methods

Sample Collection and Processing. Lake ice samples were collected from Lake Fryxell (Figure A.2) during November and December of 2001. Ice cores were collected with a SIPRE (Snow, Ice, Permafrost, Research, Establishment) auger that had been wiped 3 times with 95% ethanol. Cores were drilled to a depth of 1.9 m and penetrated the sediment layer which was around 1.5m in the Lake Fryxell ice cover at this time. Cores were removed from the SIPRE auger core tube with sterile examination gloves and placed in autoclaved containers. Ice core samples from each depth were immediately inoculated into enrichment culture media following collection (≈ 5 hours). The outermost portion of each core (≈ 2 cm) was removed with a previously autoclaved razor on 70% ethanol-treated, aluminum foil. Scraped ice cores were placed in clean autoclaved containers; half of these were placed in an aerobic jar with gas packs that created a mixed CO_2 + H_2 headspace. The remaining samples were placed in aerobic containers.

Enrichment Cultures. Anaerobic incubations and enrichments can help increase the recovery of stressed cells by eliminating stressors such as oxygen (Marthai et al., 1991). Hence, both aerobic and anaerobic sample preparations were made and allowed to melt in the cold (0.1-1º C). R2A broth (Difco), was prepared in serum vials with butyl rubber stoppers; half of these liquid cultures were sparged with CO_2 that was first passed through an oxygen trap. R2A is a lower nutrient medium designed for low temperature, longer incubations to promote the growth of stressed heterotrophic organisms from water with low cell numbers (Reasoner and Geldreich, 1979). Aerobic and anaerobic media

preparations were autoclaved and pre-chilled before inoculation. Following melting of the ice cores at low temperatures, ice melt was transferred into the R2A broth (~ 0.5 ml). Stoppers were flame sterilized before transfer and transfers were made using a sterile syringe (1 ml) with an 18 gauge needle. Cultures were incubated for ~ 1 month, at 2-4º C. Aliquots of enrichment culture (100 µl) were then transferred onto solid R2A agar plates and streaked for isolation. All agar plate isolations were done under aerobic conditions. Colonies with unique morphologies were streaked for isolation two additional times to ensure purity. Pure cultures were stored at -80º C in a 25% glycerol stock solution.

DNA extraction and sequence analysis. Four isolates were selected for 16S rDNA sequencing analysis based on depth in the ice cover, distinct colony morphology or colony pigmentation. Genomic DNA was extracted from cell pellets of pure cultures using the QIAprep Spin Miniprep Kit (Qiagen). The 16S rRNA gene was amplified using the primers listed in Table A.1. PCR product from pure cultures was prepared for sequencing using an ethanol precipitation (Shapiro, 1981). Sequences were obtained using forward and reverse primers (Table A.1). Sequences were assembled in BioEdit, Version 7.0.5 (Hall, 1999). Sequences were then aligned using ClustalX function in BioEdit, sequences were further aligned manually to related sequences obtained from GenBank using Blast (Version 2.0, National Center for Biotechnology Information; ncbi.nlm.nih.gov/BLAST/). Aligned sequences were imported into the Mega 2.1 program for phylogenetic analysis (Kumar et al., 2001). A neighbor-joining phylogenetic

tree was constructing using a Kimura 2-parameter model for estimating evolutionary distance between sequences.

Table A.1. Oligonucleotide primers used for PCR amplification and sequencing reactions of 16S rDNA from Blood Falls genomic DNA, clones and isolates. M = A or C, R = A or G, Y = C or G.

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Primer uses and name	*Direction*	*Sequence*
PCR and sequencing		
9F	Forward	GAGTTTGATCTGGCTCAG
1492R	Reverse	GGTTACCTTGTTACGACTT
Sequencing		
27F	Forward	AGAGTTTGATCCTGGCTCAG
534R	Reverse	ATTACCGCGGCTGCTGG
926R	Reverse	ACCGCTTGTGCGGGCCC
515F	Forward	GTGCCAGCMGCCGCGGTAA
1068F	Forward	GCATGGCYGYCGTCAG
1391R	Reverse	GACGGGCGGTGTGTRCA

Results

The four isolates were chosen based on depth in the ice cover and enrichment conditions (i.e. aerobic or anaerobic) (Table A.2). Cells of isolates DV1 and DV16 were rod shaped and formed long filaments. Isolate DV17 exhibited dark pink pigmentation when grown in broth and on agar; cells were rod-shaped when viewed under a microscope. Cells of isolate DV4 were coccoid and showed no distinct pigmentation.

Table A.2**.** Characteristics of isolates obtain from Lake Fryxell ice cover samples.

Isolate	***Depth in ice cover (m)***	***Original Enrichment***	***Cell Morphology***	***Pigmentation***	***Bacterial Kingdom***
DV1	1.5	Aerobic	Filamentous rods	None	*β-Proteobacteria*
DV4	1.5	Anaerobic	Cocci	None	*β-Proteobacteria*
DV16	1.7	Anaerobic	Filamentous rods	None	*β-Proteobacteria*
DV17	1.7	Aerobic	Rods	Dark pink	*CFB*

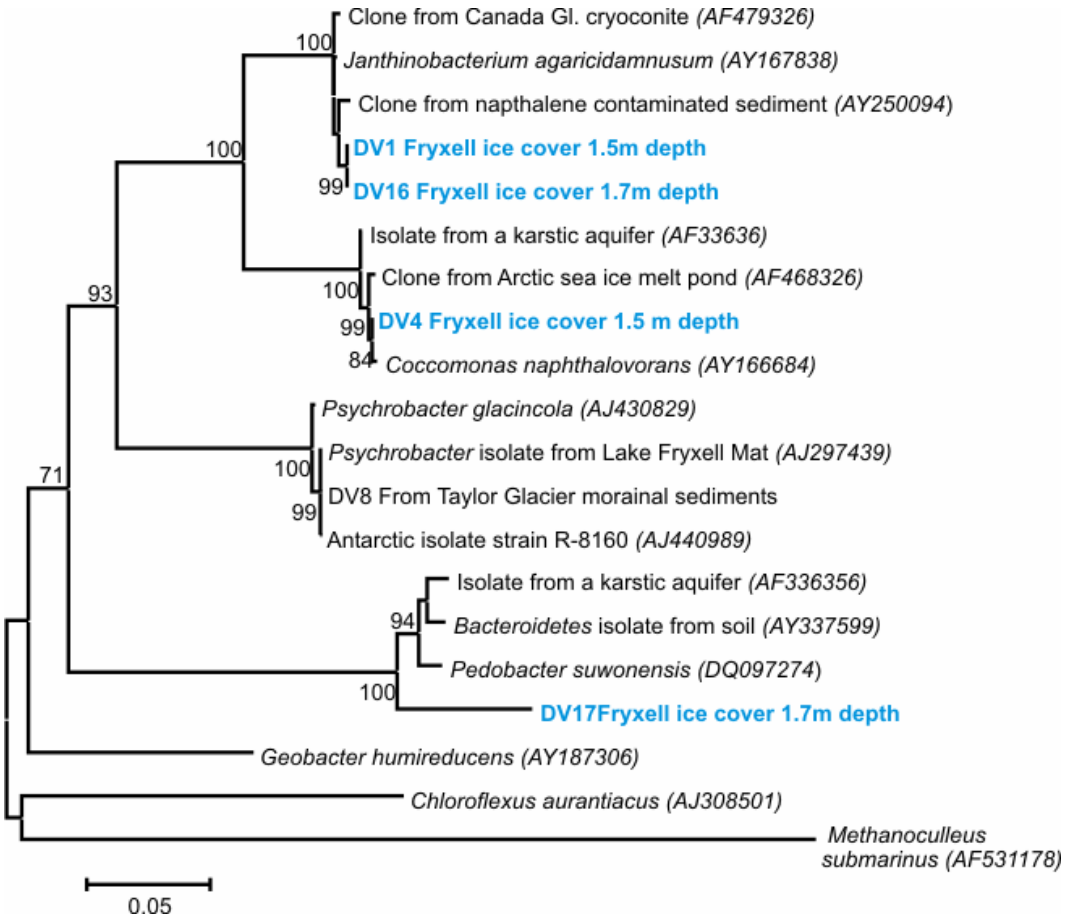


Figure A.6. Neighbor-joining phylogenetic tree of isolates obtained from the permanent ice-cover of Lake Fryxell. Isolates from Lake Fryxell ice cover are highlighted in blue. The scale bar represents 0.05 fixed substitutions per nucleotide position.

Sequence analysis revealed the presence of representatives from two Bacterial kingdoms. The isolates DV1, DV4 and DV16 were members of the *β-Proteobacteria*; DV17 was a member of the *Cytophaga-Flavobacterium-Bacteroides* kingdom (Figure A.7).

Discussion and Conclusions

Three of the four isolates selected for sequence analysis were related to potential naphthalene degrading organisms. Isolates DV1 and DV16 were 98.5% similar to clones from naphthalene contaminated sediments and DV4 shared 99.3% sequence similarity with *Coccomonas naphthalovorans* (Joen et al., 2003)Although it difficult to completely rule out contamination there are multiple lines of evidence that suggests that these isolates are indigenous to the Lake Fryxell ice-cover. The mixed enrichment culture from 1.7 meters demonstrated the ability to produce ice-binding molecules (IBMs) (Raymond, personal communication). IBMs attach to growing ice crystals to prevent structural damage to cellular components (Raymond and Fritsen, 2001). Further, these organisms are all phylogenetically related to isolates from other cold environments such as Antarctic soils (Shivaji et al., 1992) and glaciers (Margesin et al., 2003) including a closely related clone (CanDirty89) obtained from a cryoconite hole sample collected from the Canada Glacier (Christner et al., 2003).

Without a stringent sampling regime and decontamination efforts it would be significantly more difficult to rule out contamination from equipment. Because of the fuel spill the following season, rigor and control during the collection of these particular samples was imperative. Studies on the effect of these hydrocarbon compounds on the natural assemblage in the ice cover and their physical movement through the ice cover are currently underway (Priscu, personal communication). Preliminary results of the physical movement of hydrocarbon fluids in ice suggests that fuel products follow a complex pattern of movement through the ice matrix and that sediment inclusions may enhance transport through the lake cover and into the lake water column (Jepson and

Adams, 2005). The isolation of potential hydrocarbon degraders occurring naturally in the lake ice community is of major importance for these ecological studies and for bioremediation efforts since they indicate the presence of organisms capable of degrading components of the fuel spill under natural conditions.

Collecting Samples from Blood Falls

Introduction

Sample collection and processing from Blood Falls is discussed in detail relevant to the analyses of the data in each chapter and a detailed description of the site is given in Chapter 3. Here, the diverse nature of this feature will be discussed and general sampling procedures presented. Blood Falls has recently been designated as an Antarctic Specially Managed Area (ASMA) by the National Science Foundation. An ASMA designation limits activity and sample collection in specially managed areas. This work marks the first description of the ecology at Blood Falls; therefore it is appropriate to describe the environmental conditions encountered at this feature during the course of sampling for four austral summers (2001-2002, 2002-2003, 2003-2004 and 2004-2005). The following section is a field-based description of such environmental conditions.

Taylor Glacier's Subglacial Brine. Drilling through the Taylor Glacier has yet to occur but strong inference about this subglacial ecosystem can be made from outflow waters collected at the glacier snout. Blood Falls forms at the northern terminus of the Taylor Glacier, where subglacial fluid meets the surface. This outflow is a cold (-6º C) brine high in dissolved iron and sodium chloride with a conductivity approximately 2.5

times seawater (105 mS cm^{-1}). When this anaerobic water meets the atmosphere at the surface, iron and salts rapidly oxidize and precipitate forming a salt cone or crust. (Chapter 4 discusses the geochemistry of Blood Falls in detail). As the austral summer progresses, fresh surface glacial melt and flow from the Santa Fe Creek, which converges with Blood Falls at the moat of the west lobe of Lake Bonney, acts to dissolve the salt cone and dilute any subglacial outflow.

Sample collection at Blood Falls

Blood Falls was sampled for biological and geochemical analyses using a variety of methods during four austral summers (2001-2005). Subglacial outflow at Blood Falls is dynamic; each visit to the site presents a changing, unique set of sampling challenges and one must be prepared for a diversity of conditions that can range from frozen to a rapidly flowing stream of subglacial brine. Frozen conditions require drilling and/or chipping to collected frozen samples or the liquid below frozen ice, while outflow that is partially frozen (i.e. slushy ice) requires some form of sterile scrapper to collect slush. Low flow or ponding requires the use of a peristaltic pump with clean, acid-washed tubing to collect sufficient sample, and, during times of high flow rates, sterile sample collection bottles can be held directly to the falls and than transferred into other sterile sampling vessels. At the immediate ice interface, at the terminus wall, slush and icicles form (Figure A.7 A, B, C). Further downstream a salt and iron crust forms over sediments on the terminal moraine material (Figure A.7 D, E). The salt cone eventually fans out over the seasonal moat ice (Figure A.8).

