The factors affecting the distribution and dynamics of bacterioplankton biomass and productivity in Taylor Valley Lakes, Antarctica
by Cristina D Takacs

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biological Sciences
Montana State University

Abstract:
This study investigated the factors affecting summer bacterial biomass and production in Taylor Valley Lakes Fryxell, Hoare, and the east and west lobes of Lake Bonney, Antarctica, during 1993 to 1997. The major objectives of this research were to 1) define spatial and seasonal bacterial biomass and production, 2) numerically model biomass losses, and 3) determine the potential role of DOC supply, inorganic nutrients, temperature, and salinity in the regulation of summer bacterial production.

Lake Fryxell was the most productive, but variable lake, followed by Lakes Bonney and Hoare. Bacterial production, measured by 3H-thymidine uptake, ranged from 0 to 0.009 μg C ml-1d-1, and bacterial numbers, counted using epifluorescent microscopy, ranged from 3.2xl04 to 4.4xl07 cells ml-1. A forward difference model of bacterial biomass losses in the trophogenic zone and the entire water column of the lakes showed that summer loss rates reached 6.3xl014 cells m-2 d-1 and 4.16xl012 cells m-2 d-1, respectively. Lake DOC budgets indicated that bacterial carbon demand exceeded total DOC supply to the trophogenic zone and entire water column of Lakes Fryxell and Hoare, but demand and supply were approximately balanced in Lake Bonney. Inorganic nutrient bioassays did not indicate that the bacterioplankton from the primary productivity maxima of the lakes were nutrient limited. 3H-thymidine incorporation rates were 20 to 67% lower in bacterial populations incubated at in situ temperatures, compared to their optimal temperature for growth, which ranged from 10 to 20°C among the depths tested. Bacterial strains isolated from the lakes generally showed a psychrotrophic response to temperature. Strains isolated from the brackish to hypersaline deep waters of Lakes Fryxell and Bonney grew optimally at salinities ranging from 0 to 5% NaCl. The results of the study indicate that bacterial biomass in these lakes may be important to higher trophic levels through grazing and that annual bacterial production is dependent upon alternative sources of organic carbon, such as particulate organic matter decomposition. Nutrients appear to play a less important role in bacterial regulation, whereas temperature and salinity limit or even restrict bacterial production in these lakes.

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by

Cristina D. Takacs

A thesis submitted in partial fulfillment
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APPROVAL

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Cristina D. Takacs

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

John C. Priscu — Chairperson, Graduate Committee — 16 April 99
Date

Approved for the Department of Biology

Ernest R. Vyse — Department Head — 4/16/99
Date

Approved for the College of Graduate Studies

Bruce McLeod — Graduate Dean — 4-20-99
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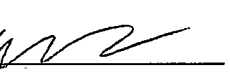
Signature 

Date 16 April, 1999

For my brother, Jack

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ABSTRACT

This study investigated the factors affecting summer bacterial biomass and production in Taylor Valley Lakes Fryxell, Hoare, and the east and west lobes of Lake Bonney, Antarctica, during 1993 to 1997. The major objectives of this research were to 1) define spatial and seasonal bacterial biomass and production, 2) numerically model biomass losses, and 3) determine the potential role of DOC supply, inorganic nutrients, temperature, and salinity in the regulation of summer bacterial production.

Lake Fryxell was the most productive, but variable lake, followed by Lakes Bonney and Hoare. Bacterial production, measured by ^{3}H-thymidine uptake, ranged from 0 to 0.009 μg C ml^{-1} d^{-1}, and bacterial numbers, counted using epifluorescent microscopy, ranged from $3.2x10^{4}$ to $4.4x10^{7}$ cells ml^{-1}. A forward difference model of bacterial biomass losses in the trophogenic zone and the entire water column of the lakes showed that summer loss rates reached $6.3x10^{14}$ cells m^{-2} d^{-1} and $4.16x10^{12}$ cells m^{-2} d^{-1}, respectively. Lake DOC budgets indicated that bacterial carbon demand exceeded total DOC supply to the trophogenic zone and entire water column of Lakes Fryxell and Hoare, but demand and supply were approximately balanced in Lake Bonney. Inorganic nutrient bioassays did not indicate that the bacterioplankton from the primary productivity maxima of the lakes were nutrient limited. ^{3}H-thymidine incorporation rates were 20 to 67% lower in bacterial populations incubated at *in situ* temperatures, compared to their optimal temperature for growth, which ranged from 10 to 20°C among the depths tested. Bacterial strains isolated from the lakes generally showed a psychrotrophic response to temperature. Strains isolated from the brackish to hypersaline deep waters of Lakes Fryxell and Bonney grew optimally at salinities ranging from 0 to 5% NaCl. The results of the study indicate that bacterial biomass in these lakes may be important to higher trophic levels through grazing and that annual bacterial production is dependent upon alternative sources of organic carbon, such as particulate organic matter decomposition. Nutrients appear to play a less important role in bacterial regulation, whereas temperature and salinity limit or even restrict bacterial production in these lakes.

