DIVERSITY, PRODUCTIVITY, AND PHYSIOLOGY OF MICROORGANISMS IN THE STREAM-MOAT-LAKE TRANSITION OF LAKE BONNEY, ANTARCTICA

by

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ABSTRACT

Air temperatures exceeding 0ºC in Taylor Valley, Antarctica 17-25 degree days each summer and constant solar irradiance melt glacial and lake ice to from liquid water moats at the edges of permanently ice-covered lakes. Moats are fed by glacial streams and interact with comparatively large volumes of ice-covered lake water. This study investigated stream influence on moat chemistry and microbial biomass, productivity and diversity in the moat of East Lake Bonney (ELB) and compared the moat to the ice-covered portion of ELB. Stream inflow was a source of dissolved ions, inorganic carbon (DIC) inorganic nitrogen (DIN), and soluble reactive phosphorus (SRP) to the moat. SRP was rapidly removed in the moat near the stream inflow. Melted ELB ice and biological uptake reduced concentrations of DIN and DIC, resulting in a negative relationship to the inflow. Stream nutrients were correlated with high chlorophyll *a* and bacterial biomass near the inflow, were positively correlated with bacterial diversity, and negatively correlated with phytoplankton diversity. Correlations between nutrient availability and microbial biomass suggest resource limitation with respect to DIN and SRP, and infer dependence of heterotrophic bacterioplankton on primary productivity. The data suggest that spatial heterogeneity in the ELB moat is caused by stream inflow, which is influenced primarily by temperature and solar irradiance, and by other factors that could not be determined in this study.

This study also investigated the extracellular release (DPR) of dissolved organic carbon (DOC) by phytoplankton and compared photosynthetic light acclimation of phytoplankton from the moat, and from two depths in four Taylor Valley lakes. DPR incorporated 0.17-2.8 $\mu gC \cdot L^{-1}$, representing 14-58% of total photosynthetic $DI^{14}C$ incorporation. Phytoplankton from the deepest lake samples demonstrated higher acclimation to low irradiance than ice-water interface phytoplankton, determined by measuring primary productivity and DPR at a range of irradiance values. Moat phytoplankton demonstrated less acclimation to low irradiance than both lake strata. DPR was responsive to irradiance, but was less responsive than net primary productivity. Phytoplankton DOC release proceeds at values of irradiance where photosynthesis stops. DOC release is an important aspect of phytoplankton-bacterioplankton coupling in these lakes, where allochthonous inputs are small.

CHAPTER 1

INTRODUCTION

Microbial Ecology: Studies Linking Physical, Chemical, and Biological Parameters in Lotic and Lentic Environments

Wetzel (2001) defines limnology as the "study of the structural and functional interrelationships of organisms of inland waters as they are affected by their dynamic physical, chemical, and biotic environments". The structure of aquatic habitats is defined by physical elements such as heat, light, and mixing by waves and currents. Chemical factors, such as availability of oxygen, nutrients and other dissolved and particulate materials, as well as pH, are equally important components of ecosystem structure. Ecology in aquatic environments combines the structural aspects of physical and chemical limnology with the distribution of aquatic organisms, recognizing that aquatic organisms influence the chemical and physical environment as well as being influenced by it (Horne and Goldman 1994).

The importance of photosynthetic microorganisms in transforming solar energy to chemical energy has been apparent to, and well studied by, limnologists and marine biologists for a number of decades (Wetzel 2001; Castro and Huber 2003). Microorganisms that are not strictly photosynthetic, however, have previously been poorly understood, and thought to be relatively unimportant in natural aquatic environments until recently (Baines and Pace 1991; Kirchman 2000; Cotner and

Biddanda 2002). The fields of limnology and microbial ecology continue to merge as the importance of all microbial life in aquatic habitats is uncovered (e.g. Nüble et al. 2000; Crump et al. 2003; Yannerell and Triplett 2004), and as techniques continue to become available for investigating microbial ecologies in natural environments (e.g. Muyzer and Smalla 1998; Beutler et al. 2002; Fromin et al. 2002; Van Mooy et al. 2004).

The field of microbial ecology has expanded significantly since the time Von Leeuwenhoek first observed microorganisms under his newly invented microscope. Kirchman (2000) referred to the highly controlled culture studies of microorganisms, applying Koch's approach to identifying disease-causing physiology, as microbial autecology. Microbial autecology, focusing on individual instead of microbial group (i.e. synecological) interactions with the environment, has been useful in determining the responses of microbes in well-defined environments (Madigan et al. 2003); though examination of microbial ecology using controlled culture studies alone has severe limitations. One such limitation is that cultivable bacteria often represent less than 1% of actual microbial diversity (Pace 1997). Additionally, researchers such as Brock (1966) realized that microbial ecology could not be accurately described without considering interactions between organisms reacting to multiple environmental variables.

The scope of microbial ecology has expanded in recent years, employing new techniques that are integrated into more classical studies of aquatic ecology (Fromin et al. 2002; Cotner and Biddanda 2002; Beutler et al. 2002; Crump et al. 2003). These new techniques allow researchers to gain understanding of microbial function and diversity under realistic environmental conditions in a variety of habitats (e.g. Head et al. 1998;

Christner et al. 2003; Bernard et al. 2005). Technological advances in detecting algal pigment diversity have led to advanced instrumentation that allows instantaneous quantification of phytoplankton diversity and abundance *in-situ* (Beutler et al. 2002). The advent of the Polymerase Chain Reaction (PCR) has been pivotal in the development of a number of molecular techniques which allow ecologists to describe patterns of microbial diversity (Madigan et al. 2003).

Muyzer et al. (1993) introduced Denaturing Gradient Gel Electrophoresis (DGGE) for detection of the diversity of molecular phylotypes in environmental samples. DGGE as a molecular fingerprinting technique is especially useful in ecology when it is utilized in combination with the study of habitat structure to elucidate important ecological drivers to diversity and distribution (Fromin et al. 2002). DGGE and molecular cloning have been used in conjunction with physical and chemical limnology and marine sciences to compare bacterioplankton community composition to ecosystem structure in estuaries, riverine, and coastal environments (Crump et al. 2004). Microbial diversity has also been compared using DGGE to various parameters across biogeographical gradients in lakes that are influenced by stream inflows (Mašín et al. 2003; Lindström et al. 2006; Crump et al. 2003) or in lakes situated in chains across longitudinal watershed gradients (Sorrano et al. 1999). These studies have found that patterns of diversity correlate to hydraulic residence time (Sorrano et al 1999; Crump et al. 2004; Lindström et al. 2005), doubling time and metabolic capacities (Mašín et al. 2003; Crump et al. 2004), primary productivity (Yannerell and Triplett 2004), salinity (Nübel et al. 2000; Bernard et al. 2005), concentration of organic matter (Mašín et al. 2003; Yannerell and Triplett 2004),

lability of dissolved organic matter (Crump et al. 2003), nutrient availability (Sorrano et al 1999; Mašín et al. 2003), and rainfall (Bernard et al. 2005).

The integration of molecular and microbial techniques with limnology is perhaps nowhere more essential than in describing the aquatic ecology of Antarctica's McMurdo Dry Valleys. The relative lack of predatory control on microbial communities (Roberts et al. 2003) and limited influence of allochthonous inputs to McMurdo Dry Valleys aquatic ecosystems (Moorhead et al. 1999) provides an ideal model in which to study the influence of habitat structure on microbial community distribution and productivity. Relatively low terrestrial input of carbon also underlines the importance of carbon cycling, and particularly of photosynthetic carbon fixation as a source of chemical energy to these aquatic systems.

The availability of light is an important factor in the structure of aquatic habitats, delineating the zones of photosynthetic production (Fritsen and Priscu 1999) and influencing the metabolic physiologies of phytoplankton communities (Lizotte and Priscu 1992a). Algae provide chemical energy to aquatic ecosystems by converting solar radiation to carbon as cell material which is recycled in the system by decomposition or dissolved organic carbon (DOC) that is released by the photosynthetic organism (Wetzel 2001). Additionally, products of phytoplankton extracellular release have long been considered an important source of labile DOC for marine and freshwater bacterioplankton (Storch and Saunders 1978, Cole et al. 1982, Fogg 1983) and as such are rapidly utilized by bacterioplankton in comparison to higher molecular weight organic compounds (Nalewajko et al. 1980, Wetzel 2001). Extracellular release of DOC

is a phenomenon that deprives phytoplankton of photosynthetically fixed carbon directly, through loss of photosynthetic effort, and possibly inhibits phytoplankton growth by stimulating the growth of bacteria, which can out-compete larger phytoplankton for limiting nutrients (Currie and Kalff 1984; Cotner and Biddanda 2002).

Study Area

The McMurdo Dry Valleys region is located in eastern Antarctica adjacent to McMurdo Sound (Figure 1.1). At 4800 km^2, the McMurdo Dry Valleys is the largest ice-free region of the continent, situated in the glaciated relief of the Transantarctic Mountains. The climate of the region is that of a cold desert in the most extreme sense; the average annual temperature is ¯20ºC and the annual precipitation averages <10 $cm \cdot yr^{-1}$ (Clow et al. 1988). The region is located at latitude greater than 77 ºS, and as such experiences winter months of complete darkness and extremely cold temperatures, reaching average minimums of ¯45ºC. Summer months in the McMurdo Dry Valleys experience 24-hour sunlight and average degree days above zero (1993-2000) ranging between 17 and 75 (Doran et al. 2002a), providing energy and liquid water which creates a seasonal oasis for microbial life in an otherwise inhospitable landscape.

The McMurdo Dry Valleys landscape consists of barren soils (<0.1% organic material, Campbell et al. 1998) and exposed bedrock, permanently ice-covered lakes, glaciers, and stream beds which remain dry most of the year. Three lake basins: Fryxell, Hoare and Bonney, located in Taylor Valley, Antarctica (Figure 1.1), have been the

focus of long-term ecological research and monitoring in the McMurdo Dry Valleys for more than 12 years (Lyons et al. 2000). Despite harsh environmental conditions (Doran et al. 2002b), research has revealed the presence of microbial life in the glaciers (Christner et al. 2003), soils (Wall and Virginia 1998), rocks (Friedmann 1982), benthic stream and lake mats (Wharton et al. 1983), lakes (Lizotte and Priscu 1998), and the ice perennially covering the lakes (Priscu et al. 1998; 2005) of the McMurdo Dry Valleys.

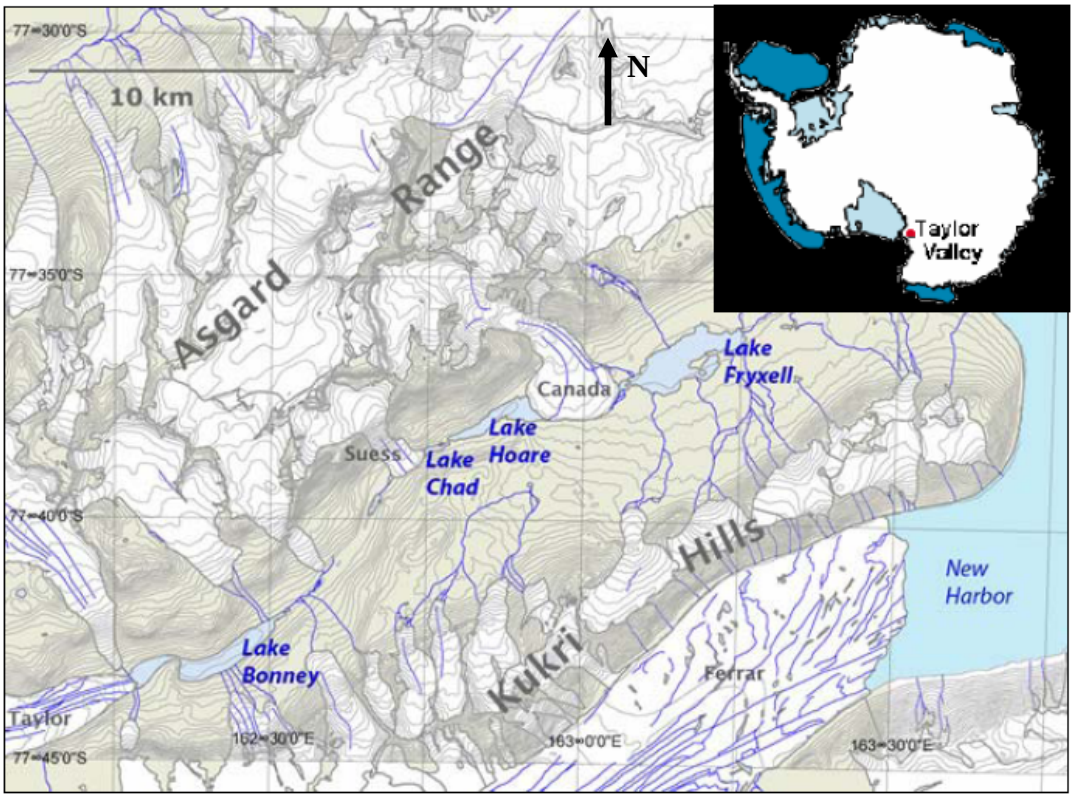

Figure 1.1. Antarctica's Taylor Valley is part of the greater McMurdo Dry Valleys region in Eastern Antarctica. Lake Bonney, Lake Chad, Lake Hoare, and Lake Fryxell are prominent features of Taylor Valley. White regions are glaciated, grey areas are not ice covered, and water bodies are shaded blue (map modified from huey.colorado.edu/diatoms/images/map_taylor_lg.jpg)

Ice-covered lake ecosystems in the McMurdo Dry Valleys region provide stability, in the availability of liquid water, throughout the year. The lakes become photosynthetically active during the austral summer (Fritsen and Priscu 1999; Priscu et al. 1999). Chemical structure is very apparent in the stable water column of these lakes, where a permanent ice-cover inhibits physical mixing and allows only molecular diffusion (Spigel and Priscu 1998). Microorganisms are acclimated in the lakes to the narrow range of available photosynthetically active radiation (Lizotte and Priscu 1992a), to limited nutrient availability in this narrow photic zone (Priscu 1995), to continually cold temperatures (i.e., between ⁻6 to ⁻1 ºC, Morgan-Kiss et al. 2006), and to the months where no solar radiation enters the system (McKnight et al. 2000).

This study focused on the eastern lobe of Lake Bonney (Figure 1.2), which will subsequently be referred to as East Lake Bonney or ELB. Lake Bonney is divided into two basins (east lobe and west lobe) connected by a narrow (20m wide) and relatively shallow (12m deep) sill which prevents mixing of the lake below 12m between the two lobes (Spigel and Priscu 1998). East Lake Bonney has a relatively large surface area (4.3 km^2) and reaches a maximum depth of about 40m. ELB is a terminal lake, having no outflows, and the below-ice water column is permanently stratified due to the aforementioned lake ice-cover and strong salinity gradients; with nutrient-rich, deep waters and a relatively nutrient-depleted, photosynthetically active photic zone (Priscu 1995; Spigel and Priscu 1998).

The Priscu Stream is a second-order stream draining the summer melt from two glaciers and is the primary stream inflow to East Lake Bonney (House et al. 1995). This

stream is 3.8km in length, flowing through a broad and sandy channel and a small pond, and is gauged approximately 120m from the stream/lake confluence (mcmlter.org). In addition to this primary inflow and the narrow connection between the two lake basins, there are three additional streams flowing into ELB; they flow intermittently during the melt season and are not regularly monitored (Bomblies et al. 2004). Flow from West Lake Bonney across the narrow sill between the west and the east lobes contributes the largest volume of water to East Lake Bonney, because stream flow entering the west lobe exceeds flow into the east lobe (Spigel and Priscu 1998).

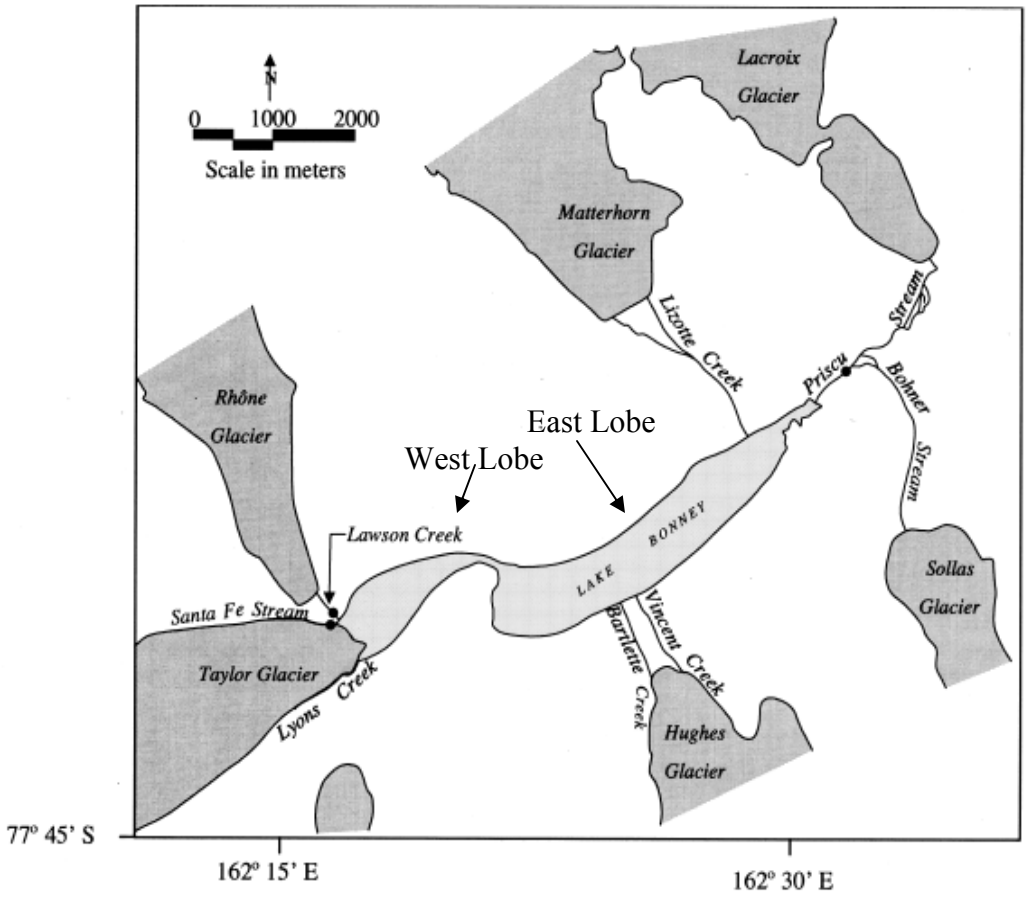

Figure 1.2. The east and west lobes of Lake Bonney are connected by a narrow, shallow sill (modified from Bomblies et al. 2004). Circles indicate stream gauging stations.

Dry Valley Moats

Though moats are features of all lakes in the Taylor Valleys during a part of the summer season, the term "lake" used in this text refers to the permanently ice covered portion of the lake water column. This distinction will be made to simplify comparison of the moat portion of lakes to the below-ice water column portion. The moats in the McMurdo Dry Valleys have received little attention compared to the main bodies of the lakes and little is known of their importance to the greater McMurdo Dry Valleys ecosystem. Moat water has been viewed as an extension of the stream ecosystem in a previous study (Miller and Aiken 1996), and as the means by which gases are exchanged between the below-ice water column and the outside environment (Wharton et al. 1986; Priscu et al. 1996). Previous investigations of moat biology focused on the benthic habitat (Wharton et al. 1983; Hawes and Schwarz 1999; Lawson et al. 2004) and have characterized the light-acclimation of benthic photosynthetic mats to light conditions at different lake depths (Wharton et al. 1983; Hawes and Schwarz 1999). Lawson et al. (2004) used natural carbon stable isotope ratios to compare benthic and pelagic productivity within the moat, underlining the importance of benthic processes to the lake/moat ecosystem. Similarities and differences in carbon and nitrogen stable isotope fractionation also revealed more extensive mixing between the moat and the lake during a warmer, wetter season (Lawson et al. 2004).

Treating the moat as a simple extension of the stream or as part of the below-ice water column appears to be an over-simplification of this feature's importance to the

greater McMurdo Dry Valleys ecosystem. Failing to recognize important moat processes or characteristics represents a gap in the understanding of stream-lake interactions and may represent a failure to consider an important and unique component of ecological interactions in the McMurdo Dry Valleys. A number of moat traits suggest that the moats embody a unique habitat compared to McMurdo Dry Valley streams. Compared to streams, moat water is a lentic environment which possibly supports unique biological populations due to differences such as temporal stability with respect to liquid water availability and aqueous chemistry. The moat is likely connected to and influenced by lake water during part of the summer season, as transfer of dissolved gases between the lake and moat is considered important in maintaining chemical equilibrium in the lake (Wharton et al. 1986; Priscu 1997).

It is likely that the moat is temporarily disconnected from the below-ice water column in the early summer season, and as such, is subject to different environmental conditions than the lake. Because moats are not persistently ice-covered, as the main McMurdo Dry Valleys lakes are, they are subject to extensive mixing with the outside environment. Such mixing includes input from seasonal stream flow and external forces such as wind (Spigel and Priscu 1998). The lack of ice-cover also allows much higher photosynthetically active radiation (PAR) in the moats than in lakes, changes temperature dynamics, and permits physical mixing that may be important to the distribution of biota, nutrients, and other important substrates (e.g. inorganic and organic carbon).

As temperatures, solar radiation, and stream flow increase during the austral summer, towards the end of November and the beginning of December, ice-free moats

form on the margins of McMurdo Dry Valleys lakes. The formation of these seasonal features and the onset of streamflow changes from year to year. Mean daily summer air temperatures fluctuate around zero, with small fluctuations having a relatively large impact on the timing and general availability of liquid water in the Dry Valleys (Fountain et al. 1999). The East Lake Bonney moat can become quite large at the east end of the lake where the primary inflow is located and where the lake basin is relatively shallow compared to the west end. The moat appears to be less extensive in the area connecting the east and west lobes of Lake Bonney (personal observation).

Thesis Statement and Research Questions

The overarching goal of this research is to describe the ELB moat in terms of microbial diversity, geochemistry, and the physiology of microorganisms in acclimating to moat conditions. This description will utilize stream and lake ecosystems as a means of cross-comparison. Focus will be placed on the following research questions:

1. How does an Antarctic stream influence physical and chemical structure in the ELB moat? What influence does this structure have on moat microbial biomass, productivity and diversity?
2. Is the moat a chemically and biologically unique ecosystem, a heterogeneous feature that varies with local conditions, or an extension of the stream or lake environment?

3. Are moat/lake phytoplankton physiologically acclimated to light conditions specific to the moat/lake, as indicated by primary production and DOC release during photosynthesis? How do these acclimations compare in different environments and what is the importance of DOC release in Antarctic lakes?

Relationships Between Chapters 2 and 3

The following chapter (Chapter 2) approaches the first two primary research questions of this work. Chapter 2 first provides a general physical description of the interaction between local climate and stream flow, and possible physical interactions between the stream and the moat related to moat melting and mixing. Following this physical description, Chapter 2 describes patterns of biogeochemistry, microbial biomass, and diversity in the moat of East Lake Bonney as influenced by the Priscu Stream. Description of stream-moat mixing is followed by a description of general moat biogeochemistry, microbial biomass, and diversity as measured in a different summer season.

One of the most obvious differences between the lake moats and the below-ice pelagic environment is the thick ice cover which restricts physical mixing of water beneath the ice (Spigel and Priscu 1998), reduces and modifies the quality of light (Howard-Williams et al. 1998), and reduces interaction with the outside environment. Chapter 3 addresses my third research question, describing the extracellular release of

dissolved organic carbon during photosynthesis. The discussion includes the importance of the cycling of organic carbon in the lake and moat, where allochthonous inputs of organic carbon are limited, making phytoplankton-bacterioplankton inter-relationships important to the cycling of carbon in the ecosystem. Chapter 3 also explores the influence that different values of irradiance, caused by light attenuation by lake ice and water, have on organic carbon production and photosynthetic DOC release in the moat and at two distinct lake depths.

The final chapter (Chapter 4) summarizes the major conclusions and importance of this study, and proposes directions for future moat research in the McMurdo Dry Valleys.

CHAPTER 2

PHYSICAL-CHEMICAL STRUCTURE AND PATTERNS OF MICROBIAL BIOMASS, PRODUCTION AND DIVERSITY IN THE EAST LAKE BONNEY MOAT

Introduction

Lakes experience various degrees of interaction with the outside environment. The physical structure of lakes is influenced by local climate variables such as wind, temperature, precipitation, and landscape shading. Lake chemistry is influenced by weather, local geology, catchment size, terrestrial input of organic matter, stream inflows and outflows, and stream productivity. The degree to which outside influences affect the physical and chemical structure of lakes depends on the magnitude of the external influence and physical extent and stability of the lake (Sorrano et al. 1999; Fietz et al. 2005; Lindström et al. 2006). The immediate area where streams interact with lakes has more recently garnered attention as aspects common to stream chemistry influence pelagic communities (Kling et al. 2000; Crump et al. 2003; MacIntyre et al. 2006). These communities, in turn, modify the chemistry of the stream water, subsequently modifying the availability of chemical inputs that influence that greater lake ecosystem (Kling et al. 2000).

McMurdo Dry Valleys glacial melt feeds streams flowing into ice-covered terminal lakes (Lyons et al. 1998), which are commonly enveloped by a moat of ice-free standing water. These moats have been studied little in comparison to the below-ice lake

environment, and contrast with the lake water column in physical and chemical interactions with the outside environment (Miller and Aiken 1996). Moats, like McMurdo Dry Valleys streams, are subject to seasonal variations of temperature and solar irradiance which strongly influence the amount of available liquid water (Conovitz et al. 1998). Compared to ice-covered lakes of the McMurdo Dry Valleys, the open water of moats allows environmental input of dissolved and particulate matter transported by wind and water, and allows wind-driven mixing, direct surface solar radiation, and free exchange of gases with the atmosphere.