Figure A.7. Diversity of sampling conditions present at Blood Falls outflow. Slushy ice and outflow mix (A), iron precipitates in ice (B) active outflow over slush (C), arrow points to tool (≈ 10 cm), iron and salt precipitates (D) salt precipitates (E).

Figure A.8. Outflow from Blood Falls on 5 Nov 2003. Outflow has fanned out over the moat area of the lake.

When subglacial fluid was actively flowing, samples were collected by holding autoclaved collection vessels underneath the water flow to capture the brine waters without contamination (Figure A.9 A). The vessels were rinsed three times with sample before collection of the final sample. Waste wash was discarded in a separate grey water waste bucket. Sample collection involved chipping ice or scraping and scooping slush ice and/or sediments when flow was not active (Figure A.9 B,C). Instruments for chipping and scraping were wrapped in aluminum foil and autoclaved, removing foil only at the time of collection. This method has some drawbacks as larger tools cannot be autoclaved and smaller instruments will eventually be contaminated by handling. If the Blood Falls outflow was flowing under the seasonal moat ice it was necessary to drill through the moat ice (up to 3 meters of ice) to open a hole for collection, brine fluid was then collected with a Niskin bottle (Figure A.9 D). Subglacial outflow may also pond

near the surface of the moat by the falls; collecting water from these small ponds or puddles (Figure A.9 E) was achieved by using a peristaltic pump with clean, acid washed tubing. Sample water was pumped through the tubing into a waste water bucket for several minutes to further clean the tubing before collecting the actual sample.

Blood Falls Sample Integrity

Any samples collected indirectly from the subglacial outflow source will inevitably be diluted with moat water and stream flow. Geochemical analysis is a clear indicator of the extent to which the water may have been altered from the subglacial source; more detail on the geochemical indicators of ancient outflow are described in Chapter 4.

Figure A.9. Diverse modes of sample collection at Blood Falls. When primitive flow was active, samples were collected directly into sterile containers (A), ice was chipped with sterile tools (B) or removed as icicles (C). When outflow occurred below the ice, the ice was drilled and samples were collected with a Niskin bottle (D). Slow, ponding outflow was collected with a peristaltic pump and tubing (E).

Sample Collection Conclusions

There is mounting evidence that life is active at temperatures well below freezing, to -20º C (Carpenter et al., 2000, Christner, 2002, Rivkina et al., 2000, Junge et al., 2004, Bakermans, et al., 2003). And environments such as deep glacial ice and other frozen environments are gaining acceptance as an important component of the Earth's biosphere (Priscu and Christner, 2004). Plans are underway to investigate Lake Vostok and other Antarctic subglacial Lakes (Priscu et al., 2003). The scientific value of unaltered, clean samples from Lake Vostok, a system potentially isolated from the atmosphere for greater than 15 million years, are significant. Exobiological targets of investigation in our solar system such as Mars and Europa are cold, icy places, samples collected from these systems will require meticulous contamination prevention protocols (NRC, 2005). While we pursue these protocols on Earthly samples; we should be applying planetary protection issues in sample collection protocols (Crawford, 2005, NRC, 2005). Given the tremendous value of samples from places like Lake Vostok, closed subglacial systems and other planets, every precaution must be taken to ensure sample integrity.

APPENDIX B

PRODUCTION OF "LIVING ICE": THE IMPORTANCE OF OUTREACH AND EDUCATION IN THE POLAR SCIENCES

APPENDIX B

PRODUCTION OF "LIVING ICE": THE IMPORTANCE OF OUTREACH AND EDUCATION IN THE POLAR SCIENCES

Abstract

The Big Sky Institute for Science and Natural History (BSI) promotes the distribution of science-based knowledge related to natural ecosystems and the human communities that depend on them. BSI awards Science and Society Fellowships semi-annually to Montana State University graduate students to promote outreach activities at the graduate level. This award aims to provide students with materials, training and opportunities to communicate science to the public at large. "Living Ice," a documentary film and laboratory activity, was the resultant educational outreach project from a BSI Fellowship (http://bsi.montana.edu/web/web). The curriculum was designed around the federal science standards (www.nist.gov) and targeted high school biology students. The hope was that an easy to use curriculum would assist high school science teachers by providing them with current, exciting tools for addressing these national standards. The McMurdo Long Term Ecological Research (LTER) limnology field team was filmed during the 2002-2003 Antarctic field season. A local Bozeman high school teacher and members of the media arts program on the MSU campus were recruited to help mold video, data and commentary into a short documentary film and laboratory experiment.

The final deliverable was called "Living Ice." Living Ice has been broadly disseminated throughout United States high schools and has received numerous positive reviews.

Introduction

Polar ecosystems play a significant role in global scale phenomena, and polar biologists are urged by the National Research Council (NRC) to communicate their discoveries widely and rapidly to the public (NRC, 2003). Despite the importance of cold environments on Earth, many textbooks do not include frozen systems in their descriptions of the biosphere (Priscu and Christner, 2004). Recommendations from the Committee of Frontiers in Polar Biology of the Polar Research Board include targeting the K-12 audience in educational and outreach efforts (NRC, 2003). Specifically, the NRC recommends expanding the coverage of polar topics in textbooks. To maintain the momentum of discovery in polar ecology, the next generation of polar scientists needs to be inspired and recruited. "Living Ice" is a film (Figure B.1) and activity designed to excite students about microbiology, Antarctic research and the importance of microbes that inhabit frozen environments.

Figure B.1. Title film clip for “Living Ice” documentary film.

The Priscu laboratory is in charge of microbial ecology studies for the National Science foundation funded Long Term Ecology Research (LTER) site in the McMurdo Dry Valleys (MCM) of Antarctica. Dr. Priscu deploys a team of scientists each austral summer (October - January) to conduct routine monitoring of the lakes in the MCM. This team typically includes one or more graduate student(s) working simultaneously on their dissertation.

The LTER Antarctic field season offers an excellent opportunity to present discovery-based science and the Ph.D. process to students. The documentary film produced for this project follows the limnology team in the field, describing their research and the “bigger picture” of the scientific questions they pursue.

The Big Sky Institute (BSI) is a research and education center that connects the public with science conducted by Montana State University (MSU) researchers working on the Yellowstone Ecosystem and ecosystems worldwide. A BSI goal is to teach graduate students how to communicate their research to the general public. The Science

and Society Fellowships were designed to provide graduate students with training and funding to construct public outreach projects based on their graduate research. Fellowship projects have helped to fulfill the BSI outreach mission by creating "learning-tool legacies" (www.bsi.montana.edu).

The "Living Ice" project was designed to meet numerous outreach goals (Table B.1 and Table B.2). The primary goal was to make a cost effective, accessible deliverable that could be used by a broad range of schools. Teachers are required to address the National Science Standards put forth by the federal government (www.nist.gov), therefore, standards addressed by this project are listed within the laboratory activity (Figure B.3).

Table B.1. Goals for the film component of the "Living Ice" project.

	Film goals:
1.	Create a film that was synergistic with the activity and curriculum
2.	Excite students about field research, microbiology and Antarctic science
3.	Easy to use for students and teachers
4.	Show 'how we know what we know'- explain the current scientific knowledge and how it was developed
5.	Answer the "So what?" q- must demonstrate why Antarctic research is important to the average person
6.	Demonstrate how microbiology plays a vital role in the advancement of scientific knowledge
7.	Introduce students to ice as an ecosystem and subjects such as Astrobiology
8.	Emphasize MSU science and the BSI mission
9.	Emphasize women as scientists
10.	Introduce students to the Ph.D. process
11.	Show the challenges of working in Antarctica

Table B.2. Goals for the laboratory activity component of the "Living Ice" project.

	Laboratory activity goals:
1.	Introduce students to the importance of cold environment microbiology
2.	Easy to use for students and teachers
3.	Provide a laboratory activity that demonstrates the ability of microorganisms to survive freezing
4.	Provide students with a platform for designing their own experimental protocol and generating logical scientific hypotheses
5.	Have students reflect on the broader implications of their experiment (i.e. medical, environmental, and exobiological implications)
6.	Address national science standards for high school level biology students
7.	Provide links for students and teachers to additional web materials on microbes and Antarctica

Methods

Filming and Equipment

Digital filming equipment was purchased for recording in Antarctica. Supplies for this project were minimal and consisted primarily of a hand held Sony MiniDV Handycam Camcorder a Sony external microphone and MiniDV tapes. All "on location" or field footage for "Living Ice" was filmed during the 2002-2003 Antarctic Field Season. A detailed film plan of desired shots was generated prior to deployment and was loosely followed as shots presented themselves. To complete the story additional footage was obtained on the MSU campus including interviews, laboratory shots and still shots. Additional footage was obtained during the summer of 2003 with the assistance of a student from the Media Arts program at Montana State University.

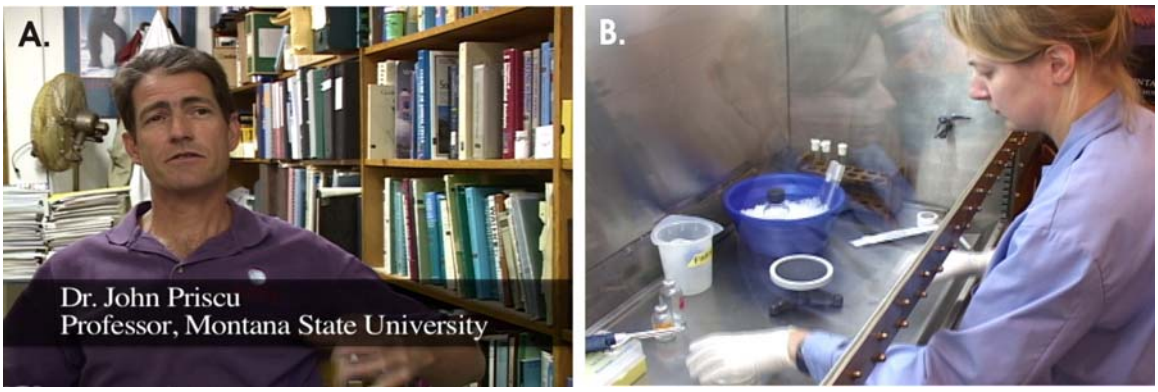


Figure B.2. Film clips of interview with Dr. John Priscu (A.) and laboratory action shot (B.).

Script Design

A script was constructed to tell the story of an Antarctic field team. Woven into the story was discussion of the scientific questions the field team was asking and a discussion of the Ph.D. process. The goals listed in Tables B.1 and B.2 were used as a guideline for the script.

Film Editing

Approximately nine hours of film footage taken in the field was cataloged and copied. A graduate student from the Media Arts program at Montana State University, Chris Kustash, was hired to edit the "Living Ice" film in partial fulfillment of his Masters degree requirements. All editing was conducted on the MSU campus in conjunction with the Natural History of Filmmaking Masters program (naturefilm.montana.edu).

Curriculum

Paul Anderson, Bozeman High School Advanced Placement Biology teacher, was recruited for expert input on high school laboratories activities. Together we designed a high school laboratory activity that met all described goals. The curriculum was

produced and converted to a pdf file and accompanies all copies of the movie "Living Ice" (Figure B.3).

Distribution and Promotion

The BSI was in charge of promotion and distribution of the "Living Ice" educational package. The package was promoted at science meetings and high schools nationwide.

Discussion

"Living Ice" presented the opportunity, at the graduate student level, to disseminate the importance of my science to the general community. There is a need in every field to excite the next generation of specialists. Not only did the "Living Ice" project address this need, it served as the foundation for future projects that will following the same design. The BSI was able to use the "Living Ice" project as a model for the recently funding NSF Graduate Teaching Fellows in GK-12 Education project; institutionalizing the role of graduate students in outreach and education (ww.bsi.montana.edu).

MSU hosted a meeting of the Nations Science Teachers Association, with representative teachers from each state. A "Living Ice" package was given to all attendees. "Living Ice" was designed to be a tool for high school teachers. The "Living Ice" tool is easy to use and inexpensive, maximizing the number of school districts able to utilize the product as a teaching resource. Several rural high schools in Montana have implemented "Living Ice" in their biology programs. Follow-up on the success of the

project in one of these high schools in Joliet, MT gave me the opportunity to interact with students first hand; captivating young minds is a truly rewarding experience.