The relative lack of top-down controls on microbial community distribution and microbial processes (Roberts et al. 2003), and the limited influence of terrestrial (allochthonous) inputs (Moorhead et al. 1999) to the aquatic ecosystem make McMurdo Dry Valleys aquatic environments informative models for the examination of physical and chemical environmental controls on microbial community structure. This research focused on the input of stream water into the East Lake Bonney moat and the influence the stream has on moat biota by supplying nutrients to the moat ecosystem. Additionally, a goal of this research was to describe general biogeochemical and biological characteristics for comparison with the lake ecosystem. The general characteristics of McMurdo Dry Valleys' aquatic environments and specifically East Lake Bonney were described previously in Chapter 1.

Streams can be sources of dissolved salts and inorganic nutrients to lake ecosystems (Baron and Campbell 1997; Sickman et al. 2003), and lake processes often transform the state (dissolved or particulate chemical species) and availability of these

stream-derived chemical inputs (Sorrano et al. 1999; Kling et al. 2000). Stream input of nutrients can result in spatial productivity gradients within lakes (MacIntyre et al. 2006). In large lakes, geographical distance alone can result in variations of aquatic chemistry which result in local peaks in production and in the regional distribution of phytoplankton species (Fietz et al. 2005). Moats may be important in modifying the availability of stream nutrients before they enter the lakes, by removing dissolved inorganic nutrients, and converting these nutrients into particulate (cell) material. Moats may introduce or influence biological diversity below permanent lake ice-covers. Chloride, a conservative geochemical tracer in the McMurdo Dry Valleys (Lyons et al. 1998), is important in the approach to determine patterns of hydrologic mixing in dry valley moats. Dissolved and particulate carbon, nitrogen, and phosphorus are compared with distribution of moat microbial biomass, and moat microbial diversity is compared to overall moat physical and chemical structure in this study.

Methods

Physical Measurements

The maximum width of McMurdo Dry Valley moats is easily distinguished when the lakes are completely frozen because ice reformed after seasonal melt is relatively smooth and flat in comparison to permanent lake ice. The maximum width of the ELB moat from the 2004-2005 season was averaged from 25 measurements of the non-permanent ice before the moat thawed during the 2005-2006 austral summer. The north

side average ELB moat width was 14.5m (SD = 4.5m), significantly larger by t-test (t = 3, $p < 0.01$) than the average width of the south shore, which measured 8.8m (SD = 3.2). The shoreline length on the south side of the lake is just over 2% larger than the length of the north shoreline. To simplify calculation of moat surface area (based on the symmetry in length of the two shorelines), the average ELB moat width of 11.1m (SD = 4.9m) was used in the calculation. Moat surface area (m^2) was estimated as the product of the moat width and the moat outer circumference (approx. 12,300m).

The ELB hypsographic curve (Doran et al. 1996) provides a relationship between surface area and depth (Figure 2.1). Using this relationship and the planar lake surface area ($A_{2.5m}$, m^2) at 2.5m (shallowest depth for which lake surface area, A_{lake}, and volume were measured), the discrete moat depth (z_{moat}) corresponding to the largest moat surface area (A_{moat}) was calculated using equation (1):

$$z_{moat} = \frac{2.5m \cdot A_{moat}}{A_{lake} - A_{2.5m}} \qquad \textbf{(1)}$$

The moat depth (z_{moat}, m^2) derived from equation one and moat surface area (A_{moat}, m^2) were used to calculate maximum moat volume (V_{moat}, m^3) as shown in equation (2). The calculation of moat volume shown in equation (2) assumes a linear slope between the lake shoreline and the calculated average of seasonal maximum moat depth.

$$V_{moat} = \frac{1}{2}\left(A_{moat} z_{moat}\right) \qquad \textbf{(2)}$$

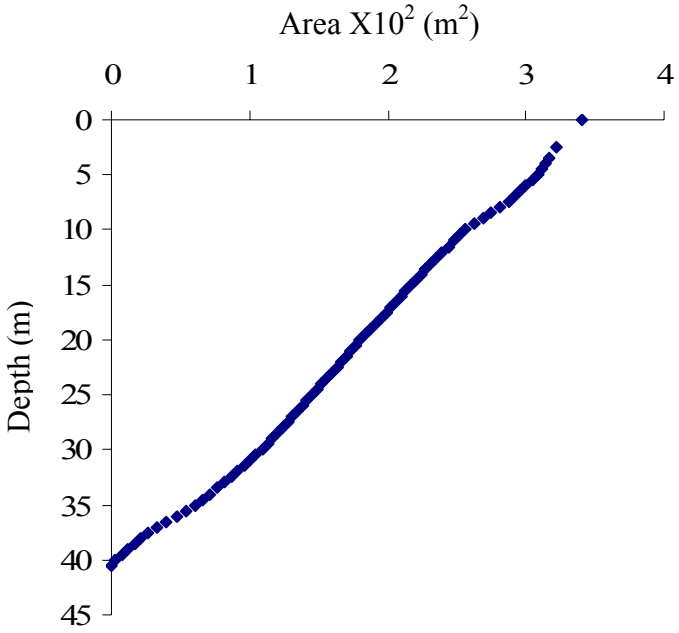

Figure 2.1. The East Lake Bonney hypsographic curve shows the relationship between lake surface area and depth.

Lake Bonney Heat Calculations

Priscu Stream discharge and temperature are logged at a gauging station located approximately 120m from the confluence of the stream and moat at 15 minute intervals during the period of stream flow each season starting in 1993 (McKnight et al. 1994). Temperature and short wave radiation at East Lake Bonney are also recorded every 15 minutes as part of routine data collection by the McMurdo Dry Valleys Long Term Ecological Research program (House et al. 1995). Stream temperature measurements were not available during the 2004-2006 seasons due to a broken temperature logger at the Priscu Stream gauging station.

The heat energy ($E_{15.i}$, J) added during the 15m periods in the stream over which temperature and discharge were logged was calculated using equation (3) as the product of the average stream temperature during the data logging interval ($T_{15.i}$, ºC), the volume of stream discharge ($V_{15.i}$, m^3) over that 15 minute interval, and the specific heat (c_w, $J \cdot g^{-1} \cdot ºC^{-1}$) and density ($\rho_w$, $g \cdot mL^{-1}$) of freshwater. The total heat added over the season ($E_{stream.tot}$, J) was calculated as the sum of heat energy over all 15m periods (equation 4) during the season.

$$E_{15.i} = c_w \rho_w V_{15.i} T_{15.i} \qquad \textbf{(3)}$$

$$E_{stream.tot} = \sum_i E_{15.i} \qquad \textbf{(4)}$$

The mass (m) of moat water as is calculated as shown in equation (5) by the moat volume (V_{moat}) and the density of fresh liquid water (ρ_w) at 0ºC ($0.99 g \cdot mL^{-1}$). The total heat required to provide the surplus moat water ($E_{melt \cdot heat}$, J), or that water which can not be accounted for by stream inflow during the 2004-2005 summer season in the ELB moat, was determined as the product of the mass of moat ice (equation 5) and the latent heat of fusion from ice to water phase ($l_{fusion} = 335 J \cdot g^{-1}$), shown in equation six (6). The temperature of the moat ice was -0.5ºC at the onset of stream flow during the 2004-2005 season (mcmlter.org), and was not included in these heat calculations because the heat required to raise the temperature of the moat ice to 0ºC was 0.0001% of the heat required to change ice from solid to liquid phase.

$$m = \rho_w V_{moat} \qquad \textbf{(5)}$$

$$E_{melt \cdot heat} = m \cdot l_{fusion} \qquad \textbf{(6)}$$

Sampling Procedures

Samples were collected from dry valley moats during the mid-austral summers of the 2004-2006 seasons. The ELB moat was sampled on 18 December 2004 and 15 December 2005, within a few days of routine lake sampling. The ELB moat was also sampled on 3 January 2005, independent of routine lake sampling. Hereafter the sampling dates may be referenced by month and year (i.e. December 2004). Stream and moat samples IDs are labeled with the habitat from which they were collected followed by the season and their proximity to the inflow (see Figure 2.2). For example, Moat 4.2 was a moat sample taken in 2004-2005, and was the second closest moat sample to the inflow.

Samples of lake water from routine sampling sites, and locations along a center line dividing ELB length-wise from the primary inflow to the routine ELB sampling hole (Figure 2.2), were collected using a Niskin bottle lowered through a 20-50cm diameter hole in the ice. HDPE plastic sampling bottles were used to collect moat water from 0.3-0.5m below the water's surface for measurement of moat chemical and biological parameters. Moat and lake sampling were completed in as short a time frame as possible; the large size and difficulty of travel on Antarctic Lakes resulting in a span of approximately 4 h from the time the first sample was collected to the last. *In-situ* spectrofluorescence measurements of algal pigment diversity were made together with moat and lake sampling (see Table B.1 in the appendix for a more detailed description of sampling locations).

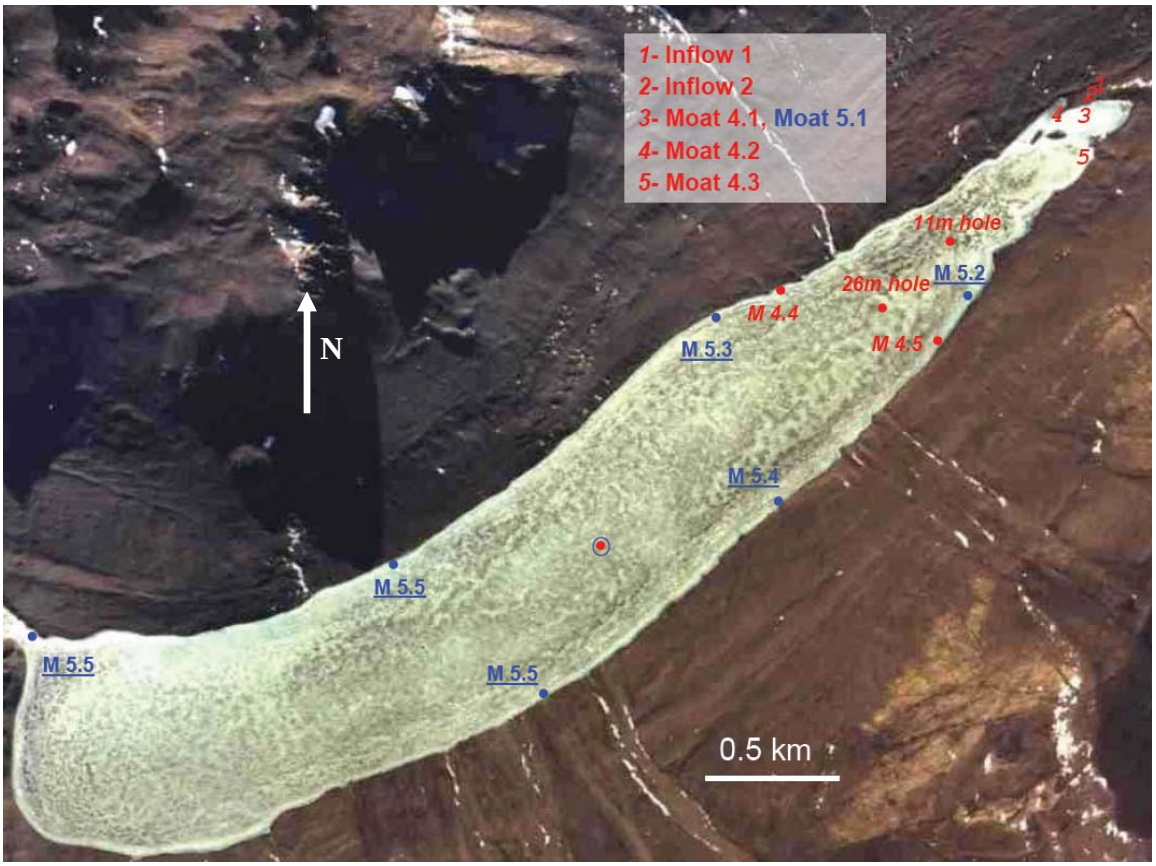

Figure 2.2. Approximate 2004-2005 (red italic) and 2005-2006 (blue underlined) sampling locations are shown on an aerial photograph of East Lake Bonney. Blue and red concentric circles mark the site of routine (>twice annual) lake sampling (photograph modified from http://huey.colorado.edu/LTER/AP9-93/3083/3083-071.jpg).

Aquatic Chemistry

Chemical analysis of stream, moat, moat ice, and lake water samples followed established procedures that have been previously documented in McMurdo Dry Valley research (Priscu 1995; Welch et al. 1996). Major anions and cations, NH_4^+, NO_3^-, NO_2^-, soluble reactive phosphate (SRP), DOC, particulate organic carbon (POC) and nitrogen (PON) from both seasons of this study and chlorophyll *a* from the 2004-2005 season were analyzed by Crary Analytical Services (2004-2005) or by the McMurdo Dry

Valleys Long Term Ecological Research Program Analytical Services (2005-2006). All other described chemical and biological analyses were performed personally. The specific measurements made and the general procedures used will be briefly outlined in the following paragraphs.

Major anions (Cl^-, SO_4^{2-}, F^- and NO_3^-) and cations (Na^+, Mg^{2+}, Ca^{2+} and K^+) were analyzed using a Dionex DX-300 ion chromatography system. Ion chromatography permits high resolution (ppb) detection of aqueous ionic species by measuring the degree to which anions and cations react with oppositely charged ion exchange columns. Ionic strength (I) was calculated for each sample location in Visual MINTEQ® by inputting the measured ionic concentrations, concentrations of dissolved inorganic carbon (DIC), and pH values. DIC and pH values were not measured in moat ice core samples. An estimate of 60μM DIC, based on previous measurements on Lake Bonney permanent ice (Priscu et al. 2005), was used to calculated charge balance and ionic strength for moat ice.

DIC was measured using a LIRA Infrared Gas Analyzer attached to an HP Integrator 3359 or to Peak Simple (v3.29). DIC was converted to $CO_{2(g)}$ by acidification and sparging with $N_{2(g)}$ then passed through the infrared gas analyzer. DIC concentrations were interpreted from a regression equation that was constructing using a $NaHCO_3$ standard curve of known concentrations. DIC concentrations were detected to 0.25mM using this method.

DOC was analyzed using a Shimadzu TOC-V analyzer. DIC was purged from samples before analysis by acidification and gassing with dry, compressed air. DOC is

oxidized to $CO_{2(g)}$ by heat, UV, and persulfate, and the $CO_{2(g)}$ is detected by infrared absorption, as described for DIC.

Particulate organic carbon and particulate nitrogen were collected on GF/F filters (filtering 1000mL of lake water), dried, encapsulated in tin, and passed through a catalyst converting combustion products to CO_2 and N_2 using a Finnegan EA 1112 Flash elemental analyzer. Following combustion of organic matter (C and N), this instrument separates resulting CO_2 and N_2 via gas chromatography, and detects the gases using a thermal conductivity detector. Measurements are standardized using known amounts of Acetanilide.

A Lachat nutrient analyzer was used to measure the concentrations of dissolved inorganic nitrogen (DIN) and soluble reactive phosphate in sample water filtered through Whatman GF/F filters. SRP was measured by the mixed molybdate method (Strickland and Parsons 1968). In this method, sample water is mixed with a solution of molybdate, ascorbic acid, and trivalent antimony. This mixture reacts with phosphate (and arsenic) compounds producing a blue color, in proportion to the SRP concentration. NH_4^+ was measured by the phenol-hypochlorite method (Solorzano 1969), in which phenol and hypochlorite react (catalyzed by sodium nitroprusside) with NH_4^+ to form indophenol blue in proportion to the NH_4^+ concentration of the sample. NO_2^- was quantified using a color-producing (diazo dye) diazotization of nitrate with sulfanilamide and diamine (Wetzel and Likens 2000). NO_3^- was measured as NO_2^- following cadmium reduction (Wood et al. 1967); NO_2^- was measured by diazotization (Wetzel and Likens 2000).

NO_2^- alone was measured on samples without cadmium reduction. NO_3^- was computed as follows:

$$NO_3^- = (NO_3^- + NO_2^-) - NO_2^- \quad \textbf{(7)}$$

Chlorophyll *a* and Primary Productivity

Organic matter from stream, moat, moat ice, and lake samples was collected onto Whatman GF/F filters; chlorophyll *a* was extracted from filtered material using a 1:1 solution of 90% Acetone and Dimethyl Sulfoxide, and measured using a Turner 10-AU-005 fluorometer. Chlorophyll *a* was distinguished from chlorophyll degradation products by acidifying samples after the initial reading, the difference after acidification represents chlorophyll *a*. The Turner fluorometer was calibrated before running samples using dilutions of a chlorophyll *a* stock solution standardized using spectrometry, which represented a range of expected environmental concentrations.

Moat water collected on 3 January 2005 was decanted into 125mL borosilicate bottles for *in-situ* measurement of particulate primary productivity (PPR). Samples were amended with $NaH^{14}CO_3$ (final concentration 0.22 $\mu Ci \cdot mL^{-1}$), and incubated in duplicate for 24 hours. A dark-bottle incubation was included to correct for dark incorporation of $^{14}CO_2$. PAR was monitored at the moat depth of incubation (0.5m) and at the surface over the course of the incubations using a spherical Li-Cor LI-193S*A* quantum sensor and a Li-Cor LI-1000 logger. Particulate matter was collected following incubation onto Whatman GF/F filters. Filters were placed in 20mL glass scintillation vials and 0.5mL 6N HCl was added to filters to remove $DI^{14}C$. Filters were subsequently dried on a

warming plate at 60ºC. ^{14}C was detected on filters with addition of scintillation cocktail by scintillation spectrometry.

In-Situ Spectral Fluorescence

A spectrophotometer (bbe Moldaenke Fluoroprobe®) was used to provide instantaneous *in-situ* measurements of algal group abundance (reported as $\mu gChl\text{-}a \cdot L^{-1}$) based on the fluorescence emission spectra of chlorophyll *a* at 685nm (Beutler et al. 2002). The instrument uses light emitting diodes at 450nm, 525nm, 570nm, 590nm, and 610nm for time-sequential fluorescence excitation and detects chlorophyll fluorescence, pressure (converted to depth), and water temperature. Fluoroprobe® software uses empirically defined algorithms to estimate the proportions of the algal spectral groups: *Chlorophyta*, C*yanophyta, Cryptophyta*, and *Chrysophyta* based on photosynthetic excitation spectra that generally describe each group according to the excitation of group-specific accessory pigments. A 370nm LED excites "yellow matter" and is used to correct for background fluorescence of DOC. *In-situ* spectral fluorescence was used to profile the distribution of algal groups based on accessory pigments during routine lake sampling and the instrument was deployed in the moat when water samples were collected.

Bacterial Enumeration and Measurement

Water samples for bacterial enumeration were immediately preserved with formalin (5% final concentration) upon collection and subsequently stored at 4ºC until

counting. A 1X TBE buffered solution of SYBR Gold was added to the water to stain the bacteria, incubated for 15 minutes in the dark, and filtered at low vacuum pressure onto a 0.2µm membrane filter. The filter with stained bacteria was mounted on a glass slide and a 0.1% solution of p-phenylenediamine was added to prevent fading. Bacterial samples were later counted at 1000X magnification in at least 10 different fields or until 300 cells were counted. A sample blank slide was prepared substituting deioniozed water filtered through 0.2µm membrane filters for the sample, to ensure that slides were not contaminated at any point during slide preparation. Cells were distinguished as coccoid, rod, and filament morphologies in counting and in measurement and calculations of biovolume. Filamentous bacteria were defined during microscopic counting by length > 5µm.

Digital images of SYBR Gold stained bacteria were processed and analyzed using SigmaScan®, which was distance-calibrated using the digital image of a micrometer scale. SigmaScan® measured major and minor cell axes, cell area, and volume. As SigmaScan® often poorly estimated, or failed to estimate, the volume of filamentous cells, volumes were often calculated using the equation of a cylinder. Bacterial biovolumes were converted to carbon biomass using a conversion of 350 $fgC \cdot \mu m^{-3}$ (Bratbak 1993).

Shannon-Weaver diversity indexes were calculated for bacterial morphology (H'_{bac}) and phytoplankton pigment diversity (H'_{phyto}) in each sampling location using cell morphology and defined phytoplankton pigment groups as operational taxonomic units (OTU's). Only three bacterial morphotypes were considered in the calculation of H'_{bac}

compared to four distinct groups of phytoplankton defined by pigment diversity in the calculation of H'_{phyto}. The proportion of the number of cells/biomass grouped according to a particular OTU (p_i) was determined by dividing the number of cells/biomass belonging to a particular OTU (n_i) by the total number of all cells/biomass in that sampling location (N), as shown in equation (8). The calculation of H' is shown in equation (9), in which s is the number of OTU's.

$$p_i = \frac{n_i}{N} \qquad \textbf{(8)}$$

$$H' = \sum_{i=1}^{s} p_i \cdot \ln(p_i) \qquad \textbf{(9)}$$

PCR/DGGE

Environmental DNA was collected on 0.2µm Pall Gelman Inc. SUPOR® filters (47mm diameter) by filtering 500-1000mL of stream, moat, or lake water. Filters were immediately preserved by freezing in liquid nitrogen. Samples were transported and stored at ⁻80ºC prior to analysis. DNA was extracted from the filtered stream, moat, and lake water samples using a MoBio Ultraclean Soil DNA kit, stored at ⁻20ºC < 30 days, and subsequently analyzed using the PCR/DGGE method described by Muyzer et al. (1993) at a later date.

Environmental DNA was amplified by the PCR using 1.5µL of Bacteria-specific primers 341F-GC (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-'3) and 534R (5'-ATTACCGCGGCTGCTGG-3'), and Eppendorf MasterTaq® kit buffers (5µL 10X buffer, 10µL 5X buffer), amended with

$MgCl_2$ to a final concentration of 3mM and *Taq* polymerase (2.5U) to yield a 50µL mixture. The primers used are those originally described in a study by Muyzer et al. (1993) containing a large G/C tail that binds denaturing double strands of DNA into branched segments. PCR amplification was performed with an Eppendorf Mastercyler gradient thermocycler using the following program: initial denaturation at 94ºC for 3 minutes, 30 cycles of denaturation/annealing /extension (94ºC for 45 seconds, 57ºC for 30 seconds, 72ºC for 1 minute), and a final extension period at 72ºC for 7 minutes. PCR products were visualized on an agarose gel before DGGE to verify the presence of amplified DNA and the purity of the PCR product.

DGGE was used to detect single base-pair mutations in the amplified, GC-clamped PCR products. 10% acrylamide gels were prepared with 40% acrylamide-bisacrylamide (37.5:1), 1X TAE (40mM Tris [pH 8.0], 20mM acetic acid, 1mM EDTA). The denaturing gradient was created in the gels by urea and formamide in a gradient of 40-60%. Upon loading samples, a Biorad DCode ^{TM} universal mutation detection system was used in the electrophoresis of amplified DNA with GC clamp at 60ºC for approximately 8.5 hours at a voltage of 70V. Following electrophoresis, DGGE banding patterns were visualized and photographed under UV light after staining of the DNA with a solution of SYBR Gold in 1X TAE (40mM Tris-acetate [pH 8.0], 1.0mM EDTA) buffer. DGGE banding patterns were used to create unweighted pair group method with arithmetic mean (UPGMA) clustering dendograms with GelCompar® software.

Statistical Analysis of Stream Influence on the Moat

In the discussion describing how stream inflow influences moat chemistry, changes in the concentration of various parameters (i.e. Cl^-, DIN, DIC) will be related to distance (m) from the stream inflow by linear correlation and regression. I chose to relate chemical concentration in the stream and moat to this distance measurement as a proxy for physical mixing parameters (i.e. turbulent mixing, diffusion, hydraulic residence time), because a more detailed description of the moat physical environment and mixing was beyond the scope of this work. Changes in chemical concentration with respect to distance to the inflow will be discussed using percentage linear decrease. Percentage linear decrease with distance from the inflow ($\%\cdot m^{-1}$) was calculated, as shown in equation (10), by dividing the slope of linear regressions relating distance from the inflow to chemical concentration ($\mu M\cdot m^{-1}$) by the y-intercept (μM). This metric will provide a means for comparing proportional changes in concentrations over distance.

$$\%\cdot m^{-1} = (\text{slope/y-intercept}) \text{ X } 100 \quad \textbf{(10)}$$

Biological variables (chlorophyll *a*, bacterial density and biomass, and Shannon-Weaver diversity measurements) were also examined using linear regression and correlation to distance from the inflow. The influence of multiple chemical parameters on predicting biological variables was determined using stepwise linear regression (Table 2.1). Stepwise regression selects independent variables to be included multiple linear regression equation from a list of candidate variables. Independent variables (predictors) were added to the equation by how well each candidate variable described variation in the data when the variable was included in the equation, determined by SigmaStat®.

SigmaStat® ignores missing data values in stepwise regression analysis, comparing only samples that are represented with a value for each parameter. In cases where candidate variables tested by stepwise linear regression were below detection limits (i.e. SRP, PON), data were first analyzed for predictive influence with the incomplete sets of variables included. If data from incomplete sets of variables were not included in predictive equations, all data was re-analyzed with the incomplete sets of candidate variable omitted, such that all candidate variables of interest were tested.

Table 2.1. Variables tested in stepwise linear regression analysis of Priscu Stream and ELB moat samples. DIC = dissolved inorganic carbon, DIN = dissolved inorganic nitrogen, SRP = soluble reactive phosphate, DOC = dissolved organic carbon, POC = particulate organic carbon, PON = particulate organic nitrogen.

Tested as Dependent Variable	Candidate variables
Chlorophyll *a*	DIC, DIN, SRP
Bacterial Density	DIN, SRP, DOC, POC, PON, Chlorophyll *a*
Bacterial Biomass	DIN, SRP, DOC, POC, PON, Chlorophyll *a*
Phytoplankton Diversity (H'_{phyto})	Distance to the inflow, DIN, SRP, Chlorophyll *a*, bacterial density, bacterial biomass
Bacterioplankton Diversity (H'_{bac})	Distance to the inflow, DIN, SRP, DOC, POC, PON, Chlorophyll *a*, bacterial density

Chlorophyll *a*, POC, and PON are inherently related because they measure, in part, chemical components of phytoplankton. Bacterial biomass is calculated from bacterial density, meaning that these measurements are also intrinsically related. The inclusion of such non-orthogonal variables in stepwise linear regression analysis only influenced the resulting predictive equations if multiple non-orthogonal variables were ultimately included in the equations. As the results will show for each test, no predictor

variables that shared an intrinsic relationship were ultimately included in stepwise regression models, thus rules of orthogonality were not violated.

Stepwise linear regression was tested as both a dependent and independent variable. Chlorophyll *a* was tested as a dependent variable to determine if there was correlation between algal biomass, using chlorophyll *a* as a proxy for biomass, and inorganic nutrients and DIC. Chlorophyll *a* was tested as a candidate variable in separate tests to reveal potential influences by algal biomass and bacterial density, bacterial biomass, and microbial (phytoplankton and bacterioplankton) diversity.

Results

Physical Measurements

The maximum surface area of the 2004 ELB moat measured 1.37×10^5 m^2. Assuming a moat depth of 1.9m, the maximum volume of the ELB moat is estimated to be $2.56 \times 10^5 m^3$. At its largest size, the 2004 ELB moat accounts for 4% of the nearly $3.4 \times 10^6 m^2$ lake surface area and only 0.35% of the 7.24×10^7 m^3 lake volume. Priscu Stream discharge (1993-2006) and heat input (1993-2002) into the ELB moat and the comparison of moat ice that is potentially melted by stream input, referenced to the 2004-2005 maximum moat volume, are reported in Table 2.2.

Priscu stream cumulative annual discharge was highly variable comparing seasons from 1993-2006, with the range spanning an order of magnitude (Table 2.2). The average of cumulative annual discharge between the 2001-2006 summer seasons was

nearly 70% greater than the average of cumulative annual discharge between the 1993-2001 seasons, though the difference is not statistically significant by t-test ($p > 0.1$). Of the two seasons during which this study took place, 2005-2006 total season discharge was nearly 25% greater than the 2004-2005 discharge. From 1993 to present, the 2000-2001 and 2001-2002 seasons' discharges represent the lowest and highest annual stream discharge measurements, respectively. The annual heat input by the stream from 1993 to 2006 spanned two orders of magnitude. The standard deviation exceeded the mean (CV>100%) for cumulative annual discharge measurements and cumulative heat input into Lake Bonney from the Priscu Stream.

Table 2.2. Priscu Stream discharge and heat input to the moat from 1993-2006. The maximum volume of moat ice which can be melted by the heat input and the percentage of lake/moat ice which could have been melted by the stream are compared to the 2004-2005 moat volume (2.56×10^5 m^3) that could not be accounted for by Priscu Stream input. Discharge alone is shown in five seasons because of missing stream temperature data.

Season	Annual Discharge (m^3)	Stream Heat Input (GJ)	Ice that can be melted by stream (m^3)	% of moat ice melted by stream
1993-94	6.35×10^4	1550	4570	2.8%
1994-95	4.12×10^4	-	-	-
1995-96	7.53×10^4	1340	3950	2.4%
1996-97	7.10×10^4	1530	4510	2.8%
1997-98	1.12×10^5	1120	3300	2.0%
1998-99	7.81×10^4	1600	4720	2.9%
1999-00	4.63×10^4	898	2650	1.6%
2000-01	2.54×10^4	517	1530	0.9%
2001-02	5.18×10^5	85700	25300	15.6%
2002-03	1.05×10^5	-	-	-
2003-04	1.86×10^5	-	-	-
2004-05	9.43×10^4	-	-	-
2005-06	1.25×10^5	-	-	-
Average (SD)	1.18×10^5 (1.27×10^5)	11800 (29900)	6320 (7750)	3.9% (4.8%)

The hydrograph shown in Figure 2.3 illustrates within- and between-season variability of the Priscu Stream flow rate and the cumulative discharge during the stream flow seasons of 2004-2005 and 2005-2006. The Priscu Stream had a relatively early pulse in discharge resulting in high stream input to the ELB moat in late November to mid-

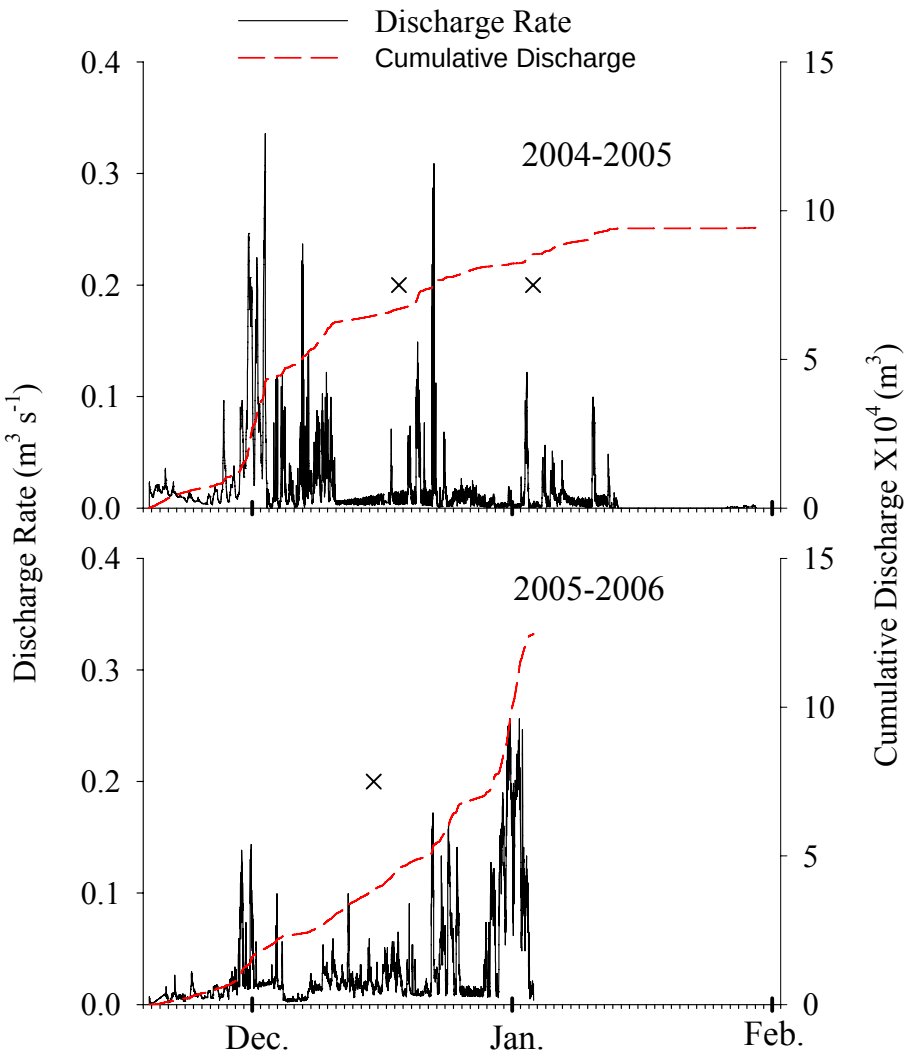

Figure 2.3. Priscu Stream discharge rate (solid) and cumulative seasonal discharge (dashed) during the summers of 2004-2005 and 2005-2006. Sampling dates are marked with ‘x’.

December 2004. There was a comparatively smaller peak in flow rate during roughly the same period of 2005 (Figure 2.3), with a later and larger peak in flow in late December 2005 to early January 2006. Cumulative discharge at the end of the 2005-2006 season exceeded cumulative discharge at the end of the 2004-2005 season by 33%.

Measurements from the Lake Bonney meteorological station were not available after 4 January 2006. Table 2.3 summarizes temperature and incoming radiation extremes and averages during the moat study seasons (2004-2006) between 1 December and 4 January of each summer season.

Table 2.3. Lake Bonney average, minimum, and maximum air temperature (ºC) and photosynthetically active radiation (PAR, µmol photon·m^{-2}·s^{-1}) between December 1 and February 1, 2004-2005 and 2005-2006.

	2004-2005		2005-2006	
	Air Temp	PAR	Air Temp	PAR
Average	-0.4	531	0.1	593
Max	7.9	1843	6.1	1657
Min	-10.4	32	-7.0	68

Moat and Stream Chemistry

Tables A.1 and A.2 (Appendix A) show concentrations of major anions and cations from stream, moat, and lake samples collected during the 2004-2005 and 2005-2006 seasons. Cl^- and Na^+ were the most abundant ions in all locations sampled at all dates, with the exception of $Ca^{2+} > Na^+$ in Moat 4.4 (3 January 2005). Concentrations of major ions varied from one sampling location to another, with ionic strength ranging from $2x10^{-4}$ to $2x10^{-3}$ (no units) for all 2004-2006 sampling dates in the ELB moat. Stream Cl^- concentrations were 1.5-4.5 times higher than all moat samples during stream

and moat sampling dates in 2004-2005, and stream chemistry was not measured during the 2005-2006 season. Ionic concentrations, as indicated by Cl^- and ionic strength, were 40-50 times higher near the ice-water interface (6m = 2.5mM Cl^-) than in the ELB moat. Total dissolved solids typically increase sharply between 15 and 25m in ELB (Spigel and Priscu 1998).

Out of 21 stream and moat locations sampled during the 2004-2006 seasons, six samples had a charge imbalance exceeding 25% (Table A.2, Appendix A). The charge difference of Moat 4.4, sampled 18 December 2004 was 47%; this sample will not be included in analysis of moat ionic concentrations. The charge difference in four samples from 3 January 2005 exceeded 25% (Inflow 1, Moat 4.2, Moat 4.4, and Moat 4.5); discussion of changes in moat ionic concentrations from this date will be limited. Comparison of major anions and cations from moat ice cores revealed charge differences between 40% and 70%. Very large charge imbalances in moat ice cores were probably the result of contamination by HCl-washed containers during ice-core processing. The discussion of moat dissolved salts will be limited to a comparison of stream inflow in determining the source(s) of moat water. Despite high chloride concentration from moat ice cores relative to other anions and cations, average ELB moat ionic strength was an order of magnitude higher on 15 December 2005 than average ionic strength of moat ice cores, though the average moat chloride concentration was only 3 times higher than average ice core [Cl^-]. The relatively low ionic strength of moat ice indicates that moat ice is not a major source of dissolved salts to the ELB moat.

The concentrations of all major ions as indicated by ionic strength was negatively related to distance from the stream/moat confluence through all moat locations (Inflow 2-Moat 4.5) sampled on 18 December 2004 as shown by linear regression in Figure 2.4 ($r^2 = 0.87$, $p = 0.02$). Figure 2.4 also illustrates the negative relationship between chloride concentration and distance from the inflow ($r^2 = 0.71$, $p = 0.07$), chloride decreasing in concentration $0.03\%{\cdot}m^{-1}$ with distance from the inflow during the 18 December 2004 sampling.

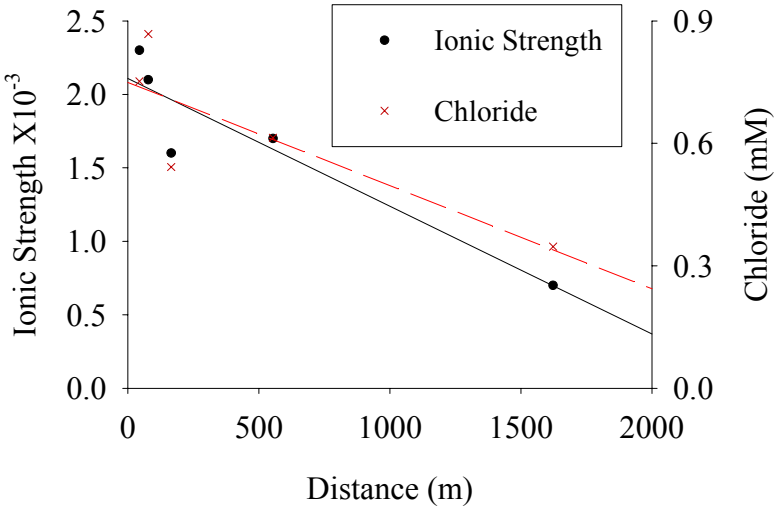

Figure 2.4. Moat ionic strength and chloride decreased linearly with distance from the inflow on 18 December 2004. Chloride linear regression ($r^2 = 0.71$) with respect to distance from the inflow is shown as a dashed line and linear regression of ionic strength ($r^2 = 0.87$) is illustrated by the solid line. Note that the left y-axis values should be $X10^{-3}$ to yield actual value of ionic strength.

No significant relationship ($p > 0.1$) could be determined between the distance from the inflow and ionic strength nor chloride when the moat was sampled on 15 December 2005 using regression analysis. In fact, the highest chloride values measured in December 2005 were not close to the inflow, but on the southeast side of the lake, 850m

(Moat 5.2) and over 3km (*Moat 5.4*) from the Priscu Stream inlet. On the west side of ELB, moat waters were relatively low in ionic strength (Moat 5.5, Moat 5.6 and Moat 5.7). Ionic strength in Moat 4.1/5.1, the only location sampled during both seasons, was 79% higher in December 2005 than in January 2005, but 29% lower than the December 2004 measurement.

Concentrations of moat dissolved salts were most variable on 15 December 2005 compared to other sample dates (see Figure 2.5). Ionic strength coefficient of variation (CV= [standard deviation/average] X 100), representing all measured ionic species, was 53% on 18 December 2004 and 61% on 15 December 2005 in the ELB moat. CVs calculated for all major ions in 6m lake samples (taken from 3 locations, Figure 2.2) were between 3.5-10.1% in mid-December 2004, revealing low spatial variation at that depth compared to spatial variation measured in the moat.

Table A.3 (Appendix A) shows stream, moat, and lake measurements of dissolved and particulate nutrients, and includes other lake parameters and measures of phytoplankton productivity (PPR), chlorophyll *a* biomass, and bacterial density (cells·mL^{-1}).

Priscu Stream was a source of phosphorus to the ELB moat during the 2004-2005 season. Inflow 1 SRP concentration was 0.77μM in December 2004 and 0.36μM in January 2005. SRP was below detection limits (<0.1μM SRP) in all moat locations when sampled in December 2004 and 2005, with the exception of Moat 5.7 (0.3μM). SRP concentrations were 1.3μM and 1.4μM for Moat 4.1 and Moat 4.5, respectively, when measured on 3 January 2005, and below detection limits (<0.1μM SRP) in other moat

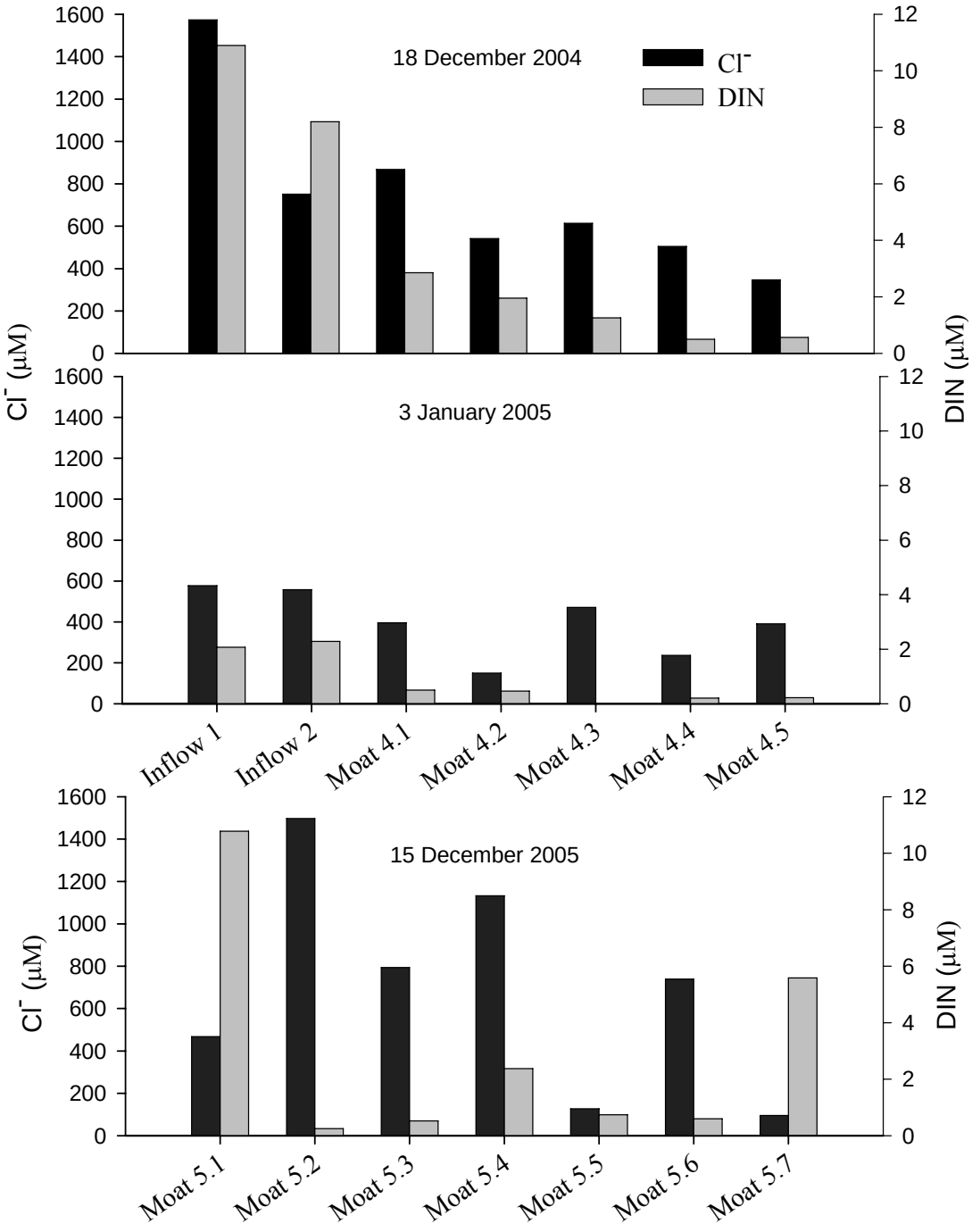

Figure 2.5. Chloride (Cl^-) and dissolved inorganic nitrogen (DIN) measured in the ELB moat during the 2004-2006 summer seasons. Note that the category axis for 15 December 2006 is different from the other two dates. Sampling locations are arranged along the x-axis from left to right in order of proximity to the confluence of Priscu Stream with the ELB moat.

locations. SRP averaged 0.2μM at 6m when sampled in December 2004 and was below detection limits (<0.1μM SRP) beneath lake ice at depths greater than 10m in January 2005, and was also below detection limits in moat ice cores.

Priscu Stream and East Lake Bonney are possible DIN sources to the ELB moat; the highest concentration of ELB stream and moat DIN was measured in the Priscu Stream on 18 December 2004 (10.9μM), within 3% of DIN concentrations measured from Moat 5.1 on 15 December 2005 and from 6m in both lake sampling holes that were sampled on 18 December 2004. Lake DIN subsequently dropped 16% to 8.9μM (11m hole) and 50% to 4.8μM (26m hole) on 3 January 2005, and DIN measured in the Priscu Stream decreased more than 80% between 18 December 2004 and 3 January 2005.

DIN generally decreased with distance from the Priscu Stream inflow on 18 December 2004 as depicted in Figure 2.5. DIN decreased between Inflow 1 and Inflow 2 from 10.9μM to 8.2μM on 18 December 2004, a decrease of $0.6\%\cdot m^{-1}$ with respect to DIN concentration at Inflow 1. The distance-related, proportional decrease in DIN at the stream/moat confluence increased to nearly $2\%\cdot m^{-1}$ between Inflow 2 *a*nd Moat 4.1. Decreasing moat DIN concentrations (Moat 4.1 through Moat 4.5) were described by a negative linear relationship to distance from the inflow ($r^2 = 0.85$, $p = 0.03$) on 18 December 2004, a proportional decrease in DIN with distance from the inflow of $0.05\%\cdot m^{-1}$.

There was no correlation ($p > 0.1$) between DIN and distance from the inflow in the 3 January 2005 ELB moat, though DIN decreased 78% over the short distance between Inflow 2 and Moat 4.1 ($2.3\%\cdot m^{-1}$ with respect to Inflow 2). High concentrations

of DIN observed in Moat 5.1 (Figure 2.5) suggest a stream influence in December 2005, but the Priscu Stream was not sampled, and the moat sampling locations were not organized in a manner (see Figure 2.2) that would allow a stream influence to be detected during the 2005-2006 season.

Priscu Stream (Inflow 1) POC concentrations were similar on 18 December 2004 (16μM) and 3 January 2005 (17μM), as shown in Figure 2.6. POC was positively correlated ($r = 0.97$, $p = 0.01$) with distance from Inflow 1 through Moat 4.3 (POC = 27μM) on 18 December 2004. PON was below method detection limits (<0.7μM) from

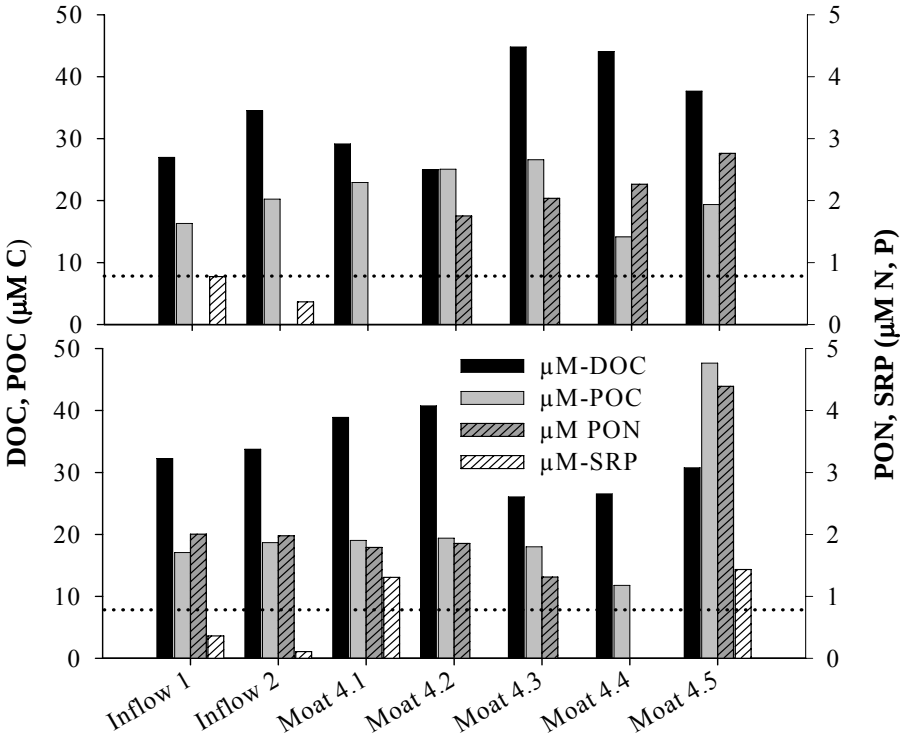

Figure 2.6. Dissolved and particulate organic carbon (DOC and POC), particulate organic nitrogen (PON), and soluble reactive phosphorus (SRP) measured in the ELB moat during the 2004-2005 summer season. Dotted horizontal line represents method detection limits for PON. Sampling locations (x-axis) are arranged from left to right in order of proximity to the confluence of Priscu Stream with the ELB moat.

Inflow 4.1 through Moat 4.1, and increased with distance from the inflow between from Moat 4.2 and Moat 4.5 ($r = 0.88$, $p = 0.1$). DIC was negatively related to distance to the inflow during December 2004 sampling ($r = -0.84$, $p = 0.02$).

Particulate organic carbon varied little from Inflow 1 though Moat 4.3 on 3 January 2005, as indicated by a 5% coefficient of variation (avg. = 18μM). DOC was positively correlated to distance from Inflow 1 through Moat 4.2 on 3 January 2005 ($r = 0.93$, $p = 0.07$). Particulate organic nitrogen was negatively correlated to distance from the inflow through Moat 4.3 on 3 January 2005 ($r = -0.97$, $p = 0.006$), dropping below method detection limits (0.7mM) in Moat 4.4.

Chlorophyll *a* and Primary Productivity

Concentration of chlorophyll *a* in the flowing water was low in the Priscu Stream (average =1.2μg·L^{-1}, SD=1.1) compared to the three moat sampling locations (average= 4.8μg·L^{-1}, SD=1.1) closest to the Priscu Stream (Moat 4.1-Moat 4.3) during the 2004-2005 season. Moat 4.1, Moat 4.2, and Moat 4.3 were 7-12 times higher in chlorophyll *a* than other moat samples in December 2004 and higher than the same samples from January 2005. In December 2004, chlorophyll *a* increased with distance (Figure 2.7) from the inflow in the ELB moat, but only in close proximity to the inflow and not described by linear correlation with statistical significance ($p = 0.18$).