Conclusions

Education and outreach is an integral component of all scientific research. Outreach and education at the graduate level can take advantage of a graduate student's enthusiasm for their research, while strengthening their interest in it by learning how to share their science with the public. Producing "Living Ice" has demonstrated that highly effective outreach tools can be generated with a minimal budget. As scientists make discoveries, they are obliged to convey that information to the public in a timely manner. To accomplish this, scientists must learn how to effectively communicate their science to a variety of audiences. Outreach projects undertaken early in one's career, such as at the graduate student level, will help to develop a life-long culture of outreach.

Figure B.3. Companion activity to the "Living Ice" documentary film.

Pond Water Popsicles

Background: Life as we know it requires liquid water, yet the Earth is a cold place. 90% of the biosphere on this planet is cold ocean <5°C, 70% of the Earth's fresh water is frozen and 20% of the surface soil is permafrost. There are many environments that undergo seasonal freezing such as the lakes and sediments at middle to high latitudes. Other environments, such as Antarctic glaciers and Russian permafrost remain permanently frozen year round. What happens to organisms that live in these environments? Does freezing damage their cells or prevent growth? Do they remain dormant and wait for warmer times? There are organisms that not only survive extremely low temperatures they love it. Psychrophiles (*psychro* is the Greek word for cold, *phile* means 'loving') are organisms that grow fastest at temperatures below 20°C (68°F). In fact, at temperatures above 20°C these organisms die. Other organisms can be classified as psychrotolerant, meaning they can grow slowly near freezing or survive freezing without growth, but overall prefer warmer temperatures.

When ice forms, the crystals can puncture and damage cell walls, therefore, many organisms that inhabit frozen environments produce protection against this type of damage in the form of antifreezes. Such organisms can secrete antifreeze molecules including proteins that bind to ice preventing further crystal formation or exopolymers that form a protective mucus-like layer around the cell. It could be that because of these cryoprotectants, psychrophilic organisms can actively grow at temperatures below the freezing point of water.

Lucky for us, not all microbes fare so well under freezing conditions. The process of sticking foods in the freezers halts most microbial activity, keeping foods fresh when thawed. It is important to realize that this freezing does not always destroy microbes and thawed meats must still be cooked thoroughly.

Exobiology (popularly known as Astrobiology), or the study of life on other planets is an exciting aspect of cold temperature microbiology. The prospective habitable planets in our solar system are very cold; understanding the survival strategies used by cold-loving microbes here on Earth will improve our quest for life beyond our home planet. This activity's companion film titled, *Life in Ice*, from the Big Sky Institute will excite students about microbiology and the challenges of discovering life in Antarctica and other extreme environments (Order film at www.bsi.montana.edu).

Target Audience: High School Biology
Advanced / AP Biology

Skills:

- Use of a **compound microscope**
- Prepare a **wet mount**
- **Heat-fix** and **stain** microorganisms
- **Quantify abundance** of organisms

Objectives:

Prepare a wet mount of pond water
Demonstrate proper technique in preparing a stain
Survey life both qualitatively and quantitatively.
Design an experiment to answer one of the following problems:

- **How does temperature affect the growth of microorganisms?**
- **How does freezing time affect the growth of organisms?**
- **How does glucose affect the growth of frozen organisms?**

Film Time: *Life in Ice*, from the Big Sky Institute, 20 minutes

Activity Time: Two 50-minute sessions or one and one-half 90-minute sessions

Materials: Have the following for each group of 2-3 students:

1 compound microscope	3 containers or beakers
4 microscope slides	1 refrigerator/freezer
2 microscope cover slips	3 thermometers
4 microbiology loops	200 ml glucose solution
1 Bunsen burner or matches	1 clothespin
1 bottle methylene blue	1 dissecting needle
1 ice cube tray	

Web Links:

http://www.bsi.montana.edu/ Order the companion film titled, *Life in Ice*, from the Big Sky Institute. This film will excite students about microbiology and the challenges of discovering life in Antarctica and other extreme environments.

http://www.microbeworld.org/ This site is one-stop-shopping for information on microbes. Their 'For Educators' link brings you to the ASM microbiology library with curriculums for a wide variety of microbiology related topics.

http://astrobio.terc.edu/ The Astrobiology Education website provides a curriculum titled, *Are There Habitable Worlds Elsewhere?, Activity 1*, that provides a nice 'thinker' follow-up to the *Pond Water Popsicle* lab exercise.

http://www.nsf.gov/od/opp/ This site is the homepage for the National Science Foundation's Office of Polar Programs (OPP), which describes the various scientific disciplines conducting research in Antarctica.

http://huey.colorado.edu/77DegreesSouth/index.html This site is hosted by the McMurdo Dry Valley Long Term Ecology Research team (LTER), which provides maps of Antarctica and additional web-page links. The best part of this site is the field team journal.

http://schoolyard.lternet.edu/ The Schoolyard LTER site provides a list of all the LTER sites currently conducting research. The 'For Educators' link lists opportunities for educators who would like to become more involved with LTER.

http://www.homepage.montana.edu/~lkbonney/ Dr. John Priscu's homepage thoroughly describes the research presented in the companion film, *Life in Ice*. This web site also offers a page of great web links for cold environment enthusiasts.

National Standards and the Activity-Objective Connections:

National Science Education Standards	Prepare a wet mount	Prepare a stain	Survey life	Design an experiment
Unifying Concepts and Processes				
Systems, order, and organization			✓	✓
Evidence, models, and explanation				✓
Change, constancy, and measurement				✓
Form and function			✓	✓
Science as Inquiry				
Abilities necessary to do scientific inquiry			✓	✓
Understanding about scientific inquiry			✓	✓
History and Nature of Science				
Science as a human endeavor			✓	✓
Nature of science	✓	✓	✓	✓
Physical Science				
Properties and changes of properties in matter				✓
Life Science				
Structure and function in living systems	✓	✓	✓	✓
Regulation and behavior			✓	✓
Populations and ecosystems			✓	✓
Diversity and adaptations of organisms			✓	✓
Science and Technology				
Understanding about science and technology	✓	✓	✓	✓
Science in Personal and Social Perspectives				
Personal health			✓	✓
Populations, resources and environments			✓	✓

National Science Education Standard, (1996), National Academy Press: Washington, D.C.

Experimental Design

Hypothesis:

Develop a hypothesis based on one of the following questions.
Your instructor may allow you to develop a hypothesis based on another question that your group has decided upon.

Question: Check One √

- ❑ How does **temperature** affect the growth of microorganisms?
- ❑ How does freezing **time** affect the growth of microorganisms?
- ❑ How does **glucose** concentration affect the growth of frozen microorganisms?
- ❑ Other:

Hypothesis: _____

_____.

Variables:

Independent variable (units): _____

Dependent variable (units): _____

Procedure:

Detail the procedure you will use to solve the above problem. You should use the laboratory techniques listed to analyze your samples before and after treatment. Data should be recorded on the data sheet. Only manipulate one variable in your experiment.

Your experiment must include a control group that is left unchanged as well as two other groups that are manipulated.

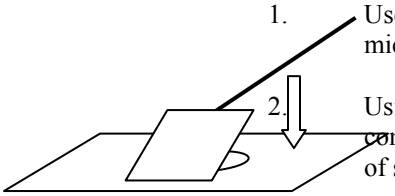
1.
2.
3.
4.
5.
6.
7.
8.
9.
10.

Procedure Approval: _____
(Supervisor's signature)

Laboratory Techniques

Observation:

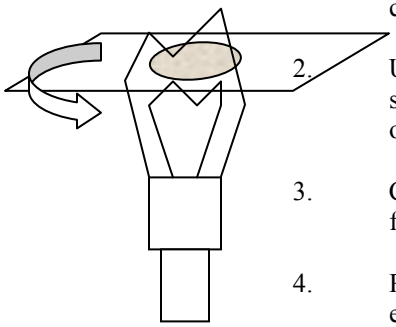
Qualitative – Wet Mount



1. Use a dropper pipette to place one drop of pond water on a clean microscope slide.
2. Using a dissecting needle angle and lower a cover slip to completely cover the water without trapping any air bubbles. The type of slide you have just created is a **wet mount**.
3. Place the wet mount on the stage of a compound microscope and use the low-power objective to focus on the sample.
4. Scan the slide for several minutes looking for evidence of life.
5. Give an overview of the life present in the drop of water in the space provided on the data sheet.
6. Choose **four** individual microorganisms and draw them in the space provided on the data sheet.

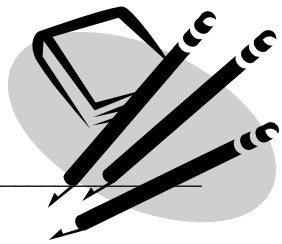
wet mount

Quantitative - Heat Fixation / Staining



1. Use a microbiology loop to place one drop of pond water on a clean slide. Use the pipette to spread the drop over an area that is approximately as large as a quarter. Allow the water to air dry completely.
2. Use a clothespin to hold the slide at one end. Quickly pass the slide three times through a Bunsen burner flame. Place the slide on a table and allow it to cool.
3. Cover the slide with several drops of methylene blue. Let the slide sit for 60 seconds.
4. Rinse the slide gently with distilled water. Tilt the slide to remove excess water and allow the slide to air dry completely.
5. Place the slide on the stage of a compound microscope. Use the low-power objective to focus on the sample. Switch to a high-power objective (preferably 1000X which will require immersion oil) and use the fine adjustment to focus the sample.
6. Count the number of cells within one field of view. Move the slide to two other fields of view and count the number of cells. Record these values as well as the average on the data sheet.

Data Sheet



Qualitative Analysis

Descriptive: Pond water _____

_____

Control _____

_____

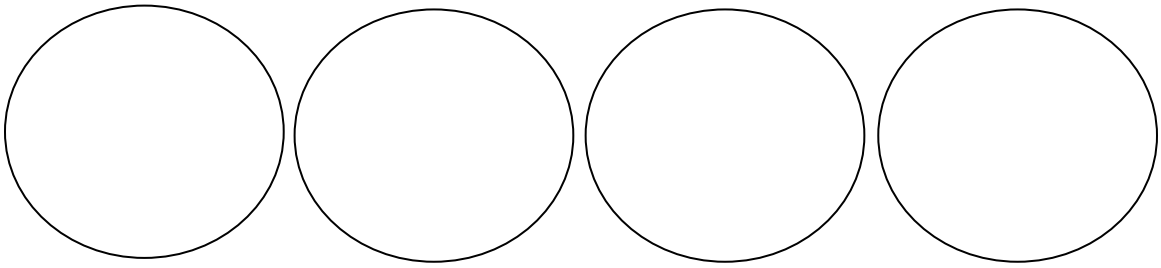
Treatment 1 _____

_____

Treatment 2 _____

_____

Pictures: Draw pictures of **four** microorganisms found in each sample.



Pond Control Treatment 1 Treatment 2

Quantitative Analysis

	Pond water	Control	Treatment 1	Treatment 2
Count 1				
Count 2				
Count 3				
Average				

Conclusions

After completing the experiment answer the following questions as a group.

1. Did you find your hypothesis to be correct? Be sure to use experimental evidence to support your claim. Discuss your control group in this section.

2. If the results were not what you expected what could have gone wrong with your experiment?

3. How could the procedure be improved if you were to perform this experiment again?

4. What are some of the implications of the results of your study in the following areas? Please refer to the **Background** section for more information.

   Ecological?

   Medical?

   Life on other planets?

APPENDIX C

MEDIA PREPERATIONS

APPENDIX C

MEDIA PREPERATIONS

Iron Reducer Medium

Iron-reducer medium (Table C.1) was adapted from MS media (Boone et al., 1989). This broth medium was prepared to enrich for organisms that use Fe-OOH as an electron acceptor, H_2 as an electron donor and CO_2 as a carbon source.

Table C.1. Iron-reducing (Fe-Rd) medium ingredients.