One of the most striking differences between December 2004 and December 2005 was the range in chlorophyll *a* from December 2004 (0.5-5.9 μgChl-a·L^{-1}) compared to the range from December 2005 (0.9-27.9 μgChl-a·L^{-1}), and the high chlorophyll *a*

concentrations of Moat 5.2 and Moat 5.4 (Figure 2.7). Moat 5.2, Moat 5.4, and Moat 5.6 were all located on the south side of ELB and averaged 17.2 $\mu g \cdot L^{-1}$ (SD=8.6), higher when compared by t-test ($p = 0.1$) than Moat 5.1, Moat 5.3, and Moat 5.5, which were located on the north side of the lake and averaged 2.9 $\mu g \cdot L^{-1}$ (SD = 3.1). There was no significant difference as revealed by t-test ($p > 0.1$) in chlorophyll *a* between the north and south shores when the moat was sampled during the 2004-2005 season.

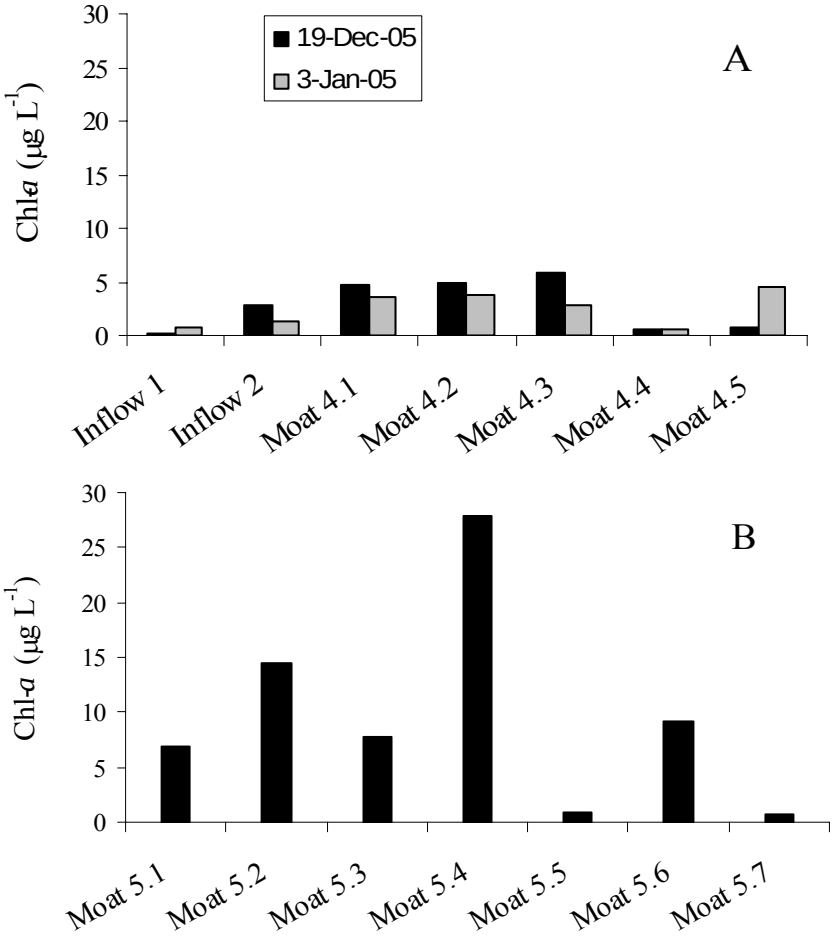

Figure 2.7. Chlorophyll *a* concentrations ($\mu gChl\text{-}a \cdot L^{-1}$) measured during the 2004-2005 season (A) and on 15 December 2005 (B). Sampling locations (x-axis) are arranged from left to right in order of proximity to the confluence of Priscu Stream with the ELB moat.

The statistical potential in DIN, DIC, and SRP to predict chlorophyll *a* concentrations in all ELB moat locations from the three sampling events was tested using a forward stepwise regression, and no significant ($p < 0.1$) predictive variable was found. Forward stepwise regression was repeated omitting December 2005 moat samples. This modified regression revealed that chlorophyll *a* concentration ($\mu gChl\text{-}a \cdot L^{-1}$) may be predicted by DIC ($\mu gC \cdot L^{-1}$) during 2004-2005 season sampling ($r^2 = 0.52$, $p = 0.02$), by the relationship in the following equation.

$$Chl\text{-}a = 0.54*DIC + 1.27 \qquad \textbf{(11)}$$

PPR rates and chlorophyll *a*-specific primary productivity (P_b) in three moat locations on 3 January 2005 are shown in Table 2.4. PPR in Moat 4.2 and Moat 4.5 was more than double the highest PPR measurement in the below-ice water column of ELB (6m) on 10 December 2004. Comparison of chlorophyll *a*-specific PPR (Table 2.4) revealed that moat phytoplankton incorporate inorganic carbon 3-5 times faster than phytoplankton at 6m in ELB. PPR calculations were based on moat DIC measurements of 3.1, 2.9, and 2.5 $mgC \cdot L^{-1}$ in Moat 4.2, Moat 4.3 and Moat 4.5, respectively. Mean *in-situ* (0.5m) PAR during the PPR incubation period was 337$\mu mol\ photon \cdot m^{-2} \cdot s^{-1}$.

Table 2.4. Net primary productivity (PPR, $\mu gC \cdot L^{-1} \cdot day^{-1}$), chlorophyll *a* ($\mu gChl\text{-}a \cdot L^{-1}$), and chlorophyll *a* biomass-specific primary productivity (PPR-P_b, $\mu gC \cdot \mu gChl\text{-}a^{-1} \cdot day^{-1}$) measured in three moat locations on 3-4 January 2005.

	PPR	Chl-a	PPR-P_b
Moat 4.2	10.2	3.7	2.9
Moat 4.3	7.1	2.9	2.5
Moat 4.5	8.3	4.6	1.8
ELB 5m	4.3	6.6	0.6

Bacterial Enumeration and Biovolume

Rod-shaped cells were the dominant morphology according to cell density in moat sampling locations, with the exception of Moat 5.1 and Moat 5.6 from December 2005 (Figure 2.8). Coccoid cells were most abundant (cells·mL^{-1}) in stream sampling locations, with the exception of Moat 5.2, on 18 December 2004. Filamentous bacteria were very low in density in some moat samples and not found in others. The average volume of an ELB filamentous bacterial cell in this study was 4.7μm^3 (SD = 7), more than 30 times larger than the average volume of either rod (average = 0.14μm^3 SD = 0.18) or coccoid cells (average = 0.13μm^3, SD = 0.22). Cell volume was converted to carbon biomass assuming a factor of 350 fgC·μm^{-3} (Bratbak 1993). Comparing bacterial cell density to biomass, filamentous bacterial cells represent a large proportion of biomass compared to the low values of filamentous cell density (i.e. Moat 5.2 shown in Figure 2.8). Conversely, rod and coccoid cells represent large proportions of cell density in some samples but represent a comparatively low cell biomass (e.g. Moat 5.6 shown in Figure 2.8).

Bacterial density in the ELB moat and Priscu stream during the 2004-2005 season was between $4x10^4$-$1.5x10^5$ cells·ml^{-1}. This range compares with, and in some locations exceeds, bacterial density from below the ice in ELB ($3.1x10^4$-$1.3x10^5$cells·ml^{-1}) for the same season (Table A.3, Appendix A). Comparing bacterial density between the two sampling dates of the 2004-2005 season shows that moat and stream bacteria were significantly more numerous as determined by t-test ($p < 0.001$) in all stream and moat sampling locations in January compared to December sampling.

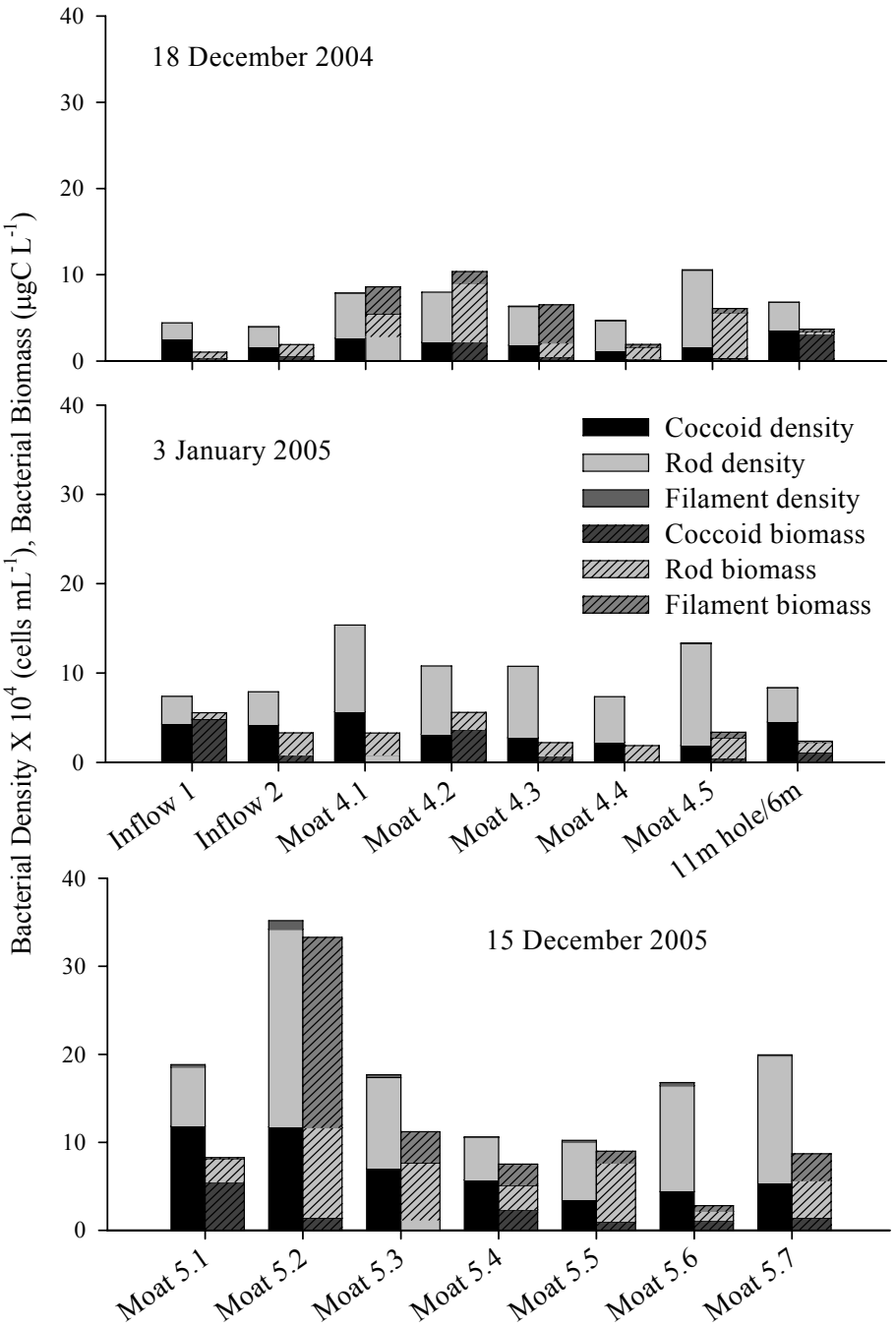

Figure 2.8. East Lake Bonney stream, moat, and lake bacterial density (cells·mL^{-1}) and bacterial biomass (µgC·L^{-1}) from sampling during the 2004-2006 season. Note that the graph showing 15 December 2005 has a different category axis. Sampling locations (x-axis) are arranged from left to right in order of proximity to the confluence of Priscu Stream with the ELB moat.

ELB moat bacterial density during December 2005 sampling was between $1.0x10^5$-$3.5x10^5$ cells·mL^{-1}, larger by t-test ($p = 0.07$) than the ELB moat bacterial density ($4.7x10^4$-$1.5x10^5$ cells·mL^{-1}) from either sampling of the 2004-2005 season (see Figure 2.8). Maximum bacterial biomass was 8.6μgC·L^{-1} (Moat 5.2) in December 2005, compared to a maximum biomass of 5.6μgC·L^{-1} (Moat 4.2) during the 2004-2005 season. Biomass values were generally higher, revealed by t-test ($p = 0.1$) in the 2005-2006 season.

There was no significant difference ($p > 0.1$) in stream and moat bacterial biomass in comparing the two sampling dates of the 2004-2005 season, though the average biomass of all stream and moat samples was actually higher in December (average = 6.7μgC·L^{-1}, SD = 3.2) than in January (average = 3.3μgC·L^{-1}, SD = 1.5). Bacterial density (cells·mL^{-1}) and bacterial biomass (μgC·L^{-1}) decreased with distance from the lake inflow through Moat 4.2 on 18 December 2004 (Figure 2.8), though these trends were not statistically significant by linear correlation ($p > 0.1$).

Neither linear correlation nor stepwise linear regression revealed any chemical variable that could be correlated with bacterial density. 18 December 2004 bacterial biomass was positively correlated with POC ($r^2 = 0.62$, $p = 0.04$). Forward stepwise linear regression showed that only DIN (μM, $r^2 = 0.71$, $p = 0.002$) was a predictive variable to stream and moat bacterial biomass (μgC·L^{-1}) during both sampling events of the 2004-2005 season (equation 11). See table 2.1 for chemical variables that were tested as predictive variables to bacterial biomass.

$$\text{Bacterial Biomass} = 2.75*\text{DIN} + 2.64 \qquad \textbf{(12)}$$

The Shannon Weaver index of bacterial diversity (H'_{bac}) significantly decreased with distance from the inflow (Figure 2.9) in the ELB moat during the 18 December 2004 moat sampling ($r = -0.69$, $p < 0.001$). H'_{bac} was positively correlated with bacterial density on 18 December 2004 ($r = 0.83$, $p = 0.02$). H'_{bac} was negatively correlated to distance from the inflow ($r = -0.73$, $p < 0.001$) and to POC concentrations ($r = -0.89$, $p < 0.001$) on 3 January 2005.

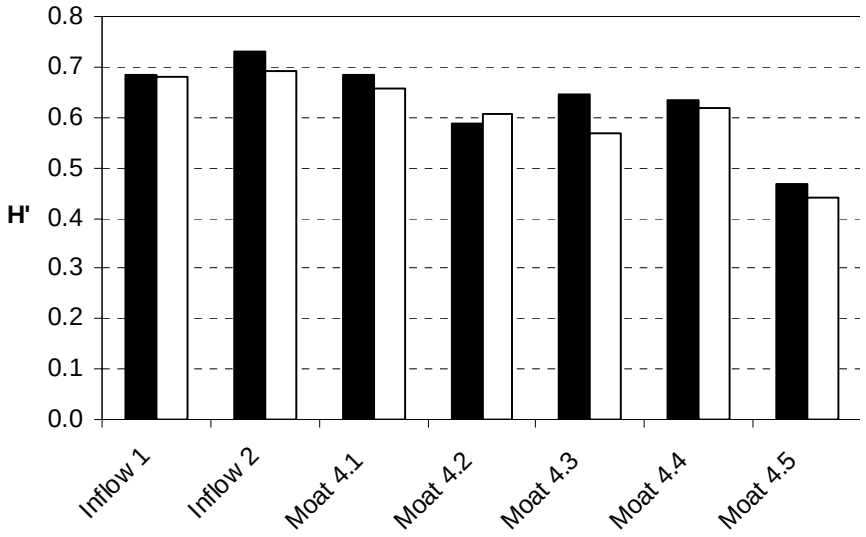

Figure 2.9. Shannon-Weaver diversity index (H') of microbial morphological diversity from 18 December 2004 (black) and 3 January 2005 (white). Sampling locations (x-axis) are arranged from left to right in order of proximity to the confluence of Priscu Stream with the ELB moat.

Phytoplankton carbon biomass was estimated from stream and moat chlorophyll *a* concentrations using a ratio of 50:1 (phytoplankton carbon mass:chlorophyll *a* mass, Cloern et al. 1995) for comparison with calculated bacterial biomass. Calculated phytoplankton carbon biomass was positively correlated with bacterioplankton biomass ($r = 0.72$, $p < 0.001$). Phytoplankton carbon biomass was significantly higher than

bacterioplankton biomass when compared by t-test ($p < 0.05$), the ratio of phytoplankton carbon biomass to bacterioplankton carbon biomass in each stream and moat sampling location was between 4:1 and 165:1 (Ave = 36:1). The phytoplankton:bacterioplankton mass ratio is useful in determining the proportion of moat POC that is phytoplankton compared to bacterioplankton.

In-Situ Spectral Fluorescence

The Fluoroprobe®, used to measure the *in-situ* abundance of algal groups based on spectral fluorescence of accessory pigments, estimates the chlorophyll *a* abundance of algal groups by extrapolating a general spectral excitation curve (specific to each measured group) from various wavelengths of the visible light spectrum. Ideally, the sum of these group chlorophyll *a* estimates would represent a 1:1 relationship with chlorophyll *a* measured by filtration and extraction. Linear regression comparing the Fluoroprobe® estimated chlorophyll *a* ($\mu gChl\text{-}a \cdot L^{-1}$, summed from all measured groups) to total (filtered) chlorophyll *a* ($\mu gChl\text{-}a \cdot L^{-1}$) in the ELB moat (dependent variable: Fluoroprobe® chlorophyll *a*) revealed a slope (no units) of 0.37 on 18 December 2004 ($r^2 = 0.53$, $p = 0.06$), and a slope of 0.05 on 15 December 2005 ($r^2 = 0.8$, $p = 0.007$). Chlorophyll *a* as interpreted by *in-situ* spectral fluorescence consistently underestimated filtered chlorophyll *a*, as the reported slopes were <1.

The underestimation of moat chlorophyll *a* by *in-situ* spectral fluorescence may have been caused by some failure in detecting the algal spectra of moat phytoplankton or by error propagated during laboratory analysis of filtered pigments. I

suggest that both causes of error are realistic explanations for the observed trends. Filtered chlorophyll *a* was underestimated by spectral pigment analysis in both years, evidence of detection insensitivity by the spectral fluorescence method.

Comparison of algal group abundance in the Priscu Stream, ELB, and the ELB moat, determined by *in-situ* spectral fluorescence and represented by chlorophyll *a* biomass, is shown in Figure 2.10. I believe that comparison of relative algal abundance using these measurements is justified by the positive correlation between Fluoroprobe®-estimated and filter-measured chlorophyll *a* despite the underestimation of total chlorophyll *a* using this device.

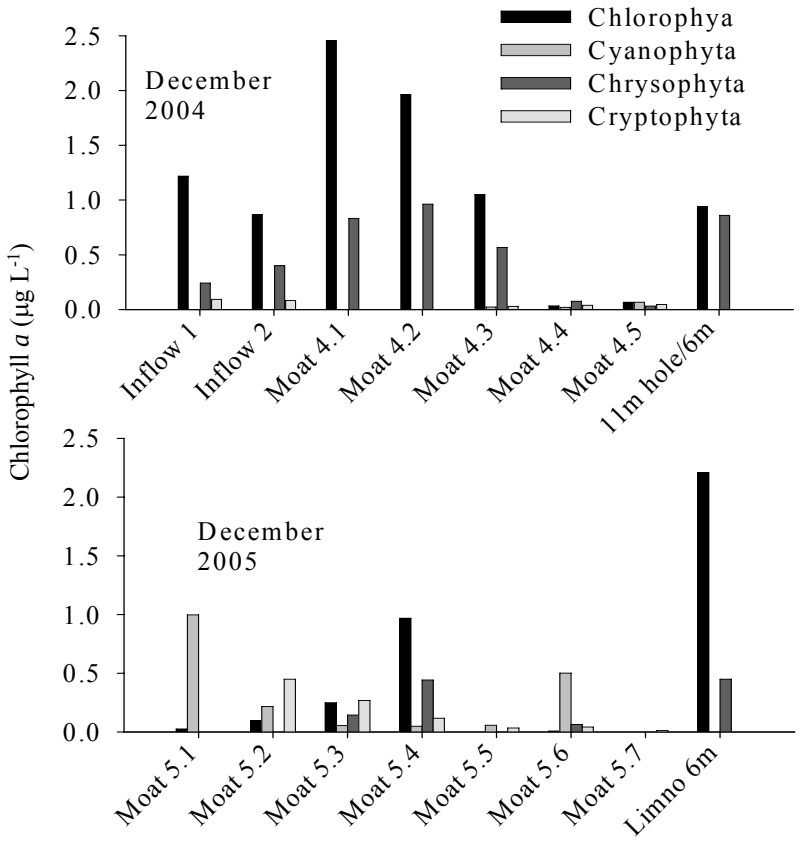

Figure 2.10. Measurements of algal pigment groups as determined by *in-situ* spectral fluorescence in the East Lake Bonney primary inflow, moat, and lake.

The abundance of each phytoplankton group with respect to total chlorophyll *a* biomass was used in the calculation of phytoplankton Shannon-Weaver diversity. The calculated Shannon-Weaver diversity index of moat phytoplankton pigment diversity (H'_{phyto}) was positively correlated to distance from the Priscu Stream inflow ($r = 0.96$, $p < 0.001$) on 18 December 2004 (Figure 2.11). Stepwise linear regression revealed that H'_{phyto} could be predicted by a linear combination of DIC ($\mu gC \cdot L^{-1}$, $r^2 = 0.47$, $p = 0.05$) and chlorophyll *a* ($\mu gChl\text{-}a \cdot L^{-1}$, $r^2 = 0.78$, $p = 0.08$). The potential influence of DIC and chlorophyll *a* on H'_{phyto} is described in equation (13).

$$H'_{phyto} = 1.35 - 0.036*DIC - 0.073*Chl\text{-}a \quad \textbf{(13)}$$

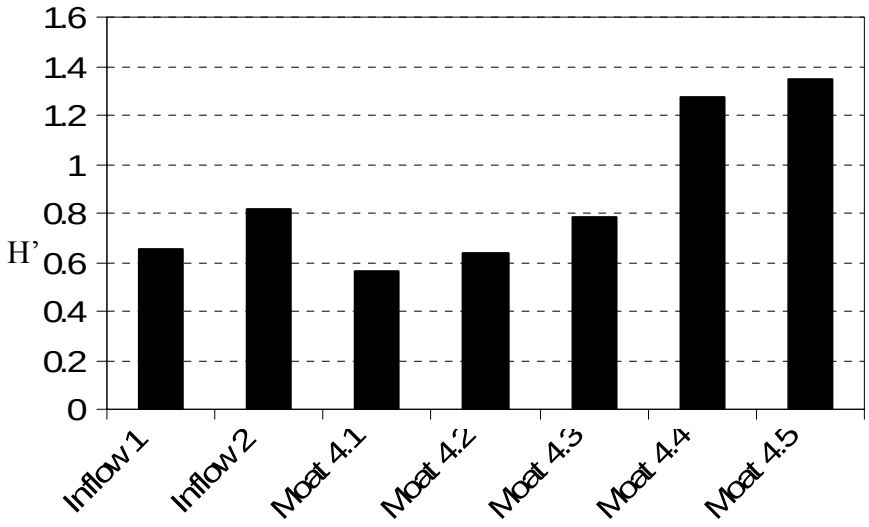

Figure 2.11. Shannon-Weaver diversity index (H') of phytoplankton pigment diversity from 18 December 2004.

DGGE

Samples from the Priscu Stream, the moat, and from below the permanent ELB ice-cover from December 2004 and December 2005 were analyzed and compared by cluster analysis using DGGE banding patterns (Figure 2.12). The comparison of similarities and differences between samples was difficult in some cases due to light bands or few bands representing some samples (Moat 4.3, Moat 5.1, and Moat 5.2), despite high bacterial abundance in these samples (Figure 2.8). Moat 4.3 had only one visible band in the DGGE gel; Moat 5.1 and Moat 5.2 had only three recognizable bands. The sampling locations with the highest band richness (12 visible bands) were Moat 4.2 and Moat 4.5 from December 2004; one sample (Moat 4.4) had 10 recognizable bands, while three samples had eight visible bands (Inflow 2, 26m hole/13m, and 26m hole/25m). Visual examination of PCR-amplified DNA segments on agarose gels before DGGE analysis revealed that similar amounts of DNA (determined visually by band strength) from Moat 4.3, Moat 5.1, and Moat 5.2, compared to other moat samples, were amplified in the near-200-base-pair location on an agarose gel (segment length verified by comparison with DNA ladders).

Visual quantification of PCR-amplified DNA in the target region suggests that biases in extraction and PCR-amplification of environmental DNA were no different for DGGE patterns with few and/or week bands than other stream, lake, and moat samples visualized in this study using DGGE, and that the lack of resolution for these samples was probably due to errors during DGGE analysis. An example of possible causes of error in DGGE analysis is using the wrong mixture of denaturing gradient, resulting in

poor DGGE band separation and the likelihood that different phylotypes may share the same visual DGGE band positions (Muyzer et al. 1993). Another inherent problem in the use of DGGE, specifically using short (~200bp) DNA segments, is the inability to detect mutations in DNA sequence outside of the target region (Muyzer and Smalla 1998).

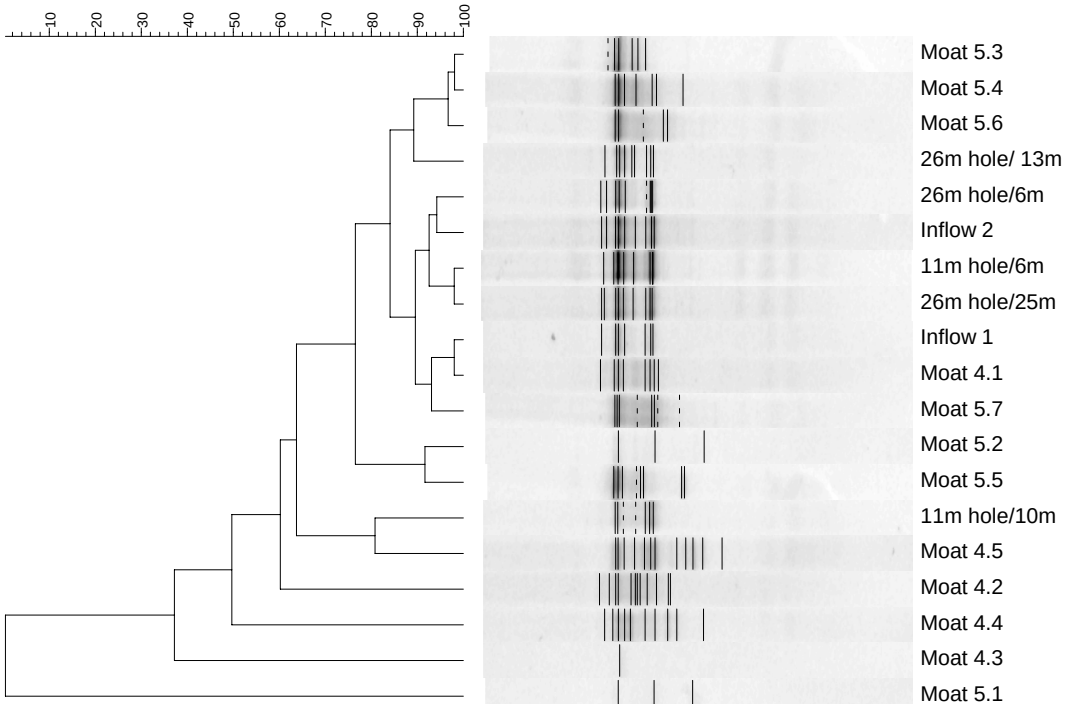

Figure 2.12. DGGE banding patterns (emphasized with lines) of ELB Moat and inflow, and ELB water column from 18 December 2004, along with samples taken from the ELB moat in December 2005. Samples from the ELB water column are labeled with the maximum lake depth at the sampling whole (11 and 26m) and the depth from which the sample was collected. The dendrogram on the left shows percent relatedness of the targeted 16S rDNA segments that were amplified for comparison by DGGE, as determined by cluster analysis.

Figure 2.12 reveals greater than 80% similarity between communities in 11 of 19 sample locations. The 11 samples sharing the highest DGGE similarity represent samples from a diversity of locations; including in and near the Priscu Stream (Inflow 1,

Inflow 2, Moat 4.1), all but two moat locations (Moat 5.2 and Moat 5.5) sampled in December 2005, and 4/5 lake samples. With the exception of Moat 4.1, ELB moat samples shared less than 65% similarity with stream and lake samples, and with moat samples from the 2005-2006 season (except Moat 5.1).

The clustering pattern depicted in Figure 2.13 reveals highest similarity (94%) in algal pigment diversity within the stream. Algal diversity from each subsequent sampling location (with reference to distance from the inflow) shares the highest similarity with the group that is nearer the inflow. For example, Moat 4.2 algal diversity is more closely related to Inflow 1 and Inflow 2 (88% similarity) than to Moat 4.3 (83% similarity). The exception to this pattern is that Moat 4.1 is not in the same clade as Moat 4.2 and Moat 4.3. This pattern describes algal diversity relationships through Moat 4.3. Moat 4.4 and Moat 4.5 make up a separate clade of community phylotypes, sharing less than 35% similarity in diversity with other moat communities.

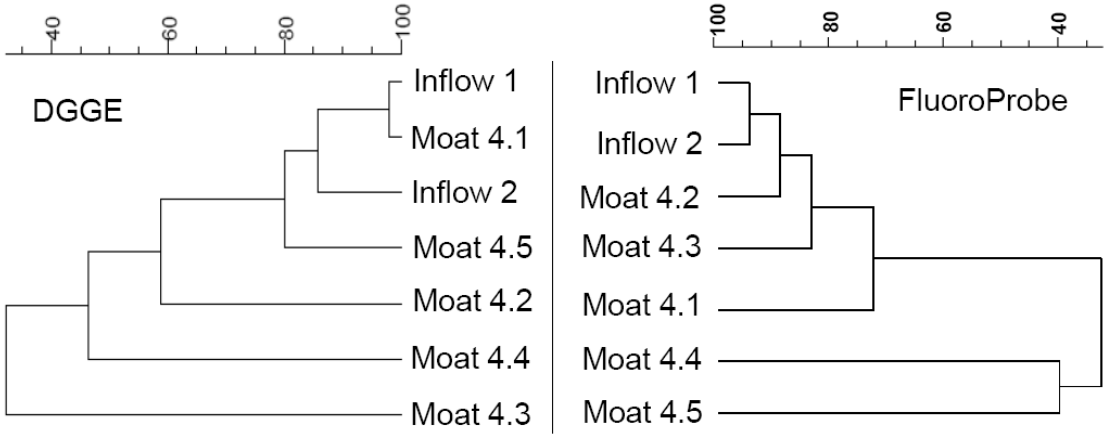

Figure 2.13. Cluster analysis showing relatedness dendograms of DGGE and *in-situ* spectral fluorescence data from the ELB moat, sampled 18 December 2004. Scales show percent relatedness.

The influence of the ELB inflow is less apparent in the clustering of the partial 16S rDNA gene segment visualized by DGGE compared to the clustering of stream and moat algal diversity (Figure 2.13) The two stream sampling sites were grouped with Moat 4.1 (>85% similarity), although Moat 4.1 was more similar to Inflow 1 than to Inflow 2, which can not be explained by the environmental data collected in this study. As was the case with pigment diversity, these samples showed high similarity (>85%) in sequence diversity despite spatial variation in aqueous chemistry (Figures 2.5 and 2.6) and phytoplankton and bacterioplankton biomass (Figures 2.7 and 2.8, respectively) in the stream and moat region between Inflow 1 and Moat 4.2.

Discussion

Physical Moat Description

Water availability in the McMurdo Dry Valleys is limited outside of a few permanently ice-covered lakes (Lyons et al. 1998). The McMurdo Dry Valleys' mean daily temperature fluctuates near freezing during the 4-12 week period when stream flow is observed in the area (Conovitz et al. 1998). The Priscu Stream seasonal hydrograph displays high variability, with unpredictable peaks in discharge and periods of low to no flow (Figure 2.3). Discharge was higher at the beginning of the austral summer than it was during the rest of the 2004-2005 summer season; while discharge was initially low and peaked at the end of the 2005-2006 season. High discharge characteristic of spring melt generally results in a high initial pulse of particulate and dissolved material in

snowmelt-fed streams (Horne and Goldman 1994; Sickman et al. 2003) and though the Priscu Stream and ELB moat were sampled on 18 December 2004 following eight days of relatively low stream flow, stream concentrations of dissolved salts and inorganic nutrients were higher than later sampling of that season (3 January 2005). The aqueous chemical concentration gradients observed in the ELB moat (i.e. Chloride, DIN, DIC) may have been showing the lasting effects of high discharge and stream loading of total dissolved solids in late November and early December 2004 (Figure 2.3).

The austral summers of 2004-2006 provided a snapshot of the Priscu Stream, and subsequently of its influence on the ELB moat, as influenced by periods of warmer temperature and relatively high melt; though the 2001-2002 season was much warmer and stream flow was comparatively high (Tables 2.2 and 2.3). The discharge of the primary East Lake Bonney inflow accounted for approximately 30% of the moat volume during the 2004-2005 season (Table 2.2), while the moat accounted for less than 0.4% of the total lake volume. Generally, the volume of liquid water habitat is higher in the ELB moat than in the Priscu Stream in terms of volume and more consistently available due to obvious differences in stream vs. moat residence times that are illustrated by variability in the stream hydrograph (Figure 2.3).

Although seasonal moat melting appears to be correlated with the onset of glacial melt and stream flow in the Taylor Valley, calculations of heat transfer from the stream show that heat from stream flow represents a relatively small (0.9-14%) potential for melting moat ice. The correlation in timing, therefore, is most likely caused by the same environmental factors that control other melting events in the area, primarily increasing

summer temperature and solar radiation (Dana et al. 1998; Fountain et al. 1998; Fountain et al. 1999).

Fountain et al. (1998) found that high rates of melting from the cliff face relative to the top of McMurdo Dry Valleys glaciers were caused by exposure to solar radiation and that proximity to dark soils that absorb and radiate heat also increases glacier melting rates. Considering the extensive amount of moat melting in shallow areas of Taylor Valley lake basins, including Lake Bonney, it appears that the factors described above also impact the pattern and magnitude of lake and moat ice melting. Dana et al. (1998) found that north-facing slopes in the McMurdo Dry Valleys receive more solar energy than south-facing slopes due to terrain effect, providing an explanation for the average moat width on the south side of East Lake Bonney being almost 40% larger than the moat on the north side of the lake.

Stream and Moat Mixing

Kling et al. (2000) noted higher concentrations of major anions and cations in arctic streams than in freshwater lakes from the same watershed. Weathering of soil and rock material in stream beds generally results in relatively large amounts of dissolved salts, with higher concentrations of major anions and cations observed in comparatively longer streams (Sorrano et al. 1999; Kling et al. 2000). Priscu Stream has high concentrations of major anions and cations, DIN, and SRP relative to the ELB moat, though these concentrations can vary temporally (e.g. Figure 2.5). The Priscu Stream is a source of dissolved salts and inorganic nutrients (DIN and SRP) to the ELB moat. The

influence of distance from the stream inflow on moat ionic strength was apparent by linear correlation (Figure 2.4) in all locations sampled in December 2004.

Chloride has been used in previous Taylor Valley studies as a conservative geochemical tracer in aquatic environments (Lyons et al. 1998; McKnight et al. 2004). Chloride and other dissolved salts and inorganic nutrients decrease in the moat as they are diluted by melted moat ice with low salt content (Figure 2.4). While dilution decreases concentrations of anions and cations that are not biologically useful or chemically active in these aquatic environments, biological processes actively remove nutrients and substrates from the dissolved pool (Lyons et al. 2003). Moat pH is 8.4-9.3 and moat temperature is consistently low indicating that the loss of dissolved nutrients and DOC is probably the result of biological uptake.

McKnight et al. (2004) found that the amount of nutrients delivered by McMurdo Dry Valleys streams depends on the amount of stream microbial mat biomass, with high mat biomass generally reducing the availability of nutrients downstream. However, the sandy stream substrate and periodic peaks in discharge of the Priscu Stream may subject microbial mats to scour, which reduces mat biomass in this stream (McKnight et al. 1998). Inflow 1 and Inflow 2 are located in close proximity to one another (Figure 2.2), but span a large physical transition from a shallow, steeper gradient streambed to a wide, slow-moving channel. Between Inflow1 and Inflow 2 on 18 December 2004, the proportional decrease of Cl^- is $1\%\cdot m^{-1}$, DIN decreased $0.6\%\cdot m^{-1}$, and SRP decreased $1.2\%\cdot m^{-1}$; implying that there was no meaningful biological nutrient uptake along this reach. A 50% reduction of chloride concentration between Inflow 1 and Inflow 2 may be

explained by mixing of the wide stream channel near Inflow 2 with the moat, as dissolved salts in the Priscu Stream exceed anions and cations in the moat ice 3-35 fold.

Chloride decreased 0.2%·m^{-1} on 18 December 2004, as mixed stream and moat water measured at Inflow 2 entered and was further diluted by the ELB moat. Over a distance of 33m between Inflow 2 and Moat 4.2, DIN decreased nearly 2%·m^{-1} and SRP decreased more than 3%·m^{-1} to below method detection limits (0.1μM). The high linear percent decrease in DIN and SRP between Inflow 2 and Moat 4.1 compared to percent decrease in chloride concentration on 18 December 2004 suggests that the uptake of inorganic nutrients exceeded the reduction in nutrients by dilution plus biotic recycling of inorganic nutrients in this part of the moat. The atomic ratio of stream DIN: SRP on 18 December 2004 (Inflow 1) is 14:1, increasing to > 22:1 at Inflow 2. The lack of strong evidence for biotic removal of DIN between Inflow 1 and Inflow 2 and 33% larger drop in SRP compared to DIN suggested increased P-uptake compared to N-uptake between Inflow 2 and Moat 4.1.

Nutrient Limitation

The P-loss near the inflow and below-detection-limit SRP concentrations in 14 of 17 ELB moat locations sampled infer moat P-limitation. SRP is often below detection limits (< 0.1μM) in most of the photic lake water column due to cycling by P-deficient phytoplankton (Dore and Priscu 2001) and SRP was undetected in lake samples in January 2005 from 10m upwards in the water column (Table A.3, appendix). P-limitation of algal growth in natural freshwater environments is a dominant paradigm in

limnology (Schindler 1977), though co-limitation (N+P-limitation), and N-limitation have been discovered in a number of freshwater environments, particularly those in the western US (Sterner and Elser 2002).

Limitation of primary productivity by phosphorus in ELB has been noted in past research (Priscu 1995; Dore and Priscu 2001). Nutrient deficiency can be estimated by comparing moat C: N: P ratios to the Redfield atomic ratio (106:16:1, Redfield et al. 1963). There were few moat locations in which SRP measurements were above the detection limits of 0.1μM-P during this study. The highest moat DIN: SRP ratios were observed at Moat 4.1/5.1 on 18 December 2004 (29:1) and 15 December 2005 (40:1). Local spikes in SRP at Moat 4.1 and Moat 4.5 on 3 January 2005, both nearly twice the 18 December inflow concentration, resulted in the lowest measured moat DIN: SRP molar ratios of 1:2.6 and 1:6.3, respectively. The heterogeneous nature of N and P availability in the ELB moat results in areas of local P abundance with respect to N, and other areas of relative P deficiency with respect N as described by the Redfield ratio. Eight of the 10 ELB stream and moat sampling locations, where PON and POC were above PON detection limits (>0.7μM), exceeded the Redfield C: N atomic ratio (6.6:1) by a factor of 1.3-2.2, suggesting moat planktonic N-deficiency with respect to C.

As stream SRP is immediately depleted upon entry into the moat and DIN consistently decreases with distance from the inflow, Moat 4.4 and Moat 4.5 may experience more acute limitation of both N and P compared to moat locations closer to the inflow. N and P co-limitation has been demonstrated in freshwater environments where diverse natural phytoplankton assemblages have different nutrient requirements,

and an algal growth response to nutrient addition is only measured in response to addition of both N and P (Dodds et al. 1989). Cycling of nutrients from particulate to dissolved form may involve continuous or temporally dynamic nutrient fluxes between the benthic and pelagic moat microenvironments, identical to nutrient spiraling in streams (Dodds et al. 2004), and further complicating the general description of moat resource limitation.

Stream Influence on Moat Microbial Biomass

Stream loading of dissolved nutrients can result in patchy ecosystem structure as planktonic communities near a nutrient source, such as a stream inflow, are often more biologically productive (Fietz et al. 2005; MacIntyre et al. 2006). Linear correlation revealed a significant negative relationship between DIC and distance from the inflow ($r = -0.84$, $p = 0.02$) in December 2004, and a possible predictive relationship between DIC and chlorophyll *a* (equation 11) during the 2004-2005 season. DIC is significantly lower in the ELB moat than in the Priscu Stream ($p = 0.01$) and below the ELB ice-cover ($p = 0.002$). Relatively low moat DIC concentrations and the predictive relationship of DIC with respect to chlorophyll *a* support postulation that DIC is also a limiting factor to moat primary production, although CO_2 exchange between the moat and the atmosphere may compensate for the low DIC concentrations in the moat. DIC limitation has been inferred by temporal deficits in carbon balances (specifically caused by summer biotic uptake) of other dry valley lakes (Neumann et al. 2001), and has resulted in measured differences in ELB moat benthic stable isotope carbon fractionation (Lawson et al. 2004).

Studies have shown that phytoplankton are an important source of DOC to bacterial communities through decomposition and extracellular release (Storch and Saunders 1978, Cole et al. 1982, Fogg 1983); bacterioplankton of the Taylor Valley lakes are no different (Priscu et al. 1999; Takacs et al. 2001). Phytoplankton benefit directly from the inflow of stream nutrients into the ELB moat; as shown by correlation of chlorophyll *a* with DIC, which is related to distance from the inflow, as described above. Heterotrophic bacteria benefit from stream nutrients directly through uptake and secondarily by phytoplankton that are stimulated by stream nutrients.

Phytoplankton derived carbon is especially important as a substrate to heterotrophic bacteria in Antarctic systems that are heavily dependent on autochthonous carbon resources (Priscu et al. 1999; Takacs et al. 2001). POC was directly related to moat bacterial biomass during the 2004-2005 season (equation 12). The ratio of phytoplankton to bacterioplankton carbon (from 4:1 to 164:1) revealed that phytoplankton dominate the ELB moat POC pool. Measurements of moat PPR, though few in number, are evidence of high rates of primary productivity in the moat compared to the lake (Table 2.4). The following chapter (Chapter 3) will discuss in greater detail the importance of photosynthetic carbon production and release in satisfying bacterial carbon demand. The large proportion of phytoplankton carbon to bacterioplankton carbon and high ELB moat primary productivity compared to the lake provide evidence of the importance of photosynthetic carbon fixation to this moat ecosystem.

Microbial Diversity

Bacterial diversity in the Priscu Stream and East Lake Bonney was examined using DGGE and the proportion of density and biomass represented by specific bacterial morphotypes (rod, coccoid, and filament) with respect to total density and biomass. Unfortunately, samples for analysis of bacterial diversity using DGGE were not collected during the 3 January 2005 sampling event. An inter-seasonal (2004-2005) shift was evident, however, in the proportion of bacterial biomass represented by each cell morphotype for some sampling locations (Figure 2.8). For example, the total bacterial biomass measured in Moat 4.1 and Moat 4.3 was largely represented by filamentous bacteria (37% and 68%, respectively) in December 2004, while no filamentous biomass was measured in January 2005. Though the cause of this shift is unclear, the data indicate that a shift in the composition of microbial communities occurs concurrently with the previously described evolution of the stream and moat chemical environment as the 2004-2005 season evolved.

Representing the diversity of bacterial morphotypes (rod, coccoid, filament) using a single value (H'_{bac}) at each sampling location permitted the comparison of physical and chemical factors with bacterial diversity by statistical correlation. The distance from a sampling location to the stream inflow was the only factor statistically correlated to the diversity of bacterial morphotypes in both December and January of the 2004-2005 austral summer ($r < -0.7$, $p < 0.001$), with bacterial diversity decreasing with distance from the stream inflow. Bacterial density was positively correlated with H'_{bac} in December 2004 ($r = 0.83$, $p = 0.02$), while bacterial diversity and POC were negatively

correlated in January 2005 ($r = -0.73$, $p < 0.001$). Other studies using molecular approaches have shown correlation between bacterial density and bacterial diversity (McGrady-Steed and Morin 2000) and researchers have also suggested relationships between bacterial diversity and primary productivity (Mašín et al. 2003; Yannerell and Triplett 2004; Lindström and Bergström 2005). Analysis of stream and moat DGGE diversity patterns in this study failed to reveal significant correlations with distance from the stream inflow or moat chemistry.

Moat phytoplankton communities are most similar near the stream inflow and become generally less similar in sampling locations further from the Priscu Stream; though a correlation between distance from the inflow and percent similarity was not statistically significant ($p > 0.1$). The similarity in phytoplankton composition described by in-situ spectral fluorescence between Moat 4.4 and Moat 4.5 (December 2004) is evidence of unique local moat communities and illustrates the potential for lateral (distance related) spatial heterogeneity in moat phytoplankton diversity (Figure 2.10). Moat 4.4 and Moat 4.5 were the only moat sampling locations in which cyanobacterial pigments were detected on 15 December 2004. The even representation of pigment groups in terms of chlorophyll *a* concentration in Moat 4.4 and Moat 4.5 relative to other moat sample locations further distinguished these sites, resulting in the highest values of moat H'_{phyto} on that date. Shannon-Weaver diversity indices representing algal pigment diversity (H'_{phyto}) revealed that, contrary to the trend in bacterial morphotypic diversity, algal pigment diversity increased with distance from the inflow on 18 December 2004 with a strong correlation ($r = 0.96$, $p < 0.001$). H'_{phyto} was positively related to

chlorophyll *a* and negatively related to DIC as predictive variables through stepwise linear regression (equation 13).

The correlations concerning microbial diversity (bacterial and algal) mentioned above merely suggest factors that may be important in determining microbial diversity, but cause-effect relationships between resource availability and microbial diversity are not made in this discussion because the microbial inhabitants of the East Bonney moat are unknown as are their ecological resource requirements. It should also be noted that the separation of phytoplankton into only five pigment groups and the separation of bacteria into three groups according to morphotype in this study provides only very coarse resolution of bacterial and phytoplankton diversity, and the trends and correlations described above should be interpreted with this consideration in mind.

December 2005 Moat Description

The goal of sampling during the 2004-2005 season was to determine the influence of the Priscu Stream on ELB moat biogeochemical and biological ecosystem structure in the moat. The goal in dispersing December 2005 sampling locations evenly around the ELB moat (Figure 2.2) was to describe general biogeochemical and biological ecosystem structure in the moat. A general ELB moat description would allow, in turn, comparison of moat chemistry, microbial productivity, biomass, and diversity with the same lake parameters. The most striking observations in comparing ELB moat conditions from 2004-2005 to 2005-2006 is the large degree of spatial heterogeneity around the moat

chlorophyll *a*, bacterial biomass, and bacterial density values that far exceeded similar measurements from the 2004-2005 season.

Lawson et al. (2004) found that the $\delta^{13}C$ composition of ELB moat water was enriched compared to water sampled below the Lake Bonney permanent ice-cover. This pattern of stable isotope fractionation provided evidence that moat and lake water mixed more extensively in warmer years with higher melt. The basin morphometry of Lake Bonney has littoral regions with steep slopes in some locations and shallower slopes in other locations (Doran et al. 1996), and one might expect that mixing of the moat with the below-ice moat is higher in the parts of the moat that have steep littoral slopes. The austral summer of 2005-2006 was warmer than the summer of 2004-2005 in the Lake Bonney basin (Tables 2.1 and 2.2); it is possible that the observed high variability and comparatively high moat concentrations of anions and cations can be explained by different degrees of lake mixing caused by more melting in 2005-2006. In support of this hypothesis, bacterial communities identified with DGGE banding patterns from 2005-2006 ELB moat locations grouped more closely with below-ice communities than they did with moat samples from the previous year (Figure 2.12), with the exception of Moat 5.1. These similarities indicate exchange between the moat and below-ice water column, and appear different from the snapshot of bacterial diversity from the previous season.

Conclusion

Despite high spatial variability and the inability to unequivocally demonstrate cause-effect relationships describing moat diversity away from the influence of the Priscu

Stream, this study illustrated that physical, chemical, and biological interactions between the Priscu Stream and the ELB moat are important aspects of moat ecology, especially near to the inflow. The moat habitat was influenced by physical and chemical conditions that were unique to the ELB moat in comparison with the stream and lake. Moat phytoplankton, in response to an abundance of light and localized availability of nutrients, produced organic carbon at a much faster rate than lake phytoplankton and in locations had chlorophyll *a* concentrations 10 times greater than below the ice cover.

Moat environments in the McMurdo Dry Valleys are constantly changing in response to subtle variations in climate. The role of these environments in the greater McMurdo Dry Valleys ecosystem also varies temporally; the three sampling dates discussed in this chapter provide mere snapshots in time during the austral summer. During the early part of the 2004-2005 season, the moat showed strong gradients with respect to stream chemistry. Only two weeks later, the influence of stream inflow on ELB moat chemistry and biological productivity was less evident, and the diversity of bacterial morphotypes shifted. December 2005 sampling provided a very different snapshot of the ELB moat; concentrations of anions and cations were very high relative to other moat locations and could not be explained by the proximity to stream input. Warmer temperatures and higher average PAR resulted in a warmer, wetter summer that may have increased the mixing between lake and moat, and increased moat environmental variation and spatial heterogeneity during the 2005-2006 summer..

CHAPTER 3

EXTRACELLULAR RELEASE OF DISSOLVED ORGANIC CARBON AND PRODUCTION BY LAKE AND MOAT PHYTOPLANKTON

Introduction

Lakes of the McMurdo Dry Valleys, Antarctica share unique characteristics underlining the importance of carbon cycling. These characteristics include limited terrestrial input of nutrients (Aiken et al. 1996), an exclusively microbial food web (Priscu et al. 1999), a permanent ice cover that prevents physical mixing (Spigel and Priscu 1998) and severely attenuates light to the water column (Howard-Williams et al. 1998), and seasonal light/dark cycles symptomatic of high latitude environments. Because of the paucity of terrestrial organic carbon inputs, the exudation of photosynthetic products by phytoplankton represents an important source of organic carbon to the heterotrophic component of these ecosystems and a loss of harvested energy from the photosynthetic organism.

Products of phytoplankton extracellular release have long been considered an important source of labile DOC to marine and freshwater bacterioplankton (Storch and Saunders 1978; Cole et al. 1982; Fogg 1983) and as such are rapidly utilized by bacterioplankton in comparison to organic compounds of higher molecular weight (Nalewajko et al. 1980; Wetzel 2001; Kritzberg et al. 2005). Dissolved organic carbon production (DPR; Moran et al. 2001) deprives phytoplankton of fixed carbon directly,

through loss of photosynthetic effort, and may actually inhibit phytoplankton growth by stimulating the growth of bacteria, which can out-compete larger phytoplankton for limiting nutrients (Currie and Kalff 1984; Roberts and Howarth 2006). Extracellular release of dissolved organic carbon can represent a significant loss of photosynthetic effort by fixing carbon that will not be incorporated into cell matter (Sharp 1977).