Ingredient	*Per L*
Sodium hydroxide	4.0 g
Ammonium chloride	1.0 g
Postassium dibasic phosphate trihydrate	0.4 g
Magnesium chloride hexahydrate	1.0 g
Calcium chloride dehydrate	0.4 g
Sodium EDTA dehydrate	0.1 g
Cobalt (I) chloride hexahydrate	28.8 g
Manganous chloride tetrahydrate	1.0 mg
Ferrous sulfate heptahydrate	1.0 mg
Zinc chloride	1.0 mg
Alumium chloride hexahydrate	0.4 mg
Sodium tungstate dehydrate	0.3 mg
Cupric chloride dehydrate	0.2 mg
Nickel (II) sulfate hexahydrate	0.2 mg
Selenous acid	0.1 mg
Boric acid	0.1 mg
Soidum molybdate dehydrate	0.1 mg
Reazurin (0.1% w/v)	1.0 mg
Fe-OOH sludge	2 ml

The sodium hydroxide was dissolved and equilibrated with CO_2 gas. The gas was made oxygen-free by first flushing gas through a copper catalyst and using butyl rubber

tubing, which has low oxygen permeability, to deliver the gas to the medium. Sodium hydroxide buffer was equilibrated before any other ingredients were added. Amorphous iron (III) oxide was prepared by neutralizing a 0.4 M solution of $FeCl_3 \bullet 6H_2O$ with 1 M NaOH and washing with distilled water. The Fe(III) gel was stored cold (4º C) and added to the bulk media by sterile pipette (Lovley and Phillips, 1986). Medium was mixed with a magnetic stirrer as it was dispensed (5 ml) anaerobically into CO_2-spared, 10 ml serum vials. The medium was crimp-sealed with butyl rubber stoppers and autoclaved (30 min). All media was pre-chilled (2º C) prior to inoculation.

Thiosulfate-oxidizer Medium

Agar plates were prepared enrich for organisms able to use thiosulfate as an electron donor (Table C.2). However, organisms capable of using agar for growth could also be enriched with these plates.

Table C.2. Thiosulfate oxidizing (S-Ox) media ingredients.

Ingredient	*Per L*
Ammonium chloride	0.2 g
Postassium dibasic phosphate trihydrate	0.4 g
Potassium Dihydrogen Phosphate	0.1 g
Magnesium chloride hexahydrate	0.2 g
Calcium chloride dehydrate	0.04 g
Vitamin solution	1 ml
Trace mineral solution	1ml
Agar	15 g

Vitamin (cat. no. MD-VS) and trace mineral (cat. no. MD-TMS) solutions were purchased from the American Type Culture Collection (ATCC). Thiosulfate (3 mM final concentration) was added (10 ml) from a 300 mM stock solution. The media was tested

for pH (final pH = 7) and adjusted with NaOH (0.5 M), autoclaved, and aseptically transferred into Petri dishes. All media was pre-chilled (2º C) prior to inoculation.

APPENDIX D

DISTANCE MEASURES

APPENDIX D

DISTANCE MEASURES

Table D.1. Pearson's correlation values of similarity between geochemical parameters of each transect sample point (BFT1-17) and Blood Falls outflow samples from ancient flow (2004) and contemporary melt (2003).

Case	Correlation between Vectors of Values																		
	BF-03BA	BFT1	BFT2	BFT3	BFT4	BFT5	BFT6	BFT7	BFT8	BFT9	BFT10	BFT11	BFT12	BFT13	BF14	BFT15	BFT16	BFT17	BF-04
BF-03	1.00	.885	.476	-.283	-.693	-.700	-.763	.451	.054	-.703	-.754	-.791	.737	.458	-.702	-.663	-.804	-.804	-.528
BFT1	.885	1.00	.817	.007	-.743	-.740	-.822	.796	.360	-.748	-.904	-.876	.953	.797	-.743	-.831	-.895	-.871	-.757
BFT2	.476	.817	1.00	.419	-.520	-.506	-.592	.979	.688	-.517	-.793	-.713	.942	.994	-.510	-.787	-.734	-.699	-.737
BFT3	-.283	.007	.419	1.00	.553	.566	.463	.315	.932	.552	.069	.292	.254	.499	.558	-.150	.246	.303	-.398
BFT4	-.693	-.743	-.520	.553	1.00	1.00	.987	-.598	.248	.999	.803	.924	-.619	-.438	.999	.590	.902	.916	.322
BFT5	-.700	-.740	-.506	.566	1.00	1.00	.987	-.585	.261	.999	.800	.924	-.611	-.424	.999	.585	.902	.917	.309
BFT6	-.763	-.822	-.592	.463	.987	.987	1.00	-.647	.137	.990	.879	.962	-.707	-.520	.989	.692	.951	.952	.412
BFT7	.451	.796	.979	.315	-.598	-.585	-.647	1.00	.582	-.594	-.762	-.745	.902	.956	-.587	-.696	-.749	-.734	-.642
:BFT8	.054	.360	.688	.932	.248	.261	.137	.582	1.00	.246	-.267	-.056	.583	.756	.254	-.448	-.105	-.047	-.612
:BFT9	-.703	-.748	-.517	.552	.999	.999	.990	-.594	.246	1.00	.809	.929	-.620	-.435	1.000	.596	.908	.920	.321
BFT10	-.754	-.904	-.793	.069	.803	.800	.879	-.762	-.267	.809	1.00	.928	-.889	-.761	.807	.949	.955	.909	.661
BFT11	-.791	-.876	-.713	.292	.924	.924	.962	-.745	-.056	.929	.928	1.00	-.819	-.657	.928	.779	.996	.998	.433
BFT12	.737	.953	.942	.254	-.619	-.611	-.707	.902	.583	-.620	-.889	-.819	1.00	.937	-.615	-.871	-.846	-.812	-.786
BFT13	.458	.797	.994	.499	-.438	-.424	-.520	.956	.756	-.435	-.761	-.657	.937	1.00	-.429	-.784	-.685	-.645	-.759
BFT14	-.702	-.743	-.510	.558	.999	.999	.989	-.587	.254	1.000	.807	.928	-.615	-.429	1.00	.594	.907	.919	.316
BFT15	-.663	-.831	-.787	-.150	.590	.585	.692	-.696	-.448	.596	.949	.779	-.871	-.784	.594	1.00	.832	.756	.748
BFT16	-.804	-.895	-.734	.246	.902	.902	.951	-.749	-.105	.908	.955	.996	-.846	-.685	.907	.832	1.00	.991	.491
BFT17	-.804	-.871	-.699	.303	.916	.917	.952	-.734	-.047	.920	.909	.998	-.812	-.645	.919	.756	.991	1.00	.402
BF-04	-.528	-.757	-.737	-.398	.322	.309	.412	-.642	-.612	.321	.661	.433	-.786	-.759	.316	.748	.491	.402	1.0

Table D.2. Jaccard measures based on presence/absence of TGGE bands in PCR amplified DNA of environmental samples from a transect from Blood Falls across WLB, Blood Falls outflow during ancient discharge (BF-99), and contemporary melt (BF-03).

Case	Jaccard Measure																		
	BF-99	BF-03	BFT1	BFT2	BFT3	BFT4	BFT5	BFT6	BFT7	BFT8	BFT9	BFT10	BFT11	BFT12	BFT13	BF14	BFT15	BFT16	BFT17
BF-99	1.000	.231	.310	.323	.321	.267	.400	.360	.333	.235	.308	.259	.286	.290	.286	.320	.320	.226	.212
BF-03	.231	1.000	.529	.447	.417	.368	.361	.500	.385	.514	.371	.333	.351	.459	.316	.382	.343	.444	.385
BFT1	.310	.529	1.000	.607	.323	.400	.444	.520	.467	.438	.407	.407	.538	.375	.333	.370	.480	.500	.467
BFT2	.323	.447	.607	1.000	.419	.500	.556	.464	.679	.441	.414	.464	.593	.516	.483	.379	.379	.500	.516
BFT3	.321	.417	.323	.419	1.000	.577	.520	.480	.536	.452	.480	.423	.500	.593	.500	.333	.286	.323	.344
BFT4	.267	.368	.400	.500	.577	1.000	.560	.407	.571	.394	.407	.357	.429	.467	.481	.321	.370	.313	.375
BFT5	.400	.361	.444	.556	.520	.560	1.000	.458	.577	.387	.400	.458	.480	.414	.480	.417	.417	.393	.414
BFT6	.360	.500	.520	.464	.480	.407	.458	1.000	.379	.400	.545	.545	.440	.429	.333	.435	.435	.407	.333
BFT7	.333	.385	.467	.679	.536	.571	.577	.379	1.000	.412	.481	.429	.615	.586	.556	.500	.444	.571	.643
:BFT8	.235	.514	.438	.441	.452	.394	.387	.400	.412	1.000	.355	.355	.375	.548	.571	.323	.414	.438	.412
:BFT9	.308	.371	.407	.414	.480	.407	.400	.545	.481	.355	1.000	.700	.440	.481	.440	.375	.375	.310	.333
BFT10	.259	.333	.407	.464	.423	.357	.458	.545	.429	.355	.700	1.000	.565	.481	.440	.375	.375	.357	.379
BFT11	.286	.351	.538	.593	.500	.429	.480	.440	.615	.375	.440	.565	1.000	.556	.462	.346	.400	.538	.556
BFT12	.290	.459	.375	.516	.593	.467	.414	.429	.586	.548	.481	.481	.556	1.000	.750	.345	.300	.419	.438
BFT13	.286	.316	.333	.483	.500	.481	.480	.333	.556	.571	.440	.440	.462	.750	1.000	.346	.400	.379	.400
BFT14	.320	.382	.370	.379	.333	.321	.417	.435	.500	.323	.375	.375	.346	.345	.346	1.000	.600	.542	.444
BFT15	.320	.343	.480	.379	.286	.370	.417	.435	.444	.414	.375	.375	.400	.300	.400	.600	1.000	.609	.500
BFT16	.226	.444	.500	.500	.323	.313	.393	.407	.571	.438	.310	.357	.538	.419	.379	.542	.609	1.000	.760
BFT17	.212	.385	.467	.516	.344	.375	.414	.333	.643	.412	.333	.379	.556	.438	.400	.444	.500	.760	1.000

REFERENCES

1992. Standards Methods for the Examination of Water and Wastewater, 18th ed.

Abraham, W.-R., C. Strompl, H. Meyer, S. Lindholst, E. R. B. Moore, R. Christ, M. vancanneyt, B. J. Tindall, A. Bennasar, J. Smith, and M. Tesar. 1999. Phylogeny and polyphasic tazonomy of *Caulobacter* species. Proposal of *Maricaulis* gen. nov. with *Maricaulis maris* (Poindexter) comb. nov. as the type species, and emended description of the genera *Brevundimonas* and *Caulobacter*. International Journal of Systematic and Evolutionary Bacteriology 49.

Abyzov, S. S., I. N. Mitskevich, and M. N. Poglazova. 1998. Microflora of the Deep Glacier Horizons of cnetral Antarctica. Microbioly 67:547-555.

Adams, E. E., J. C. Priscu, C. H. Fritsen, S. R. Smith, and S. L. Brackman. 1998. Permanent Ice Covers of the McMurdo Dry Valley Lakes, Antarctica: Bubble Formation and Metamorphism, p. 281-295. *In* J. C. Priscu (ed.), Ecosystem Dynamics in a Polar Desert: The McMurdo Dry Valleys, Antarctica, vol. 72. American Geophysical Union, New York.

Alexander, S. P., and W. L. Stockton. 2003. Lake Fryxell 79U crash site: Initial hydrocarbon monitoring program. Technical report produced by: Raytheon Polar Services.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403-410.

Anderson, T. M., S. J. McNaughton, and M. E. Ritchie. 2004. Scale-dependent Relationships between the spatial distribution of limiting resource and plant species diversity in an African grassland ecosystem. Oecologia 139.

Angino, E. E., K. B. Armitage, and J. C. Tash. 1964. Physiochemical limnology of Lake Bonney, Antarctica. Limnology and Oceanography 9:207-217.

Atlas, R. M. 1984. Diveristy of microbial communities. Advances in Microbial Ecology 7:1-47.

Atlas, R. M. 1992. Detection and Enumeration of Microorganisms Based on Phenotype, p. 29-43. *In* M. A. Levin, R. J. Seidler, and M. Rogul (ed.), Introduction to Microbial Ecology Principles, Methods and Applications. McGraw-Hill, Inc, New York.

Atlas, R. M., A. Horowitz, M. Krichevsky, and A. K. Bej. 1991. Response of Microbial Populations to Environmental Disturbance. Microbial Ecology 22:249-256.

Bakermans, C., A. I. Tsapin, V. Souza-Egipsy, D. A. Gilichinsky, and K. H. Nealson. 2003. Reproduction and metabolism at 10°C of bacteria isolated from Siberian permafrost. Environmental Microbiology 5:321-326.

Barnes, R. S. K. 1984. Estuarine Biology, 2nd ed. Edward Arnold, Cambridge.

Benn, D. I., and D. J. A. Evans. 1998. Glaciers and Glaciation. Oxford University Press Inc., New York.

Black, R. F., M. L. Jackson, and T. E. Berg. 1965. Saline discharge from Taylor Glacier, Victoria Land, Antarctica. Journal of Geology 74:175-181.

Black, R. F., and C. J. Bowser. 1968. Presented at the Reports and Discussions International Association of Scientific Hydrology, General Assembly of Gern, 25 September 1967.