The increased importance of carbon cycling via the microbial loop in Antarctic lakes (Priscu et al. 1999) due to the factors previously mentioned, and the known importance of DPR as a carbon source in marine and freshwater oligotrophic environments reveals the potential significance of DPR in Antarctic lakes and moats. Partial DOC budgets for Lakes Fryxell, Hoare, and the East and West lobes of Lake Bonney show that annual respiration exceeds primary production in the trophogenic zone of these lakes (Priscu et al 1999; Takacs et al. 2001). The calculations of Takacs et al. (2001), based on a 25% estimate of the percent extracellular release (PER= DPR/total primary production; Moran et al. 2001) for all lakes, indicate that DPR represents a significant source (9-56%) of DOC to the annual pool. As discussed in the previous chapter, DOC availability is spatially variable and at times below analytical detection limits ($0.2 mg \cdot L^{-1}$) in the ELB moat, illustrating the importance of phytoplankton DOC production as a biological control to carbon availability.

Baines and Pace (1991) estimated an average marine PER of 13% based on work previously reported in literature, although this average has been questioned for reasons of methodology and failure to represent important variables influencing DPR (Morán et al. 2002; Marañón et al. 2004). Generally, average values of PER range from 10-20%, but

large deviations from these values are common, since variation in PER is influenced by a number of factors (see review by Nagata 2000). PER can be highly variable within an ecosystem. In a Denmark fjord, PER was measured as low as 2% at a time when nutrients were available and rose to as much as 68% as carbon and nitrogen availability were decoupled as a result of a phytoplankton bloom (Van der Meersche et al. 2004). The exudation of photosynthetic products by phytoplankton has been measured in a variety of marine and freshwater ecosystems as well as in culture, work which has been summarized in a number of reviews (i.e. Hellebust 1974; Nagata 2000; Wetzel 2001). However, the unique characteristics of McMurdo Dry Valley lakes and moats, as outlined above, accentuate the importance of this process in dry valley ecosystems.

The extracellular release of DOC by phytoplankton in Antarctic lakes and moats was measured to elucidate the importance of this process to lake phytoplankton, and to quantify the contribution of DPR to the pelagic DOC pool. The light-mediated DOC release of phytoplankton from different depths was compared to variable responses of phytoplankton acclimated to different light intensities (Lizotte and Priscu 1992a; 1992b), referred to as photosynthesis-irradiance (P-E) relationships. In addition, trends observed in this study were compared to long-term physical and chemical parameters in the study lakes to evaluate potential causes of DPR that have been proposed in other aquatic habitats. Light vs. dark bacterial uptake of ^{3}H-thymidine was measured in order to determine the effect of DPR on bacterial production. This study will demonstrate that without considering the process of DPR in McMurdo Dry Valleys lakes, an accurate

representation of carbon production and cycling in ice-covered lakes and moats is not possible.

Methods

Study Site

The study lakes, Lake Fryxell (FRX), Lake Hoare (HOR), and the east and west lobes of Lake Bonney (ELB and WLB, respectively) are located in Taylor Valley, Antarctica (76º30' to 78º30'S, 160º to 164ºE). The east and west lobes of Lake Bonney are connected by a narrow, relatively shallow (12m) sill and have drastically different physical, chemical, and biological properties (Priscu 1997; Lee et al. 2004). Consequently, each lobe is treated as a distinct lake. All the lakes have a permanent ice cover between 4 and 7m thick. The locations of below-ice lake experiments coincided with the deepest portion of each lake and are the ongoing sampling sites for the McMurdo Dry Valleys Long Term Ecological Research project (see Lyons et al. 2000). Total primary production (TPR), the sum of DPR and PPR, was also examined in the moat of East Lake Bonney (near Moat 5.2, see Chapter 2 for description). More than 12 years of data exist from the lakes, providing background information of phytoplankton distribution (Spaulding et al. 1994; Lizotte and Priscu 1998; Tursich 2003), nutrient limitation (Priscu 1995; Dore and Priscu 2001), carbon budgets (Priscu et al. 1999; Takacs et al. 2001; Neumann et al. 2001), bacterial dynamics (Takacs and Priscu 1998;

Lisle and Priscu 2004) spectral light availability and phytoplankton acclimation to irradiance (Lizotte and Priscu 1992a; 1992b; 1994; Lizotte et al. 1996).

In-Situ DPR Incubations

Water for experimental incubations and measurement of background parameters was collected at two depths corresponding to the deep chlorophyll maxima (DCM) and phytoplankton productivity maxima of each lake, using a Niskin bottle lowered through a 20-50cm diameter hole in the ice. Samples were collected between 30 November 2003 and 15 December 2005, the period representing the austral spring-summer marked by 24-hour sunlight.

Samples were immediately decanted under low light into 150mL borosilicate glass bottles in duplicate, amended with ^{14}C-bicarbonate (final concentration $7.5x10^{5}$-$1.5x10^{7}$ dpm·mL^{-1}, depending on initial DIC concentration of sample), and suspended at the depths from which they were collected. Following 24-hour incubations in each lake, assays were returned to nearby laboratory facilities and immediately gravity filtered in the dark through 47mm 0.4µm Nucleopore filters, such that filtering was complete within 5 hours of the end of incubation. Samples were gravity filtered to minimize potential harm to phytoplankton that may enhance DPR. The filtrate was collected in clean borosilicate bottles and acidified with 6N HCl to pH~2. Filters were placed in 20mL glass scintillation vials and 0.5mL 6N HCl was added to each filter to eliminate extraneous DIC; filters were subsequently dried on a warming plate at 60ºC.

Light Response DPR Incubations

Lake water collected between 13 and 15 December 2005 from 5 and 15m in ELB was decanted into 125mL borosilicate bottles for experimental measurement of DPR over a range of PAR (5, 20, 40, and 110 μmol photon·m^{-2}·s^{-1}). Experimental irradiance was supplied by fluorescent and incandescent lighting in a temperature-controlled environmental chamber, with experimental intensities of irradiance controlled by wrapping the capped borosilicate incubation bottles with dark screening. Experimental intensities of irradiance were chosen with the intent of providing adequate density of data at the low range of irradiance (5-40 μmol photon·m^{-2}·s^{-1}) with higher intensities chosen to model photosynthesis at near light saturating intensities (110 μmol photon·m^{-2}·s^{-1}). Samples were amended with ^{14}C-bicarbonate (final concentration $4.9x10^5$-$2.8x10^6$ dpm·mL^{-1}, depending on the initial DIC concentration of the sample), and incubated in duplicate for 24 hours at 4ºC. PAR was monitored over the course of the incubations using a spherical Li-Cor LI-193S*A* quantum sensor and a Li-Cor LI-1000 logger. Incubation temperature remained at 4±1ºC and was monitored throughout the experiment using Onset stowaway temperature loggers. Following incubations, samples were gravity filtered and acidified as previously described for *in-situ* incubations.

ELB moat water was collected on 19 December 2005 from a depth of approximately 0.5m and incubated in borosilicate glass bottles at 40, 65, 117, 210, and 423 μmol photon·m^{-2}·s^{-1}, with temperature and PAR conditions controlled as described above. Experimental intensities of irradiance for moat phytoplankton were different from those tested using lake phytoplankton based on the expectation that moat phytoplankton

are differently acclimated to irradiance than lake phytoplankton because of differences in natural irradiance caused by light attenuation by lake water and a permanent ice cover. Incubations of moat phytoplankton were terminated by gravity filtration and acidification as described above.

To illustrate the responses of lake phytoplankton DPR, PPR and TPR to irradiance intensity, and to predict photosynthesis at specific values of PAR, chlorophyll *a* biomass-specific primary productivity (P_b; dissolved, particulate, and gross) were fit, using Marquart's algorithm, to a hyperbolic tangent (htan) function to model phytoplankton photosynthesis-irradiance relationships (Jassby and Platt 1976). The general form of the hyperbolic tangent functions is shown in equation eight (14) where P_b is primary productivity per unit chlorophyll *a* biomass, P_{bmax} is the chlorophyll *a* biomass-specific primary productivity at light-saturation, α is the initial slope of the htan curve, and R is light-independent loss due to respiration.

$$P_b = P_{b\max} \cdot \tanh(PAR \cdot \frac{\alpha}{P_{b\max}}) + R \qquad \textbf{(14)}$$

Hourly DPR and PPR were predicted at 5m and 15m using P-E relationships with ambient PAR (5m and 15m) over a cloudless day at Lake Bonney. Table A.4 (Appendix A) shows surface values of PAR at one hour intervals on 4 December 2001 (data courtesy of mcmlter.org) and PAR at 5m and 15m, calculated using extinction coefficients that were determined by an underwater profile of PAR on 10 December 2004 (Table A.5, Appendix A). The depth-specific extinction coefficients and the estimation of underwater PAR were calculated using the standard antilog relationship of light

extinction based on incident irradiance and depth (Wetzel and Likens 2000). Underwater PAR from the ice-water interface to a depth of 20m was used with P-E equations to predict DPR and PPR in East Lake Bonney to 20m. DPR and PPR were predicted from 5-12m using the 5m PPR-P_b parameters and from 13-20m using the PPR-P_b parameters from 15m.

Light vs. Dark Bacterial Productivity

Lake water for bacterial productivity measurements was incubated in fluorescent and incandescent light (423 µmol photon $m^{-2}\cdot s^{-1}$) and in the dark in an environmental chamber for 20 hours after being amended with ^{3}H-thymidine, following procedures previously described for the dry valley lakes (Takacs and Priscu 1998). Five replicates were included for each light and dark treatment, respectively. Rates of ^{3}H-thymidine incorporation (TDR, nM-thymidine$\cdot day^{-1}$) were converted to bacterial carbon productivity as described in Takacs and Priscu (1998). Calculations of bacterial carbon productivity were made based on assumptions of $2x10^{19}$ cells$\cdot mol^{-1}\cdot$thymidine and 11 fgC$\cdot cell^{-1}$.

Background Parameters

Incident PAR was measured with a Li-Cor LI-192S*A* 2π quantum sensor and underwater irradiance using a Li-Cor LI-193S*A* spherical quantum sensor. Data from both sensors was logged every 10 minutes using a Li-Cor LI-1000 during the 24-hour lake and moat incubations. Upon terminating the lake incubations, a vertical profile of

underwater light attenuation over the water column was made so PAR at individual incubation depths could be computed from the logged values. Lake water was collected from the same Niskin bottle as DPR samples for analysis of chlorophyll *a*, DIN, SRP, pH, DIC, and DOC as described by Priscu (1995) and summarized in Chapter 2, Methods.

In-Situ Spectral Fluorescence

Algal group abundance was profiled with depth in all McMurdo Dry Valleys lakes by *in-situ* spectral fluorescence. This technique, described in Chapter 2 (Methods), is used to detect the proportions of the algal spectral groups: *Chlorophyta*, C*yanophyta, Cryptophyta*, and *Chrysophyta* based on photosynthetic excitation spectra that generally describe each group.

Results

In-Situ Spectral Fluorescence

Algal abundance measured by *in-situ* spectral fluorescence is reported as chlorophyll *a* biomass ($\mu gChl\text{-}a \cdot L^{-1}$) in Table 3.1. This algal identification method recognizes the algal spectral groups: *Chlorophyta*, *Cyanophyta, Cryptophyta,* and *Chrysophyta*. *Cyanophyta* were not detected at any lake location sampled in this study. The chlorophyll *a* biomass and relative abundance of each of the four algal pigment groups were tested as potential predictive variables to DPR and then to PER by stepwise

linear regression (described in Chapter 2). Stepwise regression failed to recognize any of these inputs as predictive variables ($p < 0.1$) to DPR nor to PER.

Table 3.1. Algal group chlorophyll *a* biomass ($\mu gChl\text{-}a \cdot L^{-1}$) from sampling locations was measured using in-situ spectral fluorescence.

Lake-Strata	*Chlorophyta*	*Chrysophyta*	*Cryptophyta*
Fryxell-5m	0.11	0.03	0
Fryxell-DCM	1.03	6.04	3.74
Hoare-5m	0.65	0.05	0
Hoare-DCM	0.22	1.81	1.22
West Bonney-5m	3.11	0.8	0
West Bonney-DCM	3.04	3.06	0
East Bonney-5m	2.34	0.48	0
East Bonney-DCM	0.82	0.76	0

Background Limnological Parameters

Physical, chemical, and biological measurements used in describing putative influences to DPR, PPR, and TPR at 5m and at the DCM of the study lakes are shown in Table 3.2**.**

Table 3.2. Limnological data used in discussion of Lake Fryxell, Lake Hoare, ELB and WLB. Z = depth (m), Chl-a = Chlorophyll *a* ($\mu gChl\text{-}a \cdot L^{-1}$), DOC and DIC = dissolved organic/inorganic carbon (μM), DIN = dissolved inorganic nitrogen (μM), SRP = soluble reactive phosphate (μM). ND = below method detection limits (0.2μM DIN)

Lake	**Z**	**pH**	**Chl-a**	**DIC**	**DOC**	**DIN**	**SRP**
Fryxell	5	7.6	4.8	2951	192	ND	0.17
Fryxell	9	7.7	17.6	7341	487	ND	0.64
Hoare	5	8.7	2.4	1636	102	ND	0.11
Hoare	14	7.8	2.7	5676	174	ND	0.31
ELB	5	8.4	3.6	1026	79	16	0.03
ELB	15	6.7	2.6	8266	408	48	0.02
WLB	5	8.7	4.5	1030	53	7	0.05
WLB	14	6.4	3.7	39785	592	105	0.06

In-Situ DPR

DPR, PPR, and PER in all lakes and depths as measured during the 2003 season are shown in Table 3.3. The lowest rates of extracellular DOC production were measured in ELB (0.17 and 0.14μgC $L^{-1}d^{-1}$ at 5 and 15m, respectively). The highest rate of DPR was measured in the adjoining west lobe of Lake Bonney (2.75μgC·L^{-1}·d^{-1} fixed at 14m and 0.28 μgC·L^{-1}·d^{-1} at 5m). Phytoplankton from the DCM showed higher PER than phytoplankton from the ice-water interface (5m) at all experimental light conditions. ELB moat TPR was measured *in-situ*, averaging 3.3μgC·L^{-1}·d^{-1} (SD = 1.8).

Table 3.3. Ranges (n=2) of dissolved primary production (DPR), particulate primary production (PPR), and average percent extra-cellular release (PER) in Lake Fryxell, Lake Hoare, East Lobe Bonney (ELB), and West Lobe Bonney (WLB) as measured mid-summer 2003. Deep chlorophyll maximum (DCM) depths are 9m in Lake Fryxell, 14m in Lakes Hoare and WLB, and 15m in ELB. Units: DPR and PPR, μgC $L^{-1}d^{-1}$; PER = %.

	5m			DCM		
Lake	DPR	PPR	PER	DPR	PPR	PER
Fryxell	0.32-0.65	1.23-1.30	27.0	0.36-0.50	0.52-0.58	43.4
Hoare	0.30-0.57	1.18-2.02	21.2	0.70-0.74	0.78-0.89	46.3
ELB	0.17-0.18	1.05-1.09	14.0	0.10-0.18	0.42-0.64	21.3
WLB	0.27-0.30	0.14-0.33	57.0	2.73-2.78	1.47-2.55	58.5

In-situ incubations for determining DPR were performed under different ambient light conditions for each lake. To facilitate comparison of DPR between lakes, average values of DPR were normalized to ambient irradiance over the 24-hour incubation period (DPR*), and are shown in Table 3.4.

A two-way analysis of variance revealed significant ($p < 0.05$) differences in comparing lake vs. PER, and strata (below ice and DCM) vs. PER. The interaction of the

factors lake and strata did not significantly influence PER ($p = 0.19$). *Post-hoc* comparison of PER calculations by the Holm-Sidak method for multiple comparisons revealed significant differences ($p < 0.05$) between each lake, with the exception of comparison between Lake Hoare and Lake Fryxell PER ($p = 0.8$).

Table 3.4. Surface and sample depth 24-hour average PAR (μmol photon·m^{-2}·s^{-1}) for Lake Fryxell, Lake Hoare, East Lobe Bonney (ELB), and West Lobe Bonney (WLB). Deep chlorophyll maximum (DCM) depths correspond to 9m in Lake Fryxell, 14m in Lake Hoare and WLB, and 15m in ELB. DPR* = Average DPR normalized to ambient (sample depth) 24-hour average PAR (ngC·μmol photon^{-1}·m^{-1}).

		5m		DCM	
Lake	Surface	PAR	DPR*	PAR	DPR*
Fryxell	431	7.0	0.73	0.81	5.71
Hoare	648	11.7	0.40	0.43	18.70
ELB	697	14.7	0.14	0.14	5.72
WLB	576	13.3	0.20	0.25	214.73

DIC was positively correlated to DPR ($r = 0.96$, $p < 0.001$). Forward stepwise linear regression analysis was used to determine lake variables that may be important in describing measured *in-situ* DPR beneath the ice cover of McMurdo Dry Valleys lakes. The variables tested were PAR, pH, bacterial productivity, chlorophyll *a*, DIC, DOC, DIN, and SRP. DPR (μgC·L^{-1}·d^{-1}) could be predicted, as revealed using stepwise regression, by a linear combination of DIC (μM, $r^2 = 0.92$), DIN (μM, $r^2 = 0.99$), and DOC (μM, $r^2 = 0.96$, $p < 0.001$ for all variables), as shown in equation (15). It should be noted that DIN was only tested against DPR by stepwise regression in Lake Bonney because DIN was below detection limits for all Lake Fryxell and Lake Hoare samples (Table 3.2).

$$DPR = 0.00011*DIC+0.0015*DOC+0.011*DIN+0.35 \quad \textbf{(15)}$$

Light Response of DPR and PPR

ELB lake and moat water were incubated at various light intensities to determine the P-E relationship of phytoplankton from different general light conditions. As chlorophyll *a* concentration is variable with depth in the trophogenic zone of the dry valley lakes (Figure 3.1), photosynthetic response of 5m and 15m phytoplankton in ELB was compared by normalizing DPR, PPR, and TPR to chlorophyll *a* biomass.

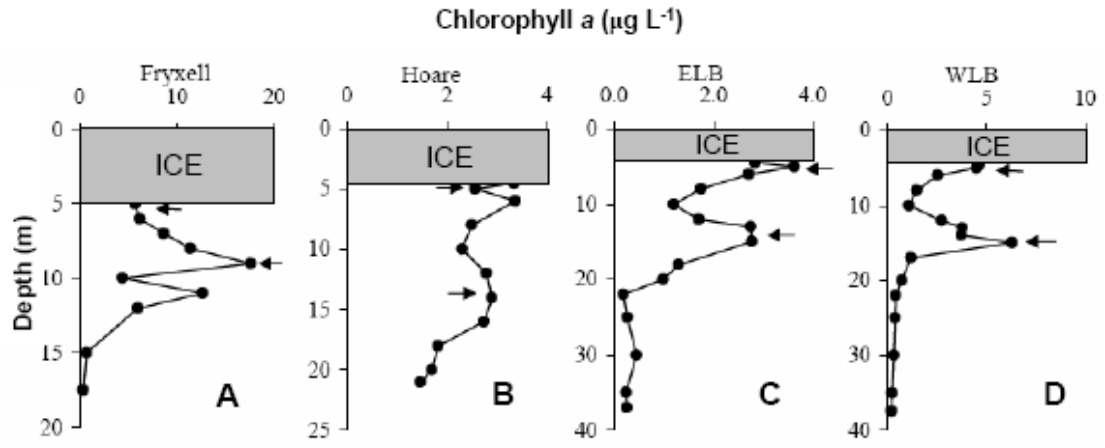

Figure 3.1. Profiles of chlorophyll *a* in Lake Fryxell (A), Lake Hoare (B), ELB (C) and WLB (D) with arrow indicating sample location and shaded area representing ice-cover. Note different scales on depth axis.

The relationship of the response variables, chlorophyll *a*-specific TPR, PPR, and DPR and at 5 and 15m in ELB were compared to values of irradiance, as illustrated in Figure 3.2 and summarized in Table 3.5. Table 3.5 also summarizes hyperbolic tangent parameters for the ELB moat, and Figure 3.3 depicts the photosynthesis-irradiance relationship in the moat. Both DPR and PPR generally increased with increasing PAR in all ELB locations, but the difference in organic carbon produced from the highest to the

lowest intensity of irradiance is an order of magnitude larger for PPR than for DPR. The hyperbolic tangent equation (equation 14) described the light response of DPR, PPR, and TPR for both 5m and 15m in ELB ($r^2 > 0.97$, all curves). Hyperbolic tangent curves also represented the relationship between moat TPR-P_b and irradiance ($r^2 = 0.91$). PPR-P_b and irradiance were not well described by the htan function ($r^2 = 0.74$) due to large variation in P-E response values (Figure 3.3), while high variability in DPR-P_b resulted in a relationship with irradiance that was poorly described by the htan function ($r^2 = 0.43$).

Table 3.5. Hyperbolic tangent parameters (see Figures 3.2 and 3.3) showing the chlorophyll *a*-specific primary productivity response to irradiance in ELB. TPR-P_b, PPR-P_b, DPR-P_b = gross, particulate, and dissolved chlorophyll *a*-specific primary productivity, respectively ($\mu gC \cdot \mu gChl\text{-}a^{-1} \cdot h^{-1}$, $p < 0.001$); Chl-a = chlorophyll *a* ($\mu gChl\text{-}a \cdot L^{-1}$), $P_{b\text{-}max}$ = chlorophyll *a*-specific primary productivity at light saturation, α = initial slope of htan function ($\mu gC \cdot m^2 \cdot \mu gChl\text{-}a^{-1} \cdot \mu mol\ photon^{-1}$), R = respiration ($\mu gC \cdot \mu gChl\text{-}a^{-1} \cdot h^{-1}$). Parameters not significant at $p < 0.1$ are italicized; p-values for each parameter are shown in parentheses.

Loc	P_b fraction	Chl-a	r^2	$P_{b\text{-}max}$	α	R
15m	TPR	1.2	0.99	6.1 (0.04)	0.18 (0.04)	*-0.045 (0.9)*
15m	PPR		0.98	5.5 (0.01)	0.16 (0.08)	-0.67 (0.5)
15m	DPR		0.98	0.57 (0.09)	*0.015 (0.4)*	0.64 (0.06)
5m	TPR	5.8	0.98	0.98 (0.0003)	0.016 (0.01)	*-0.048 (0.6)*
5m	PPR		0.97	0.92 (0.0006)	0.015 (0.02)	*-0.070 (0.4)*
5m	DPR		*0.97*	0.06 (0.008)	*0.001 (0.1)*	0.022 (0.08)
Moat	TPR	14.5	0.92	*1.4 (0.74)*	*0.04 (0.82)*	*-0.79 (0.8)*
Moat	PPR		0.74	*4.3 (0.96)*	*0.21 (0.97)*	*-3.9 (1)*
Moat	DPR		0.43	*-0.23 (0.66)*	-1987 (0.0001)	*0.53 (0.5)*

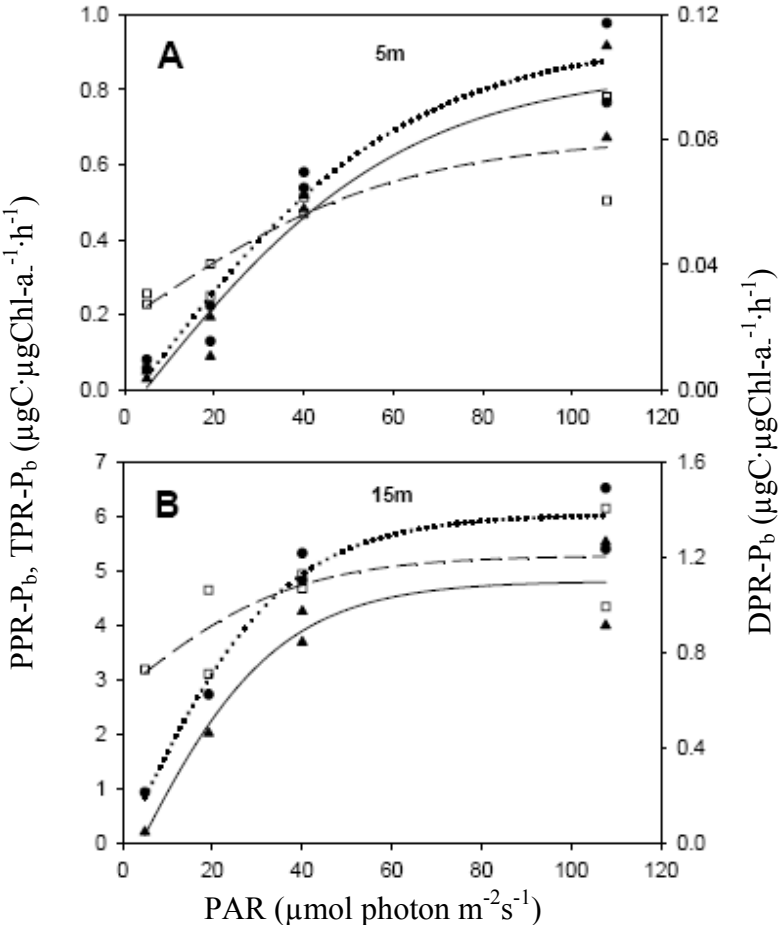

Figure 3.2. Measurements of East Lake Bonney chlorophyll *a*-specific primary productivity (P_b) response to PAR at 5m (A) and 15m (B). P_b values are fit to hyperbolic tangent curves (parameters shown in Table 3.5). DPR-dashed, PPR-solid, TPR-dotted. Points indicate measured values (DPR-open squares, PPR-solid triangles, TPR-solid circles). DPR-P_b plotted on right hand axis, note differences of scale on y-axes.

The maximum chlorophyll *a*-specific photosynthetic rate was 15 times higher for PPR compared to DPR at 5m, and 10 times higher at 15m. Light-saturated biomass-specific TPR (TPR-$P_{b\text{-}max}$) at 15m was more than 6 times TPR-$P_{b\text{-}max}$ at 5m and more than 10 times greater than TPR-$P_{b\text{-}max}$ in the ELB moat. The initial response of 15m TPR-P_b to increasing irradiance, described by TPR-α, was more than 10 times TPR-α at 5m and was

18 times TPR-α in the ELB moat. ELB moat DPR-P_b was not determined by the htan relationship (see Table 3.5), nor could DPR be predicted by linear regression with respect to PAR ($r^2 = 0.34$) in the moat, preventing comparison of moat DPR parameters with ELB 5m and 15m hyperbolic tangent parameters.

The y-intercept (or htan parameter for respiration) was only predicted with statistical significance for 5m DPR-P_b (Table 3.5), with the exception of moat DPR-P_b for which the htan function poorly described the data ($r^2 = 0.34$). The 5m DPR-P_b y-intercept was 0.02 ($p = 0.08$), compared to the PPR-P_b y-intercept of -0.07 ($p = 0.44$). These differences in y-intercepts between DPR and PPR suggest that DOC release continues at values of PAR where active photosynthesis ceases, though such a generalization can not be made with confidence due to the high p-value (0.4) of R for PPR-P_b. $P_{b\text{-}max}$ reveals differences in light acclimation of DPR and PPR between the sample depths. DPR-α was an order of magnitude higher at 15m than at 5m, while α_{15m} was more than an order of magnitude greater than α_{5m}.

ELB moat DPR was not measured *in-situ*. The DPR incubation at 423μmol photon$\cdot m^{-2}\cdot s^{-1}$ (fluorescent and incandescent light sources) provides the best estimate of ELB moat DPR and PER, though diel variation in natural irradiance causes small changes in DPR relative to PPR as I will discuss. The two DPR measurements at 423μmol photon $m^{-2}\cdot s^{-1}$ spanned an order of magnitude (0.8 and 7.5 $\mu gC\cdot L^{-1}\cdot d^{-1}$), and represented the highest variation in all photosynthesis-irradiance incubations. ELB moat incubations at various irradiance intensities did not reveal a relationship that could be useful in predicting DPR at various light conditions, as was possible with incubations of lake water

from 5m and 15m in ELB (using P-E relationships), meaning that DPR at moat irradiance could not be accurately estimated in this study.

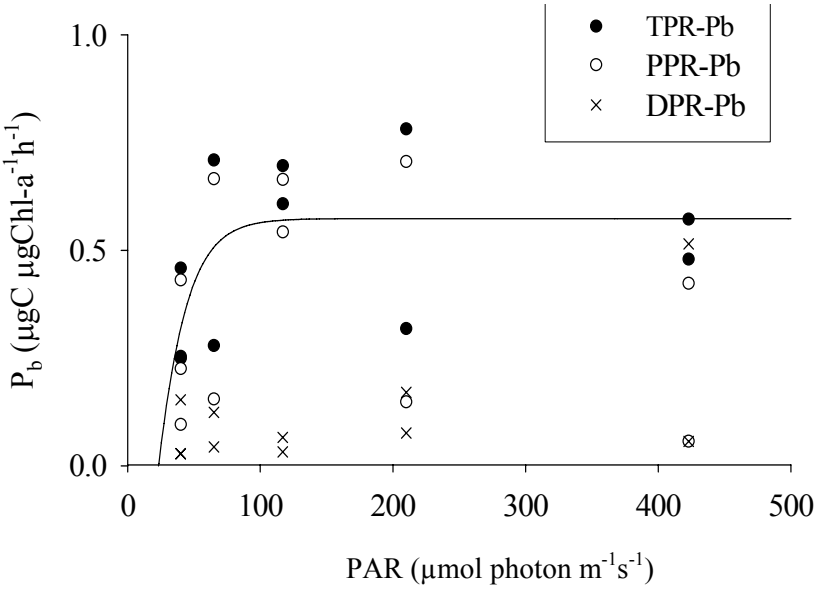

Figure 3.3. Measurements of East Lake Bonney moat total, particulate, and dissolved chlorophyll *a* biomass-specific primary productivity (TPR-P_b, PPR-P_b, and DPR-P_b, respectively) in response to photosynthetically active radiation (PAR). TPR-P_b is fit (r^2 = 92) to the hyperbolic tangent (htan) function. PPR-P_b and DPR-P_b htan functions are not shown because data was poorly fit (r^2 = 0.74 and 0.43, respectively) due to large variance. See Table 3.5 for htan parameters.

Light vs. Dark Bacterial Production

Bacterial incorporation of TCA-insoluble ^{3}H-thymidine incorporation (assumed to primarily represent DNA synthesis, Riemann et al. 1982) was converted to bacterial carbon productivity (BP) as previously described. An ANOVA comparing ^{3}H-thymidine incorporation in all lakes and depths revealed significant differences comparing all BP data between strata ($p < 0.001$), lakes ($p < 0.001$), and a less probable influence of light

vs. dark incubation ($p = 0.1$). Comparison of light vs. dark ^{3}H-thymidine incorporation at 5m in each lake revealed 3-14% higher BP in light incubations than in dark incubations (Figure 3.4), though light stimulation of bacterial productivity was only statistically higher when compared by t-test in Lake Fryxell ($t = 2.8$, $p = 0.05$). Conversely, dark BP was 12-52% higher than light BP at depths representing the DCM in all lakes except WLB, where productivity was essentially equal in light and dark incubations (<0.5% difference).

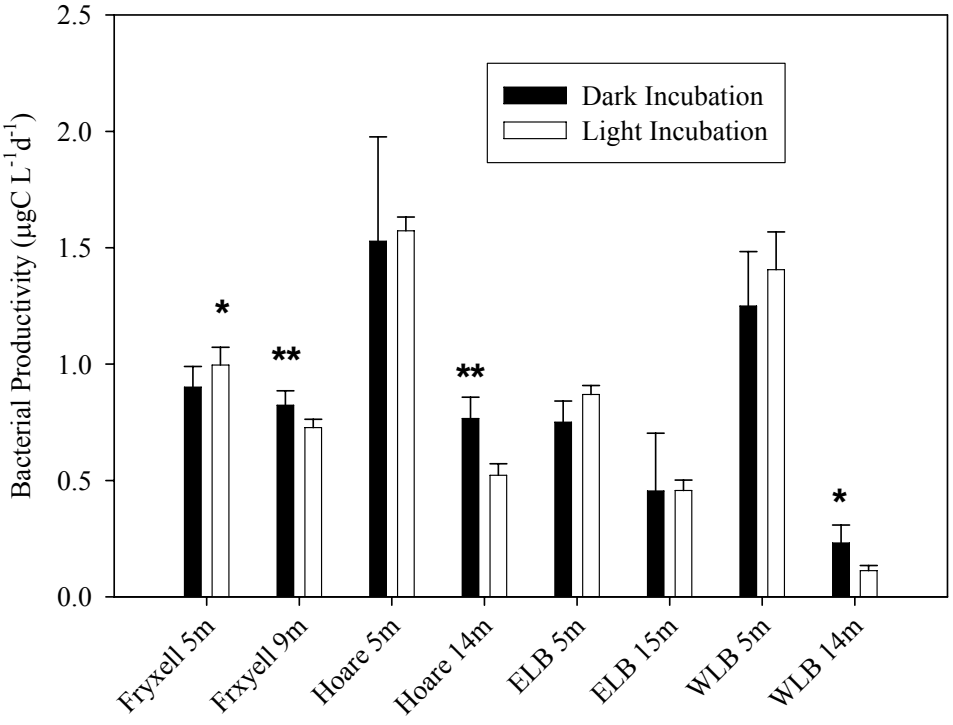

Figure 3.4**.** Light and Dark bacterial production at two depths in lakes of the McMurdo Dry Valleys. Error bars represent one standard deviation (n=5). Statistical significance by t-test between treatments at each lake and depth is shown at $p \leq 0.05$ with a single asterisk (*) and at $p < 0.01$ with double asterisks (**).

The bacterial carbon demand (BCD, $\mu gC \cdot L^{-1} \cdot day$) at 5m and at the DCM of four McMurdo Dry Valleys lakes is equal to bacterial carbon production, shown in Figure 3.4. Table 3.6 compares BCD to DPR, describing the amount of bacterial carbon demand that can be satisfied by phytoplankton extracellular release (DPR:BCD). DPR:BCD was significantly larger at the DCM than at 5m ($p = 0.07$) as revealed in a t-test comparing the ratio in all lakes with BP (or BCD) determined from both light and dark incubations. DPR:BCD from 5m only exceeded DPR:BCD at the DCM in the Lake Fryxell dark incubation, though there was less than 1% difference between the two ratios. DPR was very high at the WLB DCM compared to other measurement in this study and BCD was lower in this same location than all other measurements, the resulting ratio of DPR:BCD reveals that DPR exceeds BCD by a factor >25 in the WLB DCM. In other study locations (HOR 5m, WLB 5m, and ELB5m), BCD could not be met by DPR alone.

Table 3.6. Phytoplankton extracellular release of DOC (DPR, $\mu gC \cdot L^{-1} \cdot day$), light and dark bacterial carbon demand (BCD, $\mu gC \cdot L^{-1} \cdot day$), and DPR:BCD are compared in lakes and from different lake strata.

Lake	Z	DPR	BCD	DPR:BCD Light	BCD	DPR:BCD Dark
Fryxell	5	0.44	0.42	107%	0.38	118%
Fryxell	9	0.40	0.30	132%	0.34	117%
Hoare	5	0.41	0.66	62%	0.64	64%
Hoare	14	0.69	0.22	319%	0.32	218%
W. Bonney	5	0.26	0.59	44%	0.52	50%
W. Bonney	14	2.60	0.05	5544%	0.10	2687%
E. Bonney	5	0.16	0.36	45%	0.31	52%
E. Bonney	15	0.12	0.19	65%	0.19	65%

Discussion

Parker et al. (1977) first estimated PER by Lake Bonney phytoplankton to be greater than 75%. These measurements were made with Lake Bonney phytoplankton in Lake Bonney water; however, experiments were carried out *in-vitro*, having been transported from the Antarctic to the continental United States for analysis. 2003 measurements of average PER for ELB in this study were 14% and 21% for ice/water interface (5m) and chemocline (15m) populations, respectively. These values are similar to Lake Bonney PER measurements by Sharp (1993) of 6-24% just below the ice cover and 27-30% at 13.5m (chemocline). Measurements of DPR in this study were the first made in WLB, Lake Fryxell, and Lake Hoare. Normalizing DPR to PAR (Table 3.4) revealed large variations in photosynthetic DOC release between lakes, indicating that DPR does not represent a static contribution to the DOC pools or equally influence photosynthetic processes in all McMurdo Dry Valleys lakes studied.

The average measured values of PER in all McMurdo Dry Valleys lakes at 5m (30%, range: 8-62%) are within reported *in-situ* ranges of marine (Teira et al. 2001; Morán et al. 2002; Marañón et al. 2004; Van der Meersche et al. 2004) and other freshwater systems (Sundh 1989); however, the average DCM PER (Table 3.3) is considerably higher than values published for other systems. Average values of PER in this study are consistently higher than the global marine average of 13% reported by Baines and Pace (1991). The relatively high values of PER measured in McMurdo Dry Valleys lakes which have little allochthonous DOC input indicates that DPR represents a

significant inefficiency in terms of photosynthetic production, but a potentially important source of labile carbon for bacterioplankton productivity.

The quantity of DOC released as an extracellular product of photosynthesis has been linked to phytoplankton production (Baines and Pace 1991; Morán et al. 2002; Marañón et al. 2004), light intensity (Hellebust 1965; Marañón et al. 2004), nutrient limitation (Walsby and Fogg 1975; Magaletti et al. 2004; Van der Meersche et al. 2004), algal species composition, and the average algal cell size in a population (Fogg et al. 1964; Mague et al. 1980; Larsson and Hagström 1982; Teira et al. 2001). In aquatic ecosystems, DPR may serve as an adaptive overflow mechanism by which superfluous fixed carbon is excreted from the cell when fixation is occurring either at too high a rate, due to high irradiance, or when nutrient limitation precludes complete synthesis of fixed carbon into cell material (Bjørnsen 1988; Wood and Van Valen 1990; Van der Meersche et al. 2004). Because dry valley lake phytoplankton production is generally light (Priscu et al. 1988; Lizotte and Priscu 1992a) and nutrient limited (Priscu 1995; Dore and Priscu 2001), it is doubtful that DPR represents adaptive overflow in these lakes.

While nutrient limitation certainly influences the rate of phytoplankton productivity in the McMurdo Dry Valleys lakes, its effect is more pronounced on phytoplankton populations at the ice-water interface than at the DCM (Priscu 1995; Dore and Priscu 2001). The difference in nutrient limitation between strata has been attributed to the fact that upward diffusion from nutrient-rich deeper waters provides a constant source of nutrients to the phytoplankton forming the DCM (Priscu 1995). Therefore, nutrients are more readily available at the chemocline, although temporal events such as

freezing, thawing, and flood cause temporary fluxes of nutrients just below the ice cover (Foreman et al. 2004; Lawson et al. 2004). Upward diffusion of nutrients is considered the primary factor for the establishment of the previously described deep-chlorophyll/primary productivity maxima layer (Lizotte and Priscu 1994; Priscu 1995; Lizotte et al. 1996). My data show highest PER in the water just above the chemocline (Table 3.3) and a positive relationship between DIN and DPR ($r = 0.81$, $p = 0.02$), showing that the degree of nutrient deficiency in the study lakes is not a likely cause of DPR.

Prior studies have shown that the hyperbolic tangent parameters describing chlorophyll *a*-specific photosynthetic activity, $P_{b\text{-}max}$ and α, are higher for phytoplankton at the chemocline compared to 5m in Lake Bonney (Lizotte and Priscu 1992a). Higher values of α indicate photosynthetic acclimation by phytoplankton at low irradiance intensities and higher $P_{b\text{-}max}$ represents higher maximum photosynthesis at light-saturation. P-E relationships calculated for ELB (Figure 3.2 and Table 3.5) also revealed higher α and $P_{b\text{-}max}$ at the DCM than at 5m, and TPR-P_b in the moat. Although low-light gross primary productivity at the ELB DCM reveals acclimatized low-light photosynthetic efficiency relative to 5m, the high DPR at the deep chlorophyll maximum represents a higher relative photosynthetic loss to the organism.

Baines and Pace (1991) suggest that the rate of photosynthetic DOC release is intrinsically related to the rate of primary production because DPR results primarily as a passive release of photosynthate, meaning that as photosynthetic rates increases so do rates of DPR. Assuming that DPR is in fact intrinsically related to PPR, DPR is

transitively influenced by light intensity (Hellebust 1965, Marañón et al. 2004). DPR is also mathematically related to PPR in the relationship of percent extracellular release, as PER will invariably increase as PPR decreases.

PER was measured in a self-shaded coastal marine system where average PER was only 10% near the surface, but reached 40-50% near the bottom of the euphotic layer; this was primarily caused by a marked decline in particulate primary productivity with depth (Marañón et al. 2004). All phytoplankton populations below the ice cover of the McMurdo Dry Valleys lakes experience light-limited photosynthesis at *in-situ* PAR, despite low light acclimation (Lizotte and Priscu 1992a; 1992b; 1994; Lizotte et al. 1996). PER is higher at the DCM than at 5m in all dry valley lakes ($p < 0.05$), where PPR decreases with depth in all lakes with the exception of WLB. This trend is consistent with the higher below-ice DPR htan parameters discussed previously and supports a positive relationship between light deficiencies and DPR/PER in McMurdo Dry Valleys lakes. I suggest that phytoplankton are acclimated to low-light conditions; efficiently using limited solar flux beneath the ice-cover as shown previously (Lizotte and Priscu 1992a) and also benefit from the upward flux of N and P from deeper, nutrient-rich waters (Priscu 1995), but are not acclimated to prevent loss of photosynthetic effort by reducing exudation of photosynthetic DOC. I expect that DPR being intrinsically related to primary production, as suggested above, results in a higher DPR-P_b as a side-effect of light-acclimated gross productivity aided by relative nutrient abundance.

Using P-E relationships for DPR-P_b and PPR-P_b from 5m and 15m in ELB (Table 3.5), hourly primary production was predicted during a 24-hour period with ambient PAR

at 5m and 15m representative of a typical cloudless Antarctic day (Figure 3.5). Modeled DPR-P_b and PPR-P_b both increase with PAR between 05:00 and 14:00h for both depths. During the period of higher PAR, the importance of PPR to integrated production increases with respect to DPR. The relatively higher contribution of DPR to gross DOC production at 15m in low light is underlined by the relationship shown in Figure 3.5b; modeled DPR exceeded PPR from 21:00 to 07:00h, when ambient PAR decreased below 5µmol photon $m^{-2}s^{-1}$ (0.7% surface irradiance). The data show that phytoplankton DPR represents a variable proportion of gross photosynthetically fixed carbon over the diel cycle, ranging from 40-86% at night to 10-33% during mid-day.

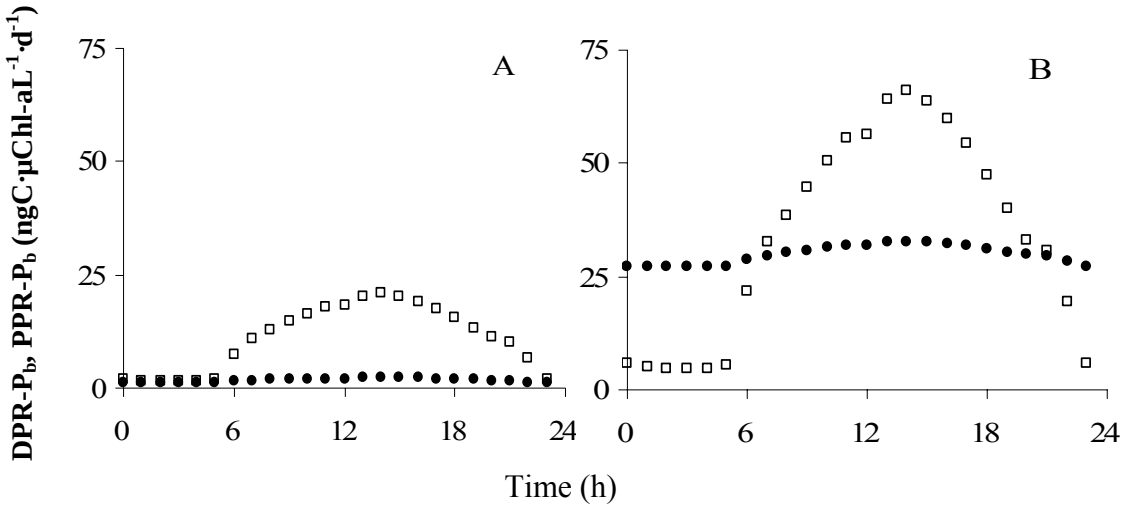

Figure 3.5. Predicted chlorophyll *a*-specific DPR (DPR-P_b, solid circles) and PPR (PPR-P_b, open squares) at during a cloudless 24-hour period in East Lake Bonney. Hourly calculations of chlorophyll *a*-specific production (ngC·µChl-a^{-1}·L^{-1}) based on ambient PAR at 5m (A) and 15m (B) over cloudless a 24-day in December.

The 5m and 15m photosynthesis-irradiance relationships shown in Figure 3.2, and represented in general form by equation (14) were used to predict DPR and PPR at 1m

intervals through the photic zone. These calculations showed that DPR and PPR spike near the observed DCM (Lizotte and Priscu 1994; Priscu 1995; Lizotte et al. 1996), while PER increased consistently with depth (Figure 3.6), illustrating that PER is largely controlled by light-dependent diel fluctuations in PPR, while DPR is much less variable with depth and over a diel cycle.

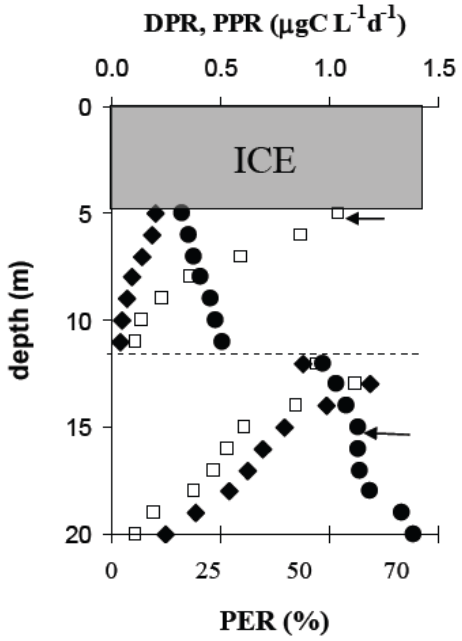

Figure 3.6. Predicted profiles of DPR (solid diamonds), PPR (open squares), and PER (solid circles) in ELB. Arrows indicate the depth from which PER was measured in-situ. Points above the dashed line were calculated by the photosynthesis-irradiance relationship of phytoplankton from 5m, while points below the dashed line were calculated using the P-E relationship of phytoplankton from 15m.

Linear correlation revealed a significant ($p < 0.001$) relationship between DIC and DPR in dry valley lakes. It is possible that abundant DIC and DIN in the DCM of WLB promoted high photosynthetic production; supporting the hypothesis that high DPR results as a by-product of photosynthesis. DIC limitation is more likely in Lake Hoare,

where there is not a large DIC pool supplying DIC to the photic zone via upward diffusion (Neumann et al. 2001), and in the ELB moat where DIC is lower than lake concentrations. However, Lake Hoare is not high in PER or DPR relative to Lakes Fryxell nor WLB (Table 3.3).

Correlations between phytoplankton community composition (Teira et al. 2001) and average algal cell size (Malinsky-Rushansky and Legrand 1996; Teira et al. 2001) have been made with respect to PER in marine environments. Quantitative comparisons of phytoplankton population structure have been made in Lake Bonney (Lizotte and Priscu 1998; Tursich 2003), describing distinct phytoplankton populations at the DCM compared to the ice-water interface. A correlation between average phytoplankton cell size and PER (see Teira et al. 2001) is unlikely in Lake Bonney, as the dominant phytoplankton species at the DCM in Lake Bonney are larger than the dominant phytoplankton species at 5m (Lizotte and Priscu 1998; Tursich 2003). I found no relationship connecting DPR and PER to the abundance of algal pigment groups, as measured using *in-situ* spectral fluorescence.

Measurements of primary productivity in the McMurdo Dry Valley lakes have focused on the particulate fraction of carbon fixed in photosynthesis (e.g. Priscu 1995; Priscu et al. 1999; Takacs et al. 2001). While measurement of particulate primary productivity provides an estimate of algal carbon biomass produced (i.e. net photosynthesis), my data show that inclusion of DPR accounts for 14-60% more ecologically available organic carbon. Addition of DPR to PPR accounts for an increase in organic carbon availability from approximately 15% at 5m in ELB to more than 100%

at the DCM of WLB. Consideration of the variable and sometimes large input of DPR may be significant to the understanding of lake productivity, carbon cycling, quantification and relative importance of carbon sources, and coupling of autotrophy and heterotrophy. For example, in the carbon budget of WLB described previously (Takacs et al. 2001), increasing PER from the estimate of 25% to 58% as measured in this study would increase the estimated input of DPR as a source of DOC to the carbon budget from 56% to nearly 75%.

DOC released by phytoplankton can be a significant labile carbon source for heterotrophic bacterioplankton (Lancelot 1984; Norrman et al. 1995; Baines and Pace 1991; Amon et al. 2001; Moran et al. 2002; Kritzberg et al. 2005). Extracellular DOC release can represent more than 80% of the variation in bacterial carbon demand in Antarctic marine systems (Moran et al. 2001). I have shown that bacterial carbon demand can exceed net primary production at some depths within McMurdo Dry Valleys lakes, and that inclusion of extracellular DOC release can reduce this deficit by 13-53%. DPR alone could potentially satisfy BCD in 50% of the locations of this study where both DPR and BP were measured at the time of measurement.

Measurements of bacterial productivity in the light generally exceeded those in the dark at 5m in my study (Figure 3.4), a depth where DOC concentrations were much lower than at 15m (Takacs et al. 2001), and where dark bacterial productivity was higher than bacterial productivity in the light. These results indicate a close coupling of autotrophy and heterotrophy at 5m in all of the study lakes.

Although specific DPR products have not been identified and subsequently quantified in the McMurdo Dry Valleys lakes, proteins and polysaccharides are the primary end products of PPR (Priscu et al. 1988). These photosynthetic products and their intermediates are often responsible for the majority of DPR in other systems (Fogg 1964; 1983; Bjørnsen 1988; Wetzel 2001). In general, most photosynthetically produced DOC is quickly utilized by heterotrophic bacteria in marine and freshwater environments (Cole et al. 1982; Sundh and Bell 1992; Moran et al. 2001), and rates of bacterial productivity have been shown to correlate closely with rates of primary production and DPR (Chróst and Siuda 2006). Another DOC source that may satisfy the heterotrophic carbon demand in the trophogenic zone above the chemocline in the study lakes is upward diffusion from the large DOC pool existing in the bottom waters beneath the chemocline (Priscu 1995; Priscu et al. 1999; Takacs et al. 2001). Sources of DOC to the dry valley lakes also include stream input (see Chapter 2) and degradation of particulate matter (Priscu et al. 1999; Takacs et al. 2001).