Blankenship, R., M. T. Madigan, and C. Bauer. 1996. Anoxygenic photosynthetic bacteria. Chapter 13, The Prokaryotes. Kluwer, Boston.

Block, W. 1984. Terrestrial microbiology, invertebrates and ecosystems, p. 163-236. *In* R. M. Laws (ed.), Antarctic Ecology, vol. 1. Academic Press Inc., London.

Bottrell, S. H., and M. Tranter. 2002. Sulphide oxidation under partially anoxic conditions at the bed of the Haut Glacier d'Arolla, Switzerland. Hydrological Processes 16:2363-2368.

Bowman, J. P., S. A. McCammon, D. S. Nichols, J. H. Skerratt, S. M. Rea, P. D. Nicols, and T. A. McMeekin. 1997. *Shewanella gelidimarina* sp. nov. and *Shewanella frigidimarina* sp. nov., novel Antarctic species with the ability to produce eicosapentaenoic acid (20:5w3) and grow anaerobically by dissimilatory Fe(III) reduction. International Journal of Systematic Bacteriology 47:1040-1047.

Bowman, J. P., S. M. Rea, S. A. McCammon, and T. A. McMeekin. 2000. Diversity and community structure within anoxic sediment from marine salinity meromictic lakes and a coastal meromictic marine basin, Vestfold Hills, Eastern Antarctica. Environmental Microbiology 2:227-237.

Brambilla, E., H. Hippe, A. Hagelstein, B. J. Tindall, and E. Stackebrandt. 2001. 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica. Extremophiles 5:23-33.

Brinkhoff, T., and G. Muyzer. 1997. Increased species diversity and extended habitat range of sulfur-oxidizing *Thiomicrospira spp.* Applied and Environmental Microbiology 63:3789-3796.

Brinkmeyer, R. K. Knittel, J. Jurgens, H. Weyland, R. Amann and E. Helmke. 2003. Diversity and structure of bacterial communities in Arctic versus Antarctic Pack Ice. Applied and Environmental Microbiology 69:6610-6619.

Brown, M. V., and J. P. Bowman. 2001. A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). FEMS Microbiology Ecology 1226:1-9.

Brune, A., P. Frenzel, and H. Cypionka. 2000. Life at the oxic-anoxic interface: microbial activities and adaptations. FEMS Microbiology Reviews 24:691-710.

Bull, A. T. 2004. Biotechnology, the Art of Exploiting Biology, p. 3-10. *In* A. T. Bull (ed.), Microbial Diversity and Bioprospecting. ASM Press, Washington, D.C.

Bull, A. T., and J. E. M. Stach. 2004. An overview of biodiversity-estimating the scale, p. 15-27. *In* A. T. Bull (ed.), Microbial Diversity and Bioprospecting. ASM Press, Washington, D.C.

Burt, D. M. and Knauth L. P. 2003. Electrically conducting, Ca-rich brines, rather than water, expected in the Martian subsurface. Journal of Geophysical Research 108: 8026-8034.

Cameron, R. E., R. C. Honour, and F. A. Morelli. 1976. Antarctic microbiology-preperation for Mars life detection, quarantine, and back contamination. *In* M. R. Heinrich (ed.), Extreme Environments Mechanisms of Microbial Adaptation. Academic Press, Inc., New York.

Carpenter, E. J., S. Lin, and D. G. Capone. 2000. Bacterial activity in South Pole snow. Applied and Environmental Microbiology 66:4514-4517.

Carr, M. H. 1987. Water on Mars. 326:30-35.

Chillrud, D. N., F. L. Pedrozo, P. F. Temporetti, H. F. Planas, and P. N. Froelich. 1994. Chemcial weathering of phosphate and germanium in glacial meltwater streams: effects of subglacial pyrite oxidation. Limnology and Oceanography 39:1130-1140.

Chinn, T. J. 1993. Physical hydrology of the Dry Valley lakes. p. 1-51. *In* W. J. Green and E. I. Friedmann (ed.), Physical and Biogeochemical Processes in Antarctic Lakes, vol. 59. Antarctic Research Series.

Christner, B. C., E. Mosley-Thompson, L. G. Thompson, and J. N. Reeve. 2001. Isolation of bacteria and 16S rDNAs from Lake Vostok accretion ice. Environmental Microbiology 3:570-577.

Christner, B. C. 2002. Incorporation of DNA and protein precursors into macromolecules by bacteria at ¯15 degrees Celsius. Applied and Environmental Microbiology 68:6435-6438.

Christner, B. C., B. H. Kvitko, J. N. Reeve. 2003. "Molecular identification of Bacteria and Eukarya inhabiting an Antarctic cryoconite hole." Extremophiles 7: 177-183.

Christner, B. C., J. A. Mikucki, C. M. Foreman, J. Denson, and J. C. Priscu. 2005. Glacial ice cores: A model system for developing extraterrestrial decontamination protocols. Icarus 174:572-584.

Chyba, C. F., and C. B. Phillips. 2001. Possible ecosystems and the search for life on Europa? Proceedings of the National Academy of Science 98:801-804.

Claypool, G. E., and K. A. Kvenvolden. 1983. Methane and other hydrocarbon gases in marine sediments. Annual Review of Earth and Planetary Sciences 11:299-327.

Clifford, S. M., J. A. George, and C. R. Stoker. 2003. Introduction to the special section: Geophysical detection of subsurface water on Mars. Journal of Geophysical Research 108:8020.

Cole, J. R., B. Chai, T. L. Marsh, R. J. Farris, Q. Wang, S. A. Kulam, S. Chandra, D. M. McGrrell, T. M. Schmidt, G. M. Garrity, and J. M. Tiedje. 2003. The Ribosomal Database Project (RDP-II): preview a new autoaligner that allows regular updates and the new prokaryotic taxonomy. Nucleic Acids Research 31:442-443.

Crawford, R. L. 2005. Microbial diversity and its relationships to planetary protection. Applied and Environmental Microbiology 71:4163-4168.

Day, J. W., C. A. S. Hall, W. M. Kemp, and A. Yanes-Arancibia. 1989. Estuarine Ecology. New York, Wiley-Interscience Publication.

Deming, J. W. 2002. Psychrophiles and polar regions. Current Opinion in Microbiology 5:301-309.

Denton, G. H., D. E. Sugden, D. R. Marchant, B. L. Hall, and T. I. Wilch. 1993. East Antarctic Ice Sheet sensitivity to Pliocene climatic change from a Dry Valleys perspective. Geografiska Annaler 75:155-204.

Donachie, S. P., J. P. Bowman, and M. Alam. 2004. *Psychroflexus tropicus* sp. nov., an obligately halophilic *Cytophaga-Flavobacterium-Bacterioides* group bacterium from an Hawaiian hyersaline lake. International Journal of Systematic and Evolutionary Bacteriology 54:935-940.

Doran, P. T., C. H. Fritsen, C. P. McKay, J. C. Priscu, and E. E. Adams. 2003. Formation and character of an ancient 19-m ice cover and underlying trapped brine in an "ice-sealed" east Antarctic lake. Proceedings of the National Academy of Science 100:26-31.

Doran, P. T., J. C. Priscu, W. B. Lyons, J. E. Walsh, A. G. Fountain, D. M. McKnight, D. L. Moorhead, R. A. Virginia, D. H. Wall, G. D. Clow, C. H. Fritsen, C. P. McKay, and A. N. Parsons. 2002. Antarctic climate cooling and terrestrial ecosystem response. Nature 415:517-520.

Dore, J. E., and J. C. Priscu. 2001. Phytoplankton nutrient deficiency and alkaline phosphatase activity in McMurdo Dry Valley lakes, Antarctica. Limnology and Oceanography 46:1331-1346.

Douterelo, I., E. Perona, and P. Mateo. 2004. Use of cyanobacteria to assess water quality in running waters. Environmental Pollution 127:377-384.

Dunbar, J., S. Takala, S. M. Barns, J. A. Davis, and C. R. Kuske. 1999. Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. Applied and Environmental Microbiology 65:1662-1669.

Eilters, H., J. Pernthaler, F. O. Glockner, and R. Amann. 2000. Culturability and *in situ* abundance of pelagic bacteria from the North Sea. Applied and Environmental Microbiology 66:3044-3051.

Elser, J., L. Stabler, and P. R. Hassett. 1995. Nutrient limitation of bacterial growth and rates of bacterivory in lakes and oceans: A comparative study. Applied and Environmental Microbiology 9: 9129-9140.

Elston, D. P., and S. L. Bressler. 1981. Magnetic stratigraphy of DVDP drill cores and late Cenozoic history of Taylor Valley, Transantarctic Mountains, Antarctica. Dry Valley Drill Project, Antarctic Research Series 33:413-426.

Falkowski, P., R. J. Scholes, E. Boyle, J. Candell, D. Canfield, J. Elser, N. Gruber, K. Hibbard, P. Hogberg, S. Linder, F. T. Mackenzie, B. Moore, T. Pedersen, Y. Rosenthal, S. Seitzinger, V. Smetacek, and W. Steffen. 2000. The Global Carbon Cycle: A test of our knowledge of the Earth as a system. Science 290: 291-296.

Farrelly, V., F. A. Rainey, and E. Stackebrandt. 1005. Effect of genome size and *rrn* gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. Applied and Environmental Microbiology 61:2798-2801.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. Journal of Molecular Evolution 17:368-376.

Felsenstein, J. 1993. DNADIST: a program to compute distance matrix from nucleotide sequences. Version 3.5c. University of Washington.

Finster, K., W. Liesack, and B. Thamdrup. 1998. Elemental sulfur and thiosulfate disproportionation by *Desulfocapsa sulfoexigens* sp. nov., a new anaerobic

bacterium isolated from marine surface sediment. Applied and Environmental Microbiology 3:119-125.

Floyd, M. M., J. Tang, M. Kane, and D. Emerson. 2005. Captured diversity in a culture collection: case study of the geographic and habitat distributions of environmental isolates held at the American Type Culture Collection. Applied and Environmental Microbiology 71:2813-2823.

Foght, J., J. Aislabie, S. Turner, C. E. Brown, J. Ryburn, D. J. Saul, and W. Lawson. 2004. Culturable bacteria in subglacial sediment and ice from two southern hemisphere glaciers. Microbial Ecology 47:329-340.

Foreman, C. M., C. F. Wolf, and J. C. Priscu. 2004. Impact of Episodic warming events on the physical, chemical and biological relationships of lakes in the McMurdo Dry Valleys, Antarctica. Aquatic Geochemistry 10:239-268.

Formisano, V., S. Atreya, T. Encrenaz, N. Ignatiev, and M. Giuranna. 2004. Detection of methane in the atmosphere of Mars. Science 306:1758-1761.

Fountain, A. G., W. B. Lyons, M. B. Burkins, G. L. Dana, P. T. Doran, K. J. Lewis, D. M. McKnight, D. L. Moorhead, A. N. Parsons, J. C. Priscu, D. H. Wall, R. A. W. Jr., and R. A. Virginia. 1999. Physical controls on the Taylor Valley ecosystem, Antarctica. Bioscience 49:961-971.

Freckman, D. W. and R. A. Virginia. 1997. Low-diversity Antarctic soil nematode communities: distribution and response to disturbance. Ecology 78:363-369.

Fritsen, C. H., and J. C. Priscu. 1999. Seasonal change in the optical properties of the permanent ice cover on Lake Bonney, Antarctica: Consequences for lake productivity and phytoplankton dynamics. Limnology and Oceanography 44:447-454.

Fulton, J. R., D. McKnight, C. Foreman, R. Cory, C. Stedman, and E. Blunt. 2004. Changes in fulvic acid redox state through the oxycline of a permanently ice-covered Antarctic lake. Aquatic Science 66:1-20.

Gaidos, E. J., B. Lanoil, T. Thorsteinsson, A. Graham, M. Skidmore, S.-K. Han, T. Rust, and B. Popp. 2004. A viable microbial community in a subglacial volcanic crater lake, Iceland. Astrobiology 4:327-344.

Gilichinsky, D. A., and S. Wagener. 1995. Microbial life in permafrost: a historical review. Periglacial Processes 6:243-250.

Gold, T. 1992. The deep, hot biosphere. Proceedings of the National Academy of Science 89:6045-6049.

Goldman, C. R., D. T. Mason, and J. E. Hobbie. 1967. Two Antarctic desert lakes. Limnology and Oceanography 12:295-310.

Hall, T. A. 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 85/98/NT. Nucleic Acids Symposium Series 41:95-98.

Hamilton, W. L., I. C. Frost, and P. T. Hayes. 1962. Saline Features of a Small Ice Platform in Taylor Valley, Antarctica, p. B73-76, US Geological Survey, Professional Paper, vol. 450B. US Geological Survey.

Hendy, C. H., A. T. Wilson, K. B. Popplewell, and D. A. House. 1977. Dating of geochemical events in Lake Bonney, Antarctica and their relation to glacial and climate changes. New Zealand Journal of Geology and Geophysics 20:1103-22.

Hendy, C. H. 2000. Late quaternary lakes in the McMurdo Sound region of Antarctica. Geografiska Annaler 75:205-220.

Hodell, J. P. K. a. D. A. 1993. Evidence for relative climatic stability of Antarctica during the early Pliocene: a marine perspective. Geografiska Annaler 75:205-220.

Hodgkins, R., M. Tranter, and J. A. Dowdeswell. 1998. The hydrochemistry of runoff from a 'cold-based' glacier in the high Arctic (Scott Turnerbreen, Svalbard), p. 47-64. *In* M. Sharp, K. S. Richards, and M. Tranter (ed.), Glacier Hydrology and Hydrochemistry. John Wiley and Sons, New York.

Hoffman, P. F., A. J. Kaufman, G. P. Halverson, and D. P. Schrag. 1998. A neoproterozoic snowball earth. Science 281:1342-1346.

Hoffman, P. F., and D. P. Schrag. 2002. The snowball earth hypothesis: Testing the limits of global change. Terra Nova 14:129-155.

Holmes, D. E., J. S. Nicoll, D. R. Bond, and D. R. Lovley. 2004. Potential role of a novel psychrotolerant member of the family *Geobacteraceae, Geopsychrobacter electrodiphilus* gen. nov., sp. nov., in electricity production by a marine sediment fuel cell. Applied Environmental Microbiology 70:6023-6030.

Hubbard, A., W. Lawson, B. Anderson, B. Hubbard, and H. Blatter. 2004. Evidence for subglacial ponding across Taylor Glacier, Dry Valleys, Antarctica. Annals of Glaciology 39:79-84.

Huber, T., G. Faulkner, and P. Hugenholtz. 2004. Bellerophon; a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics 20:2317-2319.

Hugenholtz, P., C. Pitulle, K. L. Hershberger, and N. R. Pace. 1998. Novel division level bacterial diversity in a Yellowstone hot spring. Journal of Bacteriology 180:366-376.

Hughes, J. B., J. J. Hellmann, T. H. Ricketts, and B. J. M. Bohannan. 2001. Counting the Uncountable: statistical approaches to estimating microbial diversity. Applied and Environmental Microbiology 67:4399-4406.

Hungate, R. E. 1969. A roll tube method for cultivation of strict anaerobes, p. 117-132. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in Microbiology, vol. 3B. Academic Press, New York.

Hungate, B. A., R. J. Naiman, M. Apps, J. J. Cole, B. Moldan, K. Satake, J. W. B. Steward, R. Victora, P. M. Vitousek. (2003). Distrubance and Element Interactions. Interactions of hte Major Biogeochemical Cycles: Glabal Change and Human Impacts. J. M. Melillo, C. B. Field and B. Moldan. Washington, Island Press. 1: 47-62.

Hyde, W. T., T. J. Crowley, S. K. Baum, and W. R. Peltier. 2000. Neoproterozoic 'snowball Earth' simulations with a coupled climate/ice-sheet model. Nature 405:425-429.

Inagaki, F., M. Suzuki, K. Takai, H. Oida, T. Sakamoto, K. Aoki, K. H. Nealson, and K. Horikoshi. 2003. Microbial communitites associated with geological horizons in coastal subseafloor sediment from the Sea of Okhotsk. Applied and Environmental Microbiology 69:7224-7235.

Jakosky, B. M., and E. L. Shock. 1998. The biological potential of Mars, the early Earth, and Europa. Journal of Geophysical Research 103:19,359-19364.

Jeon, C. O., W. Park, P. Padmanabhan, C. DeRito, J. R. Snape, and E. L. Madsen. 2003. Discovery of a bacterium, with distinctive dioxygenase, that is responsible for *in situ* biodegradation in contaminated sediment. Proceedings of the National Academy of Science 100:13591-1595.

Jepson, S. M. and Adams, E. A. 2005. Experimental study of hydrocarbon transport mechanisms in the Lake Fryxell ice cover, McMurdo Dry Valleys, Antarctica. Poster presentation at the McMurdo LTER site meeting, Portland, OR.

Johnson, D. B. 195. Mineral cycling by microorganisms: iron bacteria, p. 185-198. *In* D. Allsopp, R. R. Colwell, and D. L. Hawksworth (ed.), Microbial Diversity and Ecosystem Function. University Press, Cambridge.

Junge, K., J. J. Gosink, H. G. Hoppe and J. T. Staley. 1998. *Arthrobacter*, *Brachybacterium* and *Planococcus* isolates identified from Antarctic sea ice brine. Description of *Planococcus mcmeekenii*, *sp*. *nov*. Systematic and Applied Microbiology 21:306-314.

Junge, K., H. Eicken, and J. W. Deming. 2004. Bacterial Activity at -2 to -20 degrees Celsius in arctic wintertime sea ice. Applied and Environmental Microbiology 70:550-557.

Karl, D. M. 1986. Determination of *in situ* microbial biomass, viability, metabolism and growth, p. 85-176. *In* J. S. Poindexter and E. R. Leadbetter (ed.), Methods and Special Applications in Bacterial Ecology, vol. 2. Plenum Press, New York.

Karl, D. M. 1993. Microbial processes in the Southern Oceans, p. 1-64. *In* E. I. Friedmann (ed.), Antarctic Microbiology. Wiley-Liss, New York.

Karl, D. M., D. F. Bird, K. Bjorkman, T. Houlihan, R. Shackelford, and L. Tupas. 1999. Microorganisms in the accreted ice of Lake Vostok, Antarctica. Science 286:2144-2147.

Kemp, P. F., and J. Y. Aller. 2004. Estimating prokaryotic diversity: when are 16S rDNA libraries large enough? Limnology and Oceanography: Methods 2:114-125.

Kennet, J. P. and D. A. Hodell. 1993. Evidence for relative climatic stability of Antarctica during the early Pliocene: a marine perspective. Geografiska Annaler 75:205-220.

Keys, J. R. 1979. Saline discharge at the terminus of the Taylor Glacier. Antarctic Journal of the United States 14:82-85.

Keys, J. R. 1980. Salts and their distribution in the McMurdo region, Antarctica. Ph.D. Victoria University, Wellington, NZ.

Kirchman, D. L. 2002. The ecology of *Cytophaga-Flavobacteria* in aquatic environments. FEMS Microbiology Ecology 39:91-100.

Kirschvink, J. L. 1992. Late Proterozoic low-latitude global glaciation: the Snowball Earth. *In* J. W. Schopt, C. Klein, and D. D. Maris (ed.), The Proterozoic Biosphere: A Multidisciplinary Study. Cambridge University Press, Cambridge.

Kirschvink, J. L., E. J. Gaidos, L. E. Bertani, N. J. Beukes, J. Gutzmer, L. N. Maepa, and R. E. Steinberger. 2000. Paleoproterozoic snowball Earth: Extreme climatic and geochemical global change and its biological consequences. Proceedings of the National Academy of Science 97:1400-1405.

Klappenbach, J. A., P. R. Saxman, J. R. Cole, and T. M. Schmidt. 2001. rrndb: the Ribosomal RNA Operon Copy Number Database. Nucleic Acids Research 29:181-184.

Knight, P. G. 1998. The basal ice layer of glaciers and ice sheets. Quarternary Science Research 16:975-997.

Knittel, K., J. Kuever, A. Meyerdierks, R. Meinke, R. Amann, and T. Brinkhoff. 2004. *Thiomicrospira arctica* sp. nov. and *Thiomicrospira psychrophila* sp. nov. psychrophilic obligatory chemolithoautotrophic sulfur-oxidizing bacteria isolated from marine Arctic sediments. International Journal of Systematic and Evolutionary Bacteriology.

Knoll, A. H. 2003. The geological consequences of evolution. Geobiology 1:3-14.

Kumar, S., K. Tamura and M. Nei. 1993. MEGA: Molecular Evolutionary Genetics Analysis. Version 2.1. Pennsylvania State University, University Park, PA.

Kuzmin, R. O. 2005. Ground ice in the Martian regiolith, p. 155-189. *In* T. Tokano (ed.), Water on Mars and Life. Springer-Verlag, Berlin.

Lawson, J., P.T. Doran, F. Kenig, D. J. Des marais, and J. C. Priscu. 2004. Stable carbon and nitrogen isotopic composition of benthic and pelagic organic matter in lakes of the McMurdo Dry Valleys, Antarctica. Aquatic Geocemistry 10:269-301.

Lead Technologies. 2004. SPSS Software Version 13.0. SPSS Inc., Apache Software Foundation. Chicago, Illinois.

Lee, P. A., J. A. Mikucki, C. M. Foreman, J. C. Priscu, G. R. DiTullio, S. F. Riseman, S. J. de Mora, C. F. Wolf, and L. Kester. 2004. Thermodynamic constraints on microbially mediated processes in lakes of the McMurdo Dry Valleys, Antarctica. Geomicrobiology Journal 21:221-237.

Levin, M. A., and R. J. Seidler. 1992. Introduction to microbial ecology principles, methods and applications, p. 1-10. *In* M. A. Levin, R. J. Seidler, and M. Rogul (ed.), Microbial Ecology Principles, Methods, and Applications. McGraw-Hill, Inc., New York.

Lisle, J. T., and J. C. Priscu. 2004. The occurrence of lysogenic bacteria and microbial aggregates in the lake of the McMurdo Dry Valleys, Antarctica. Microbial Ecology 47:427-439.

Lizotte, M. P., T. R. Sharp, and J. C. Priscu. 1996. Phytoplankton dynamics in the stratified water column of Lake Bonney, Antarctica. I. Biomass and productivity during the winter-spring transition. Polar Biology 16:155-162.

Lizotte, M. P. and J. C. Priscu. 1998. Distribution, succession and fate of phytoplankton in the dry valley lakes of Antarctica, based on pigment analysis. p. 229-240. *In* J.C. Priscu (ed.) Ecosystem Dynamics in a Polar Desert: The McMurdo Dry Valleys. Antarctic Research Series, Vol 72, American Geophysical Union.

Lovley, D. R. and E. J. P. Phillips 1986. Organic Matter Mineralization with Reduction of Ferric Iron in Anaerobic Sediments. Applied and Environmental Microbiology 51: 683-689.

Luttage, A., and P. G. Conrad. 2004. Direct observations of microbial inhibition of calcite dissolution. Applied and Environmental Microbiology 70:1627-1632.

Lyons, W. B., and K. A. Welch. 1997. Lithium in waters of a polar desert. Geochimica et Cosmochimica Acta 61:4309-4319.

Lyons, W. B., K. A. Welch, and P. Sharma. 1998. Chlorine-36 in the waters of the McMurdo Dry Valley lakes, southern Victoria Land, Antarctica: Revisited. Geochimica et Cosmochimica Acta 62:185-191.

Lyons, W. B., S. K. Frape, and K. A. Welch. 1999. History of McMurdo Dry Valley Lakes, Antarctica, from stable chlorine isotope data. Geology 27:69-83.

Lyons, W. B., K. A. Welch, J. C. Priscu, J. Laybourne-Parry, D. Moorhead, D. M. McKnight, P. T. Doran, and M. Tranter. 2001. The McMurdo Dry Valleys Long-Term Ecological Research Program: new understanding of the biogeochemistry of the dry valley lakes: a review. Polar Geography 62:202-217.

Lyons, W. B., C. A. Nezat, L. V. Benson, T. D. Bulen, E. Y. Graham, J. Kidd, and K. A. Welch. 2002. Strontium isotopic signatures of the streams and lakes of Taylor Valley, Southern Victoria Land, Antarctica: Chemical weathering in a polar climate. Aquatic Geochemistry 8:75-95.

Lyons, W. B., K. A. Welch, A. G. Fountain, G. L. Dana, B. H. Vaughn, and D. M. McKnight. 2003. Surface glaciochemistry of Taylor Valley, southern Victoria Land, Antarctica and its relationship to stream chemistry. Hydrological Processes 17:115-130.

Lyons, W. B., K. A. Welch, G. Snyder, J. Olesik, E. Y. Graham, G. M. Marion, and R. J. Poreda. 2005. Halogen geochemistry of the McMurdo Dry Valleys Lakes, Antarctica: Clues to the origin of solutes and lake evolution. Geochimica et Cosmochimica Acta 69:305-323.

MacClune, K. L., A. G. Fountain, J. S. Kargel, and D. R. MacAyeal. 2003. Glaciers of the McMurdo Dry Valleys: Terrestrial analog for martian polar sublimation. Journal of Geophysical Research 108:5031-5043.