This study represents the first measurements of DPR and PER in WLB, Lake Fryxell, and Lake Hoare and the first attempt to describe the release of dissolved photosynthate products in response to variation in irradiance in all Taylor Valley lakes and in the ELB moat. Photosynthesis-irradiance relationships were previously determined in the below-ice water column of dry valley lakes (e.g. Lizotte and Priscu 1992a). This study augmented descriptions of lake productivity by detailing photosynthesis-irradiation relationships in the moat, though moat DPR measurements were highly variable. The moat description of photosynthetic light-response allows comparison of light-limited

phytoplankton from beneath the ice with moat phytoplankton, which are subject to near full-surface irradiance.

I have discussed possible variables influencing causative theories for DPR and how they might apply to the DPR values measured in dry valley lakes. These variables showed that irradiance is closely related to the amount of photosynthetically fixed carbon released from active phytoplankton. DPR was also positively correlated with DIN and DIC, variables that may stimulate photosynthesis. Examining these correlations, I again suggest that extracellular release of DOC in dry valley lakes and moats is an unfortunate by-product of photosynthesis for phytoplankton. If DPR represents an adaptive mechanism, as suggested by some researchers (Bjørnsen 1988; Wood and Van Valen 1990; Van der Meersche et al. 2004), I can not find evidence that it is adaptive in Taylor Valley lakes. I find it surprising that phytoplankton which have been shown to efficiently fix carbon despite severe light limitation in the study lakes (e.g. Lizotte and Priscu 1992) lose such a large amount of organic carbon to their surroundings through DPR. This phenomenon seems detrimental to phytoplankton as DPR represents a loss of photosynthetic effort, but appears to provide important substrate to satisfy bacterial carbon demand in lakes and moats of the McMurdo Dry Valleys.

CHAPTER 4

CONCLUSIONS

Past studies on the ice-free moats of the McMurdo Dry Valleys have focused on benthic microbial communities and processes (i.e. Wharton et al. 1983; Hawes and Schwarz 1999; Lawson et al. 2004), leaving moat plankton communities largely unstudied. Other studies have focused on the importance of the moat in the exchange of dissolved constituents between the lake and the greater outside environment (Neumann et al. 2001; Takacs et al. 2001; Lee et al. 2004), and the moat has also been studied in terms of selected physical properties (Miller and Aiken 1996). The work presented in Chapter 2 provides a view into pelagic moat diversity and biogeochemistry, the physical processes of mixing, and a first examination of the spatial variability in the moat including lateral chemical gradients influenced by stream flow, microbial production, algal and bacterial diversity and factors that may influence microbial distribution.

My first research question focused on describing the influence of stream mixing with the moat. The data showed that the stream represented a source of dissolved ions and nutrients to the moat and that stream/moat mixing caused significant chemical gradients with respect to the inflow. SRP was immediately removed from the pool of dissolved solids as the stream entered the moat. DIC and DIN also decreased in concentration with respect to the inflow while particulate nitrogen and phosphorus increased with distance from the Priscu Stream. My data provided evidence of

phytoplankton growth limitation caused by moat resource deficiencies (SRP > DIC > DIN). Phytoplankton biomass exceeded bacterioplankton biomass 4-165 fold, implying potential heterotrophic bacterial dependence on primary production. The diversity of bacterial morphotypes (rod, coccoid, and filament) decreased with distance from the inflow as phytoplankton pigment diversity increased.

My second research goal was to determine if the ELB moat is a chemically and biologically unique ecosystem, a heterogeneous feature that varies with local conditions, or an extension of the stream or lake environment. In the moat I found combinations of chemical and biological characteristics from both the stream and from the lake that result in a unique moat environment. Local climate variability plays an important role in McMurdo Dry Valleys moat environments. Subtle changes in temperature can cause a "ripple" effect in the amount of stream discharge entering the moat, its chemical content, physical extent, temporal duration and variability, and the extent of the below-ice water column-moat mixing. I conclude that the moat represents a dynamic ecosystem being influenced by both stream and lake environments, which are all controlled by local climate variation (primarily temperature and PAR).

My research also detailed an important aspect of algal physiology in McMurdo Dry Valleys lakes and provided a comparison of this physiology to moat phytoplankton. Localized input of DIN from stream flow, low moat concentration of DIC, high moat photosynthesis, and abundant irradiation were factors influencing DPR in the lake; I expect these factors would also have an influence on moat phytoplankton. My examination of extracellular release of dissolved organic carbon revealed the ecological

and physiological importance of this release in a completely microbially dominated system. The release of photosynthetic organic material represents a notable loss in photosynthetic effort by lake and moat phytoplankton that live in a stressed environment, and provides important potential substrate to bacterioplankton. This substrate is important because DOC is often scarce in the lake photic zone and in moat locations within the aquatic environments studied. My study of the influence of light on primary production and extracellular release (3rd research question) revealed specific acclimation in photosynthetic efficiency under three different light regimes. Phytoplankton populations in the deep chlorophyll maxima demonstrated low light photosynthetic acclimation, responding relatively quickly to an increase in light availability and fixing more carbon per unit of chlorophyll *a* at saturating PAR. Phytoplankton populations just below the ice cover were less acclimated to low light conditions, while moat phytoplankton were the least acclimated to low-light.

My study of moat environments provides three snapshots of the moat environment, each being quite different from the others. Some samples of the moat showed relatively strong gradients with respect to stream chemistry and similarity between stream microbial diversity and proximal-stream moat diversity indicated the input of stream microbes. Samples of the moat from another date revealed a more subtle stream influence, and the variation in the moat appeared to be dampened, as moat biogeochemistry and microbial productivity approached relative uniformity. Sampling during a different season provided a very different snapshot of the ELB moat, one in which the characteristics of the previous year's stream discharge were very different,

while warmer temperatures and higher average PAR resulted in more melting and streamflow, which may have increased the mixing between lake and moat. Due to the high variability in the moat environment, a higher temporal density of sampling is necessary for further comparisons of productivity, community structure and physiology.

The moat environments provide a number of promising directions for future research in microbial ecology. One future direction that should be taken is using molecular tools to examine microbial physiology with the goal of elucidating bacterial succession and providing further insight into bacterial physiological diversity with respect to *in-situ* environmental variables. The resulting information would allow meaningful experiments to be designed, testing possible reasons that observed patterns in variation exist and to determine important driving factors in the distribution of microbial communities.

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APPENDICES

APPENDIX A

DATA TABLES

Table A.1. Measured pH, concentration (μM) of major anions and dissolved inorganic carbon (DIC) and sum of anions (mmolcharge·kg^{-1}) measured during the 2004-2006 seasons in East Lake Bonney and the primary inflow. Z = depth (m), italicized values are between method detection limit and one half method detection limit, italicized ranges represents values below one half method detection limit or values that were not reported, ND* represents values that were not detected due to dilution (6m diluted 55X, 10m diluted 100X, 13m diluted 550X)for analysis of other ionic species, NM = not measured.

Date	Loc ID	Z	pH	DIC	Cl^-	SO_4^{2-}	F^-	NO_3^-	∑anions
18-Dec-04	Inflow 1	surf.	8.8	1322	1573	132	11	*2.5*	3.2
	Inflow 2	surf.	8.6	1191	752	103	8	*<1.6*	2.2
	Moat 4.1	0.2	9	537	868	102	7	*<1.6*	1.6
	Moat 4.2	0.2	9	614	542	76	5	*<1.6*	1.3
	Moat 4.3	0.2	9.1	660	614	81	6	*<1.6*	1.5
	Moat 4.4	0.2	9	*66*	505	19	3	*<1.6*	0.6
	Moat 4.5	0.2	8.4	*111*	347	23	4	*<1.6*	0.5
	11m hole	6	8.5	1459	26917	2241	ND*	ND*	30
		10	8.4	2153	45300	2718	ND*	ND*	49
	26m hole	6	8.6	1470	23888	2098	ND*	ND*	27
3-Jan-05	Inflow 1	surf.	8.7	831	577	60	5	*<1.6*	1.5
	Inflow 2	surf.	8.6	762	558	62	5	*<1.6*	1.5
	Moat 4.1	0.2	9	234	396	46	4	*<1.6*	0.7
	Moat 4.2	0.2	8.9	257	150	10	3	*<1.6*	0.4
	Moat 4.3	0.2	9	238	472	52	5	*<1.6*	0.8
	Moat 4.4	0.2	9	204	237	61	9	*0.8*	0.6
	Moat 4.5	0.2	9.1	*76*	391	74	7	*0.8*	0.6
	11m hole	6	8.8	NM	19949	1720	ND*	ND*	24
		10	8.6	NM	42937	2650	ND*	ND*	487
	26m hole	6	8.9	NM	10741	957	ND*	ND*	14
		13	7.2	NM	188474	5245	ND*	ND*	193
28-Nov-05	Core 5.1	0.2	NM	NM	265	2	*1*	*1.2*	0.0
	Core 4.3	0.2	NM	NM	151	5	*<1.3*	*2*	0.1
	Core 5.7	0.2	NM	NM	143	1	1	*2.1*	0.0
15-Dec-05	Moat 5.1	0.2	8.8	738	468	88	7	*<1.6*	1.4
	Moat 5.2	0.2	9.1	141	1498	78	6	*<1.6*	1.8
	Moat 5.3	0.2	8.7	*118*	794	95	5	*1.2*	1.1
	Moat 5.4	0.2	9.3	298	1133	69	5	*<1.6*	1.6
	Moat 5.5	0.2	8.9	*<75*	127	8	4	*<1.6*	0.2
	Moat 5.6	0.2	8.7	*<75*	740	35	4	*0.9*	0.6
	Moat 5.7	0.2	9	134	96	9	3	*<1.6*	0.3

Table A.2. Concentration of major cations (μM), sum of cations (mmolcharge·kg^{-1}), charge difference and ionic strength (I, no units) measured during the 2004-2006 seasons in East Lake Bonney and the primary inflow. Z = depth (m).

Date	Loc ID	Z	Na^+	Mg^{2+}	Ca^{2+}	K^+	∑cations	Charge Diff.	I
18-Dec-04	Inflow 1	surf.	2186	493	510	109	4.2	14%	0.0048
	Inflow 2	surf.	663	175	226	46	1.5	19%	0.0023
	Moat 4.1	0.2	391	141	338	56	1.4	9%	0.0021
	Moat 4.2	0.2	401	116	201	39	1.0	12%	0.0016
	Moat 4.3	0.2	453	125	198	40	1.1	14%	0.0017
	Moat 4.4	0.2	80	29	39	10	0.2	47%	0.0005
	Moat 4.5	0.2	228	66	68	16	0.5	0.1%	0.0007
	11m hole	6	21366	3080	2292	634	32	2%	0.0376
		10	34355	5389	2812	1052	50	1%	0.0587
	26m hole	6	19315	2747	2111	603	29	2%	0.0341
3-Jan-05	Inflow 1	surf.	423	99	129	28	0.9	26%	0.0015
	Inflow 2	surf.	429	93	132	29	0.9	23%	0.0015
	Moat 4.1	0.2	303	67	100	22	0.7	7%	0.0009
	Moat 4.2	0.2	97	33	33	10	0.2	31%	0.0004
	Moat 4.3	0.2	341	78	125	25	0.8	4%	0.0011
	Moat 4.4	0.2	221	109	319	65	1.1	32%	0.0013
	Moat 4.5	0.2	316	135	314	66	1.3	34%	0.0015
	11m hole	6	15806	2363	1741	476	24	2%	0.0283
		10	33737	5160	2854	1021	487	3%	0.0566
	26m hole	6	8929	1257	1124	298	14	5%	0.016
		13	131866	29639	6999	4016	193	3%	0.2209
28-Nov-05	Core 5.1	0.2	16	5	9	3	0	70%	0.0002
	Core 4.3	0.2	30	5	12	6	0.1	40%	0.0001
	Core 5.7	0.2	14	5	6	3	0	59%	0.0001
15-Dec-05	Moat 5.1	0.2	363	132	254	49	1.2	10%	0.0018
	Moat 5.2	0.2	717	141	113	29	1.2	19%	0.0019
	Moat 5.3	0.2	583	113	141	28	1.1	0.1%	0.0015
	Moat 5.4	0.2	634	123	107	27	1.1	19%	0.0017
	Moat 5.5	0.2	74	25	26	6	0.2	8%	0.0002
	Moat 5.6	0.2	175	63	115	24	0.8	20%	0.0009
	Moat 5.7	0.2	71	12	16	4	0.1	34%	0.0002

Table A.3. Limnological data used in discussion of East Lake Bonney stream, lake and moat. Z = depth (m), BAC = bacterial cell density (cells·mL^{-1}), Chl-a = Chlorophyll *a* (µgChl-a·L^{-1}), POC and PON = particulate organic carbon and nitrogen (µM), DOC = dissolved organic carbon (µM), NH_4^+, NO_2^-, NO_3^-, and soluble reactive phosphorus (SRP) are reported in µM, italicized values are between one half method detection limit and detection limit, an italicized range indicates values below one half reported method detection limits.

Date	Loc ID	z	Type	BAC	Chl-a	POC	DOC	NH_4^+	NO_3^-	NO_2^-	PON	pH	SRP
18-Dec-04	Inflow 1	s	Stream	4.4E+04	0.1	17	27	1.0	9.9	*<0.1*	*<0.35*		0.77
	Inflow 2	s	Stream	4.0E+04	2.8	20	34	3.7	4.5	*<0.1*	1.8		0.37
	Moat 4.1	0.2	Moat	7.9E+04	4.8	22	29	1.1	1.5	0.28	2.1		*<0.1*
	Moat 4.2	0.2	Moat	8.0E+04	4.8	25	25	0.2	1.5	0.26	2.3	9.0	*<0.1*
	Moat 4.3	0.2	Moat	6.4E+04	5.9	27	50	0.3	0.7	0.29	2.8	9.1	*<0.1*
	Moat 4.4	0.2	Moat	4.7E+04	0.5	14	44	0.5	*<0.1*	*<0.1*	*<0.35*		*<0.1*
	Moat 4.5	0.2	Moat	1.1E+05	0.7	19	37	0.3	0.2	*<0.1*	*<0.35*	*8.4*	*<0.1*
	11m hole	6m	Lake	6.8E+04	2.6	22	61	0.3	10.3	*<0.1*	*<0.35*	*8.5*	0.34
		10m	Lake	1.0E+05	1.3	18	66	0.4	9.2	*<0.1*	*<0.35*	*8.4*	0.35
	26m hole	6m	Lake	3.1E+04	3.4	22	58	0.5	10.2	*<0.1*	*<0.35*	*8.6*	0.20
3-Jan-05	Inflow 1	0.2	Stream	7.4E+04	0.8	17	32	0.3	1.8	*<0.1*	2.0	8.7	0.36
	Inflow 2	0.2	Stream	7.9E+04	1.2	18	34	0.2	2.1	*<0.1*	2.0	8.6	*0.11*
	Moat 4.1	0.2	Moat	1.5E+05	3.5	19	39	0.3	0.2	*<0.1*	1.8	9.0	1.31
	Moat 4.2	0.2	Moat	1.1E+05	3.7	19	41	0.2	0.2	*<0.1*	1.9	8.9	*0.05*
	Moat 4.3	0.2	Moat	1.1E+05	2.9	18	26	*<0.07*	*<0.1*	*<0.1*	1.3	9.0	*0.08*
	Moat 4.4	0.2	Moat	7.3E+04	0.7	12	27	0.2	*<0.1*	*<0.1*	*<0.35*	*9.0*	*0.08*
	Moat 4.5	0.2	Moat	1.3E+05	4.6	48	31	0.2	*<0.1*	*<0.1*	4.4	9.1	1.43
	11m hole	6m	Lake	8.4E+04	3.1	18	62	0.7	8.2	*<0.1*	1.4	8.8	*<0.1*
		10m	Lake	6.5E+04	2.3	16	65	0.4	7.5	*<0.1*	*<0.35*	*8.6*	*<0.1*
	26m hole	6m	Lake	1.3E+05	1.7	17	60	0.5	4.4	*<0.1*	1.3	8.9	*<0.1*
		13m	Lake		5.1	20	*<5*	1.5	14.6	*0.33*	3.3	7.2	1.58

Table A.3., Continued.

Date	Loc ID	z	Type	BAC	Chl-a	POC	DOC	NH^{4+}	NO_3^-	NO_2^-	PON	pH	SRP
28-Nov-05	Core 5.1 (T)	Top	Core				125	1.0	7.0	*<0.1*			*<0.1*
	Core 5.1 (B)	Bot	Core				83	1.7	3.4	*<0.1*			*<0.1*
	Core 4.3 (T)	Top	Core				*<9*	2.0	6.7	*<0.1*			*<0.1*
	Core 4.3 (B)	Bot	Core				183	1.2	10.0	*<0.1*			*<0.1*
	Core 5.7	All	Core				108	2.2	9.5	*<0.1*			*<0.1*
15-Dec-05	Moat 5.1	0.2	Moat	1.9E+05	6.9		*17*	0.3	10.4	*0.12*		8.8	*<0.1*
	Moat 5.2	0.2	Moat	3.5E+05	14.5		*11*	0.3	*<0.1*	*<0.1*		9.1	*<0.1*
	Moat 5.3	0.2	Moat	1.8E+05	7.7		*9*	0.3	0.2	*<0.1*		8.7	*<0.1*
	Moat 5.4	0.2	Moat	1.1E+05	27.9		166	0.3	2.1	*<0.1*		9.3	*<0.1*
	Moat 5.5	0.2	Moat	1.0E+05	0.9		*17*	0.3	0.4	*<0.1*		8.9	*<0.1*
	Moat 5.6	0.2	Moat	1.7E+05	9.2		*<9*	0.3	0.3	*<0.1*		8.7	*<0.1*
	Moat 5.7	0.2	Moat	2.0E+05	0.7		*8*	0.2	5.4	*<0.1*		9.0	0.3

Table A.4. Photosynthetically active radiation (PAR, µmol photon·m^{-2}·s^{-1}) at the surface, 5m, and 15m in East Lake Bonney over a 24-hour period on 4 December 2001, an apparently cloudless day.

Time	Ambient	5m	15m
		PAR	
0:00	124	3.2	0.86
1:00	105	2.7	0.73
2:00	100	2.6	0.69
3:00	102	2.7	0.71
4:00	95	2.5	0.66
5:00	116	3.0	0.81
6:00	459	12	3.2
7:00	694	18	4.8
8:00	820	21	5.7
9:00	955	25	6.6
10:00	1080	28	7.5
11:00	1198	31	8.3
12:00	1213	32	8.4
13:00	1388	36	9.6
14:00	1434	37	10.0
15:00	1381	36	9.6
16:00	1294	34	9.0
17:00	1168	30	8.1
18:00	1014	26	7.0
19:00	851	22	5.9
20:00	703	18	4.9
21:00	648	17	4.5
22:00	407	11	2.8
23:00	120	3.1	0.84

Table A.5. Surface and underwater (UW) irradiance (μmol photon·m^{-2}·s^{-1}) at 1m depth intervals from below the ice cover to 20m in East Lake Bonney, measured 10 December 2004.

Depth	Surface	UW
5	483	12.3
6	482	10.4
7	482	9.2
8	482	8.1
9	478	6.7
10	485	6.1
11	484	5.5
12	477	4.8
13	471	4.1
14	470	3.7
15	470	3.3
16	469	3.3
17	428	2.9
18	427	2.7
19	539	2.4
20	537	2.1

APPENDIX B

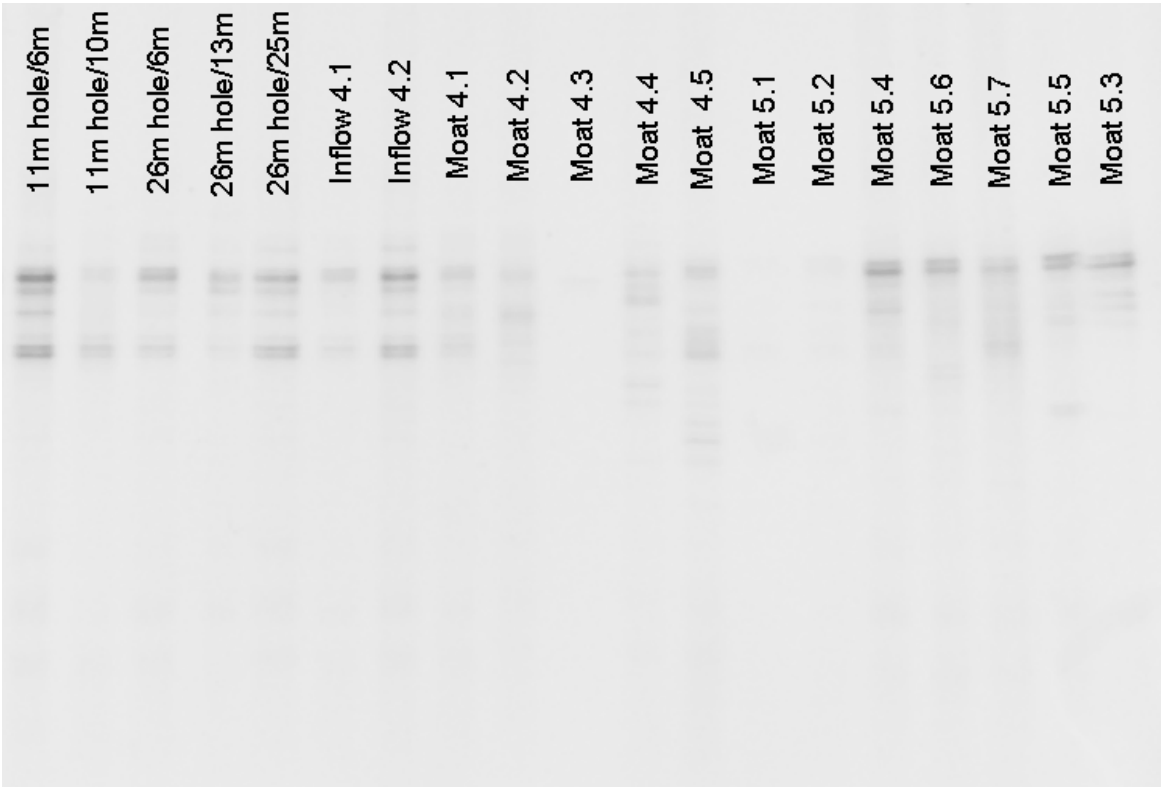
DESCRIPTION OF SAMPLE LOCATIONS

Table B.1. Names, dates, shore-line distance from Priscu Stream Inflow, and description of stream, moat, and lake sampling locations during the 2004-2006 summer seasons. See Figure 2.2 for locations shown on aerial photograph.

Sample ID	Date Sampled	Dist. from Inflow 1 (m)	Location/Physical Description (at time of initial sampling)
Inflow 1	18-Dec-04, 3-Jan-05	NA	Shallow stream bed over fine, sandy material approx. 50m upstream of stream inlet and 200m below gauging station
Inflow 2	18-Dec-04, 3-Jan-05	44	Slow flowing inlet to Lake Bonney 10-20m wide, 30-40 m long, approx. 1m at deepest. Contained floating pieces of algal mat.
Moat 4.1/5.1	18 Dec 04, 3-Jan-05, 22-Nov-05 (Ice Core), 15-Dec-05	78	Sampled from lake ice edge near inlet, approx. 10m wide and 1.5-2.0 m deep during sampling, less visible transparency
Moat 4.2	18-Dec-04, 3-Jan-05	166	North side of lake near Moat 4.1, see Moat 4.1 for attributes
Moat 4.3	18-Dec-04, 3-Jan-05, 22-Nov-05 (Ice Core)	554	South side of lake across from inlet, also approx. approx. 10m wide and 1.5-2.0 m deep. More visibly transparent.
Moat 4.4	18-Dec-04, 3-Jan-05	1613	North side of lake, visibly transparent water, approx. 1m deep
Moat 4.5	18-Dec-04, 3-Jan-05	1623	South side of lake, visibly transparent water, approx. 1.5 m deep
Limno	18-Dec-04, 3-Jan-05	2699	Routine lake sampling hole
11m hole	18-Dec-04, 3-Jan-05	764	Hole drilled in the center of the lake width-wise, 11m pieziometric depth
26m hole	18-Dec-04, 3-Jan-05	1046	Hole drilled in the center of the lake width-wise, 26m pieziometric depth
Moat 5.2	15-Dec-05	855	South side of lake, visibly transparent water, approx. 1.5 m deep
Moat 5.3	15-Dec-05	-	North side of lake, visibly transparent water, approx. 1 m deep
Moat 5.4	15-Dec-05	3294	South side of lake, visibly transparent water, approx. 1.5 m deep
Moat 5.5	15-Dec-05	4590	North side of lake, visibly transparent water, less than 1 m deep
Moat 5.6	15-Dec-05	3132	South side of lake, moat less visibly transparent and wider, within 50m of secondary stream inflow
Moat 5.7	22 Nov-05, 15-Dec-05	1644	North side of narrow channel connecting East and West Lake Bonney, largely frozen during sampling compared to other moat locations

APPENDIX C

IMAGE OF DGGE GEL


11m hole/6m
11m hole/10m
26m hole/6m
26m hole/13m
26m hole/25m
Inflow 4.1
Inflow 4.2
Moat 4.1
Moat 4.2
Moat 4.3
Moat 4.4
Moat 4.5
Moat 5.1
Moat 5.2
Moat 5.4
Moat 5.6
Moat 5.7
Moat 5.5
Moat 5.3