Madigan, M. T., J. M. Martinko, and J. Parker. 2000. Brock Biology of Microorganisms, 9th ed. Prentice Hall, Upper Saddle River, New Jersey.

Magurran, A. 1988. Ecological Diversity and Its Measurement. Princeton University Press, Princeton.

Marchant, D. R., G. H. Denton, and D. E. Sugden. 1993. Miocene glacial stratigraphy and landscape evolution in the western Asgard Range, Antarctica. Geografiska Annaler 75:269-302.

Margalef, R. 1972. Interpretation not strictly statistical of representation of biological entities in multifactorial space. Investigaci? on Pesquera 36:183-190.

Martin, S., M. C. Marquez, C. Sanchez-Porro, E. Mellado, D. R. Arahal and A. Ventosa. 2003. *Marinobacter lipolyticus* sp. nov., a novel moderate halophile with lipolytic activity. IJSEM 53:1383-1387

Marthi, B., B. T. Shaffer, B. Lighthart, and L. Ganio. 1991. Resuscitation effects of catalase on airborne bacteria. Applied and Environmental Microbiology 57:2775-2776.

McCaig, A. E., L. A. Glover, and J. I. Prosser. 1999. Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. Applied and Environmental Microbiology 65:1721-1730.

McKay, C. P. 1993. Relevance of Antarctic Microbial Ecosystems to Exobiology, p. 593-602. *In* E. I. Friedmann (ed.), Antarctic Microbiology. Wiley-Liss, New York.

McKay, C. P., D. T. Anderson, W. H. Pollard, J. L. Heldmann, P. T. Doran, C. H. Fritsen, and J. C. Priscu. 2005. Analogs for the hydrological cycle on Mars, p. 219-233. *In* T. Tokano (ed.), Water on Mars and Life. Springer-Verlag, New York.

Meyers, J. L., and R. T. Edwards. 1990. Ecosystem metabolism and turnover of organic carbon along a blackwater river continuum. Ecology 781:668-677.

Mikucki, J. A., C. M., Foreman, B. Sattler, W. B. Lyons, and J. C. Priscu. 2004. Geomicrobiology of Blood Falls: An iron-rich saline discharge at the terminus of the Taylor Glacier, Antarctica. Aquatic Geochemistry 10:199-220.

Miller, L. G., and G. R. Aiken. 1996. Effects of glacial meltwater inflows and moat freezing on mixing in an ice-covered Antarctic lake as interpreted from stable isotope and tritium distributions. Limnology and Oceanography 41:966-976.

Mock, T., and D. N. Thomas. 2005. Recent advances in sea-ice microbiology. Environmental Microbiology 7:605-619.

Moss, S. 1988. Natural History of the Antarctic Peninsula. Columbia University Press, New York.

Moyer, C. L., F. C. Dobbs, and D. M. Karl. 1994. Estimation of diversity and community structure through restriction fragment length polymophism distribution and analysis of bacterial 16S RNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. Applied and Environmental Microbiology 60:871-879.

Muyzer, G. E., E. C. D. Waal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59:695-700.

Muyzer, G. and K. Smalla 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie van Leeuwenhoek 73: 127-141.

Naish, T. R., K. J. Woolfe, P. J. Barrett, G. S. Wilson, C. Atkins, S. M. Bohaty, C. J. Bucker, M. Claps, F. J. Davey, G. B. Dunbar, A. G. Dunn, C. R. Fielding, F. Florindo, M. J. Hannah, D. M. Harwood, S. A. Henrys, L. A. Krissek, M. Lavelle, J. van der Meer, W. C. McIntosh, F. Niessen, S. Passchier, R. D. Powell, A. P. Roberts, L. Sagnotti, R. P. Scherer, C. P. Strong, F. Talarico, K. L. Verosub, G. Villa, D. K. Watkins, P.-N. Webb, and T. Wonik. 2001. Orbitally induced oscillations in the East Antarctic ice sheet at the Oligocene/Miocene boundary. Nature 413:719-723.

Nealson, K. H. 1999. Post-viking microbiology: New approaches, new data, new insights. Origins of Life and Evolution of the Biosphere 29:73-93.

Nienow, J. A., and E. I. Friedmann. 1993. Terrestrial Lithophytic (Rock) Communities, p. 343-412. *In* E. I. Friedmann (ed.), Antarctic Microbiology. Wiley-Lise, New York.

NRC. 1999. Perspectives on biodiversity: valuing its role in an ever changing world. National Academy Press. Washington, D.C.

NRC. 2003. Frontiers in polar biology in the genomic era. National Academies Press. Washington, D.C.

NRC. 2005. Preventing the Forward Contamination of Mars. National Research Council of the National Acadamies -Space Science Board. Washington, D.C.

Nubel, U., F. Garcia-Pichel, M. Kuhl, and G. Muyzer. 1999. Quantifying microbial diversity: morphotypes, 16S rRNA genes, and carotenoids of oxygeneic phototrophs in microbial mats. Applied and Environmental Microbiology 65:422-430.

Olsen, G. J., H. Matsuda, R. Hagstrom, and R. Overbeek. 1994. fastDNAml: A Tool for Construction of phylogenetic trees of DNA sequences using maximum likelihood. Computer Applications in the Biosciences 10:41-48.

Olson, J. B., T. F. Steppe, R. W. Litaker, and H. W. Paerl. 1998. Nitrogen-fixing microbial consortia associated with the ice cover of Lake Bonney, Antarctica. Microbial Ecology 36:231-238.

Pace, N. R. 1997. A molecular view of microbial diversity and the biosphere. Science 276:734-740.

Pearce, D. A. 2000. A method to study bacterioplankton community structure in Antarctic lakes. Polar Biology 23: 352-356.

Pearl, H. W., and J. C. Priscu. 1998. Microbial phototrophic, heterotrophic, and diazotrophic activities associated with aggregates in the permanent ice cover of Lake Bonney, Antarctica. Microbial Ecology 36:221-230.

Price, P. B. 2000. A habitat for psychrophiles in deep Antarctic ice. Proceedings of the National Academy of Science 97:1247-1251.

Priscu, J. C., and C. R. Goldman. 1984. The effect of temperature on photosynthetic and respiratory electron transport system activity in the shallow and deep-living phytoplankton of sub-alpine lake. Freshwater Biology 14:143-155.

Priscu, J. C., A. C. Palmisano, L. R. Priscu, and C. W. Sullivan. 1989. Temperature dependence of inorganic nitrogen uptake and assimilation in Antarctic sea-ice microalgae. Polar Biology 9:443-446.

Priscu, J. C. 1995. Phytoplankton nutrient deficiency in lakes of the McMurdo dry valleys, Antarctica. Freshwater Biology 34:215-227.

Priscu, J. C. 1997. The Biogeochemistry of nitrous oxide in permanently ice-covered lakes of the McMurdo Dry valleys, Antarctica. Global Change Biology 3:301-305.

Priscu, J. C. 1998. Ecosystem Dynamics in a Polar Desert, vol. 72. American Geophysical Union, Washington, DC.

Priscu, J. C., C. H. Fritsen, E. E. Adams, S. J. Giovannoni, H. W. Pearl, C. P. McKay, P. T. Doran, D. A. Gordon, B. D. Lanoil, and J. L. Pinckney. 1998. Perennial Antarctic Lake Ice: An oasis for life in a polar desert. Science 280:2095-2098.

Priscu, J. C., E. E. Adams, W. B. Lyons, M. A. Voytek, D. W. Mogk, R. L. Brown, C. P. McKay, C. D. Takacs, K. A. Welch, C. F. Wolf, J. D. Kirshtein, and R. Avci. 1999. Geomicrobiology of subglacial ice above Lake Vostok, Antarctica. Science 286:2141-2144.

Priscu, J. C., A. G. Fountain, D. H. Wall, W. B. Lyons, R. A. Wharton, M. B. Burkins, R. A. Virginia, G. L. Dana, P. T. Doran, K. J. Lewis, D. M. McKnight, D. M. Moorhead, and A. N. Parsons. 1999. Physical controls on the Taylor Valley Ecosystem, Antarctica. Bioscience 49: 961-971.

Priscu, J. C., C. F. Wolf, C. D. Takacs, C. H. Fritsen, J. Laybourn-Parry, E. C. Roberts, B. Sattler, and W. B. Lyons. 1999. Carbon Transformations in a Perennially Ice-Covered Antarctic Lake. Bioscience 49:997-1008.

Priscu, J. C. and C. F. Wolf. 2000. Limnological Methods for the McMurdo Long Term Ecological Research Program. www.homepage.montana.edu/~lkbonney/

Priscu, J. C., R. E. Bell, S. A. Bulat, J. C. Ellis-Evans, M. C. Kennicutt, V. V. Lukin, J.-R. Petit, R. D. Powell, M. J. Siegert, and I. Tabacco. 2003. An international plan for Antarctic subglacial lake exploration. Polar Geography 27:69-83.

Priscu, J. C., and C. C. Christner. 2004. Earth's icy biosphere. *In* A. T. Bull (ed.), Microbial Diversity and Bioprospecting. ASM Press, Washington, D.C.

Priscu, J. C., E. E. Adams, H. W. Pearl, C. H. Fritsen, J. E. Dore, J. T. Lisle, C. F. Wolf, J. A. Mikucki. 2005. Perennial Antarctic lake ice: a refuge for Cyanobacteria in an extreme environment. p. 22-49. *In* J. D. Castello and S. O. Rogers (eds.), Life in Ancient Ice. Princeton University Press, Princeton.

Psenner, R., M. Felip, J. C. Priscu, D. Wagenbach, A. Wille, and B. Sattler. 2002. Extremophiles: Ice ecosystems and biodiversity, p. 573-598, Knowledge for Sustainable Development. Encyclopedia of Life Support Systems. Eolss Publishers, Oxford.

Pugh, H. E., K. A. Welch, W. B. Lyons, J. C. Priscu, and D. M. McKnight. 2003. The Biogeochemistry of Si in the McMurdo Dry Valley Lakes, Antarctica. International Journal of Astrobiology 1:401-413.

Raiswell, R. 1984. Chemical models of solute acquisition in glacial meltwaters. Journal of Glaciology 30:49-57.

Ravenschlag, K. K. Sahm and R. Amann. 2001. Quantitative molecular analysis of the microbial community in marine Arctic sediments (Svalbard). Applied and Environmental Microbiology 67:387-395.

Raymond, J. A. and C. H. Fritsen. 2001. Semipurification and ice recrystallization inhibition activity of ice-active substances associated with Antarctic photosynthetic organisms. Cryobiology 43:63-70.

Reasoner, D. J., and E. E. Geldreich. 1979. A new medium for the enumeration and subculture of bacteria from potable water, Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. N7.

Reynolds, R. T., S. W. Squyres, D. S. Colburn, and C. P. McKay. 1983. On the habitability of Europa. Icarus 56:246-254.

Rivkin, R. B., and M. R. Anderson. 1997. Inorganic nutrient limitation of oceanic bacterioplankton. Limnology and Oceanography 42:730-740.

Rivkina, E. M., E. I. Friedmann, C. P. McKay, and D. A. Gilichinsky. 2000. Metabolic activity of permafrost bacteria below the freezing point. Applied and Environmental Microbiology 66:3230-3233.

Robinson, P. H. 1984. Ice dynamics and thermal regime of Taylor Glacier South Victoria Land, Antarctica. Journal of Glaciology 30:153-159.

Rogers, S. O., L.-J. Ma, Y. Zhao, V. Theraisnathan, S.-G. Shin, G. Zhang, C. M. Catranis, W. T. Starmer, and J. D. Castello. 2005. Recommendations for elimination of contaminants and authentication of isolates in ancient ice cores, p. 5-21. *In* J. D. Castello and S. O. Rogers (ed.), Life In Ancient Ice. Princeton University Press, Princeton.

Rogers, S. O., V. Theraisnathan, L. J. Ma, Y. Zhao, G. Zhang, S. G. Shin, J. D. Castello, and W. T. Starmer. 2004. Comparisons of protocols for decontamination of environmental ice samples for biological and molecular examinations. Applied and Environmental Microbiology 70:2540-2544.

Rosenzweig, M. L. 1995. Species Diversity in Space and Time. Cambridge University Press, New York.

Russell, N. J. 1990. Cold adaptation of microorganisms. Philosophical Translations of the Royal Society of London 326:595-611.

Sass, H., E. Wieringa, H. Cypionka, H. D. Babenzien, and J. Overmann. 1998. High Genetic and physiological diversity of sulfate-reducing bacteria isolated from an oligotrophic lake sediment. Archives of Microbiology 170:243-251.

Schlesinger, W. H. 1997. Biogeochemistry: An Analysis of Global Change, 2nd ed. Academic Press, New York.

Sea-Bird-Electronics. 1998. CTD Data Acquisition Software-Seasoft, 3.3G ed. Sea-Bird Electronic, Inc, Bellevue, Washington.

Shapiro, D. J. 1981. Quantitative ethanol precipitation of nanogram quantities of DNA and RNA. Annals of Biochemistry 110:229-231.

Sharp, M., J. Parkes, B. Cragg, I. J. Fairchild, H. Lamb, and M. Tranter. 1999. Widespread bacterial populations at glacier beds and their relationship to rock weathering and carbon cycling. Geology 27:107-110.

Shaivaji, S., M. K. Ray, N.S. Rao, L. Saisree, M. V. Jagennadham, G. S. Kumar, G. S. N. Reddy and P. M. Bhargava. (1992). "Sphingobacterium antarcticus sp. nov., a psychrotrophic bacterium from the soils of Schirmacher Oasis, Antarctica." International Journal of Systematic and Evolutionary Bacteriology 42: 102-106.

Sheridan, P. P., V. I. Miteva, and J. E. Brenchley. 2003. Phylogenetic analysis of anaerobic psychrophilic enrichment cultures obtained from a Greenland glacier ice core. Applied and Environmental Microbiology 69:2153-2160.

Siegert, M. J. 2000. Antarctic subglacial lakes. Earth-Science Reviews 50:29-50.

Sigler, W. V. and J. Zeyer. 2002. Microbial diversity and activity along the forefields of two receding glaciers. Microbial Ecology 43:397-407.

Simmons, G. M., J. R. Vestal, and R. A. Wharton. 1993. Environmental regulators of microbial activity in continental Antarctic lakes, p. 491-542. *In* E. I. Friedmann (ed.), Antarctic Microbiology. Wiley-Liss, New York.

Skidmore, M. L., J. M. Foght, and M. J. Sharp. 2000. Microbial life beneath a high arctic glacier. Applied and Environmental Microbiology 66:3214-3220.

Smith, D. C., A. J. Spivack, M. R. Fisk, S. A. Haveman, H. Staudigel, and L. S. S. Party. 2000. Methods for quantifying potential microbial contamination during deep ocean coring. ODP Technical Note, vol. 28.

Smith, R. I. L. 1984. Terrestrial Plant Biology, p. 61-162. *In* R. M. Laws (ed.), Antarctic Ecology, vol. 1. Academic Press Inc., London.

Smoot, G. F., C. L. Bennet, A. Kogut, E. L. Wright, J. Aymon, N. W. Boggess, E. S. Cheng, G. d. Amici, S. Gulkis, M. G. Hauser, G. Hinshaw, P. D. jackson, m. Janssen, E. Kaita, T. Kelsall, P. Keegstra, C. Lineweaver, K. Loewenstein, P. Lubin, J. Mather, S. S. Meyer, S. H. Moseley, T. Murdock, l. Rokke, R. F. Silverberg, L. Tenorio, R. Weiss, and D. T. Wilkinson. 1992. Structure of the COBE differential microwave radiometer first-year maps. Astrophysical Journal, Part 2 - Letters 396:L1-L5.

Sorensen, K. B., A. Lauer, and A. Teske. 2004. Archaeal phylotypes in a metal-rich and low activity deep subsurface sediment of the Peru Basin, ODP Leg 201, Site 1231. Geobiology 2:151-161.

Spiegel, R. H., and J. C. Priscu. 1996. Evolution of temperature and salt structure of Lake Bonney, a chemically stratified Antarctic lake. Hydrobiologia 321:177-190.

Spigel, R. H., and J. C. Priscu. 1998. Physical limnology of the McMurdo Dry Valleys Lakes, p. 153-187. *In* J. C. Priscu (ed.), Ecosystem dynamics in a polar desert: The McMurdo Dry Valleys, Antarctica. American Geophysical Union, Washington, D.C.

Squyers, S. W., R. T. Reynolds, P. M. Cassen, and S. J. Peale. 1983. Liquid water and active resurfacing on Europa. Nature 301:225-226.

Sugden, D. E., D. R. Marchant, and G. H. Denton. 1993. The Case for a Stable East Antarctic Ice Sheet: The Background. Geografiska Annaler 75A:151-154.

Summerfield, M. A., F. M. Stuart, H. A. P. Cockburn, D. E. Sugden, G. H. Denton, T. Dunai, and D. R. Marchant. 1999. Longterm rates of denudation in the Dry Valleys, Transantarctic Mountains, Southern Victoria Land, Antarctica based on *in-situ*-produced cosmogenic ^{21}Ne. Geomorphology 27:113-129.

Takacs, C. D., and J. C. Priscu. 1998. Bacterioplankton dynamics in the McMurdo Dry Valley Lakes, Antarctica: production and biomass loss over four seasons. Microbial Ecology 36:239-250.

Takacs, C. D. 1999. Temporal change in bacterial plankton in the McMurdo Dry Valleys. Ph.D. Montana State University, Bozeman.

Takacs, C. D., J. C. Priscu, and D. M. McKnight. 2001. Bacterial dissolved organic carbon demand in McMurdo Dry Valley Lakes, Antarctica. Limnology and Oceanography 46:1189-1194.

Talalay, P. G., and N. S. Gundestrup. 2002a. Hole fluids for deep ice core drilling. Proceedings of Ice Drilling Technology 56:148-170.

Talalay, P. G., and N. S. Gundestrup. 2002b. Hydrostatic pressure and fluid density profile in deep ice bore-holes. Proceedings of Ice Drilling Technology 56:171-181.

Taylor, G. 1922. The physiography of the McMurdo Sound and Granite Harbor Region. Harrison and Sons, Ltd., London.

Teske, A., T. Brinkhoff, G. Muyzer, D. P. Moser, J. Rethmeier, and H. W. Jannasch. 2000. Diversity of thiosulfate-oxidizing bacteria from marine sediments and hydrothermal vents. Applied and Environmental Microbiology 66:3125-3133.

Thamdrup, B., J. W. Hansen and B. B. Jorgensen. 1998. Temperature dependence of aerobic respiration in a coastal sediment. FEMS Microbial Ecology 25:189-200.

Thomas, D. N., and G. S. Dieckmann. 2002. Antarctic sea ice-a habitat for extremophiles. Science 295:641-644.

Tiedje, J. M. 1995. Approaches to the comprehensive evaluation of prokaryote diversity of a habitat. *In* D. Allsopp, R. R. Colwell, and D. L. Hawksworth (ed.), Microbial Diversity and Ecosystem Function. University Press, Cambridge.

Tindall, B. J. 2004. Prokaryotic Diversity in the Antarctic: the tip of the iceberg. Microbial Ecology 47:271-283.

Tranter, M., M. J. Sharp, G. H. brown, I. C. Willis, B. P. Hubbard, M. K. Nielsen, C. C. Smart, S. Gordon, M. Tulley, and H. R. Lamb. 1997. Variability in the chemical composition of *in situ* subglacial meltwaters. Hydrological Processes 11:59-77.

Tranter, M., G. H. Brown, A. J. Hodson, and A. M. Gurnell. 1998. Hydrochemistry as an indicator of subglacial drainage system structure: a comparison of alpine and sub-polar environments, p. 65-80. *In* M. Sharp, K. S. Richards, and M. Tranter (ed.), Glacier Hydrology and Hydrochemistry. John Wiley and Sons, New York.

Tranter, M., M. J. Sharp, H. R. Lamb, G. H. Brown, B. P. Hubbard, and I. C. Willis. 2002. Geochemical weathering at the bed of Haut Glacier d'Arolla, Switzerland- a new model. Hydrological Processes 16:959-993.

Tranter, M., A. G. Fountain, C. H. Fritsen, W. B. Lyons, J. C. Priscu, P. J. Statham, and K. A. Welch. 2004. Extreme hydrochemical conditions in natural microcosms entombed within Antarctic ice. Hydrological Processes 18:379-387.

Tranter, M., M. Skidmore, and J. Wadham. 2005. Hydrological controls on microbial communities in subglacial environments. Hydrological Processes 19:995-998.

Vincent, W. F. 1988. Microbial Ecosystems of Antarctica. Cambridge University Press, New York.

Vincent, W. F., R. W. Castenholz, M. T. Downes, and C. Howard-Williams. 1993. Antarctic Cyanobacteria: light, nutrients, and photosynthesis in the microbial mat environment. Journal of Phycology 29:745-755.

Vincent, W. F., J. A. E. Gibson, R. Pienitz, V. Villeneuve, P. A. Broady, P. B. Hamilton, and C. Howard-Williams. 2000. Ice shelf microbial ecosystems in the high Arctic and implications for life on Snowball Earth. Naturwissenschaften 87:137-141.

Vincent, W. F., and C. Howard-Williams. 2000. Life on Snowball Earth. Science 287:2421.

Vishniac, W. K. C. Atwood, R. M. Bock, H. Gaffron, T. H. Jukes, A. D. McLaren, C. Sagan and H. Spinrad. 1966. A model of Martian ecology. *In* C. S. Pittendrigh, W. Vishniac and J. P. T. Pearman (eds.), Biology and Exploration of Mars. Nactional Research Council, Washington, D.C.

Wadham, J. L., A. J. Hodson, M. Tranter, and J. A. Dowdeswell. 1998. The hydrochemistry of meltwaters draining a polythermal-based, high Arctic glacier, south Svalbard: I. The ablation seaosn. Hydrological Processes 12:1825-1849.

Wadham, J. L., A. J. Hodson, M. Tranter, and J. A. Dowdeswell. 2002a. The hydrochemsitry of meltwaters draining a high arctic, polythermal-based glacier, South Svalbard: I. The Ablation Season. Hydrological Processes 12:1825-1849.

Wadham, J. L., M. Tranter, and J. A. Dowdeswell. 2002b. Hydrochemistry of meltwaters draining a polythermal-based, high-Arctic glacier, south Svalbard: II. winter and early spring. Hydrological Processes 14.

Wadham, J. L., S. Bottrell, M. Tranter, and R. Raiswell. 2004. Stable isotope evidence for microbial sulphate reduction at the bed of a polythermal high Arctic glacier. Earth and Planetary Science Letters 219:341-355.

Walther, G. R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J.-M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. Nature 416:389-395.

Walton, D. W. H. 1984. The terrestrial environment, p. 1-60. *In* R. M. Laws (ed.), Antarctic Ecology, vol. 1. Academic Press, New York.

Ward, B. B., and J. C. Priscu. 1997. Detection and characterization of denitrifying bacteria form a permanently ice-covered Antarctic Lake. Hydrobiologia 347:57-68.

Ward, B. B., J. Granger, M. T. Maldonado, and M. L. Wells. 2003. What limits bacterial production in the suboxic region of permanently ice-covered Lake Bonney, Antarctica? Aquatic Microbial Ecology 31:33-47.

Welch, K. A., W. B. Lyons, E. Graham, K. Neumann, J. M. Thomas, and D. Mikesell. 1996. Determination of major element chemistry in terrestrial waters from Antarctica by ion chromatography. Journal of Chromatography 739:257-263.

Welch, S. A., and W. J. Ullman. 1999. The effect of microbial glucose metabolism on bytownite feldspar dissolution rates between 5 degrees and 35 degrees Celsius. Geochimica et Cosmochimica Acta 63:3247-3259.

Wery, N., U. Gerike, A. Sharman, J. B. Chaudhuri, D. W. Hough, and M. J. Danson. 2003. Use of a Packed-Column Bioreactor for Isolation of Diverse Protease-Producing Bacteria from Antarctic Soil. Applied and Environmental Microbiology 69:1457-1464.

Wharton, R. A., C. P. McKay, G. M. Simmons, and B. C. Parker. 1985. Cryoconite holes on glaciers. Bioscience 35:499-503.

White, D. 2000. The Physiology and Biochemistry of Prokaryotes, 2 ed. Oxford University Press, New York.

Williams, P. J. l. B. 2000. Heterotrophic bacteria and the dynamic of dissolved organic material, p. 153-200. *In* D. L. Kirchman (ed.), Microbial Ecology of the Oceans. John Wiley and Sons, Inc., Publ., New York.

Wilson, E. O. 1992. The Diversity of Life. Harvard University Press, Cambridge.

Wintzingerode, F. v., U. B. Gobel, and E. Stackebrandt. 1997. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiology Reviews 21:213-229.

Zimmer, C., K. K. Khurana, and M. G. Kivelson. 2000. Subsurface oceans on Europa and Callisto: constraints from Galileo magnetometer observations. Icarus 147:329-347.