CHAPTER 1

FACTORS AFFECTING THE DISTRIBUTION AND DYNAMICS OF BACTERIOPLANKTON BIOMASS AND PRODUCTIVITY IN TAYLOR VALLEY LAKES, ANTARCTICA: INTRODUCTION

Microbial Ecology and the Study of Bacteria in Natural Waters

The term "microbial ecology" was first defined and used by Ernest Haeckel over 140 years ago, but did not come into frequent use until the 1960s (Atlas and Bartha 1987). It was not until Winogradsky and Beijernick and their development of enrichment culture techniques that microbiology began to appreciate the diversity and importance of bacteria in the natural environment (Atlas and Bartha 1987; Madigan et al. 1997). However, because of Koch's Postulates, microbiological research primarily focused on understanding bacterial physiology in pure cultures. It was not until the general importance of bacteria in global geochemical cycles was acknowledged that their ecology was widely investigated (Atlas and Bartha 1987).

The ecological role of aquatic heterotrophic bacteria has received increasing attention in the past thirty years (Pomeroy 1974; Van Es and Mayer Reil 1982; Cho and Azam 1988; Pomeroy 1984; Robarts and Wicks 1990; Riemann and Sondergaard 1986; Whitman et al. 1998). Researchers from the fields of limnology and oceanography were perhaps the first to undertake true *in situ* microbial studies, an approach that is essential for meaningful ecological studies. Initially, aquatic microbial studies were primarily

concerned with primary production, which resulted in the development of radioisotopic techniques (Steeman-Nielsen 1952; Goldman 1963). These techniques eventually were transferred to bacterial studies (Parsons and Strickland 1962; Wright and Hobbie 1965, 1966; Fuhrman and Azam 1980). It is now clear that bacteria are important not only in the regeneration of nutrients and organic matter decomposition (Pomeroy 1974), but also as a source of carbon to higher trophic levels in the ocean (Azam et al. 1983; Pomeroy 1984) and in freshwater systems (Porter 1984; Christoffersen et al. 1990; Lyche et al. 1996). The development of techniques to measure bacterial biomass (Hobbie et al. 1977), bacterial production (Fuhrman and Azam 1980, Karl 1986, Simon and Azam 1989), and determine phylogeny (Olsen and Woese 1993) have demonstrated the importance of bacteria in the environment in terms of overall biomass, production, and diversity (Whitman et al. 1998; Giovannoni et al. 1990). However, questions remain concerning the factors regulating bacterial biomass and production, and interactions with other members of the food web.

Because of the complexity of physical, chemical, and biological interactions in most aquatic systems, microbial ecology studies are undertaken more easily in relatively simple, natural systems (Ward et al 1994; Hooper et al. 1998; Eichner et al. 1999). The lakes of the McMurdo Dry Valleys provide unique systems in which to study microbial ecology. The lakes contain permanent ice-covers, which prevent wind-driven mixing, and are comprised of a primarily microbial biota.

The lakes were first described at the beginning of the century (e.g., Scott 1905), but were not extensively studied until the 1960s when the first quantitative physical,

chemical, and biological measurements were made (Armitage and House 1962; Angino and Armitage 1963; Goldman 1964; Goldman et al. 1967). However, a majority of these studies focused on the phytoplankton (Goldman et al. 1967; Koob and Leister 1972; Parker et al. 1977; Vincent 1981; Lizotte et al. 1996); the bacterioplankton have been relatively unexplored. Of the studies that have been concerned with the bacterioplankton, biomass and production were largely underestimated. Essentially all of these studies were conducted during a time when bacterial colony plate counts were the standard technique of determining bacterial abundance (Koob and Leister 1972; Mikell et al. 1984). It is now clear that plate counts are an inefficient means of enumerating bacteria from the natural environment because not all bacteria are able to grow on enriched agar, resulting in underestimates of total bacterial biomass and diversity (Ward et al. 1990; Button et al. 1993). A clear picture of seasonal and inter-annual bacterial biomass and production dynamics has not been possible from previous studies because of the lack of a long-term continuous dataset. The effects of increased anthropogenic impact from both researchers and tourists, and the sensitivity of the polar regions to global climate change require long-term monitoring in this region to delineate impacts. The objectives of this study were to describe the spatial and seasonal distribution of bacterial biomass and determine the factors affecting bacterial production in McMurdo Dry Valley Lakes Fryxell, Hoare, and Bonney during four Austral summers.

Description of Study Site

The McMurdo Dry Valleys forms a 4800 km^2 region of Antarctica's southern Victoria Land that has been ice free for approximately the last 3.5 million years (Prentice et al. 1998). This region has often been described as one of the harshest places on earth because of the low mean annual temperatures (-20°C) and precipitation (<10 cm y^{-1}), coupled with the high mean wind speed. Organic material comprises less than 0.1% of the soil (Horowitz 1972; Fritsen et al. In press), which is permanently frozen 10 to 30 cm below the surface (Freckman and Virginia 1998).

The streams of the valley are fed by glacial meltwater when summer temperatures in the valley approach or exceed freezing. Stream flow is highly variable in the valley on both a daily and annual basis (Conovitz et al. 1998). Taylor Valley stream nutrients are labile and are derived from the microbial mats that inhabit the stream beds (Vincent et al. 1993; Aiken et al. 1996). Detailed descriptions of the lakes and streams may be found in Green and Friedmann (1993) and Priscu (1998).

This study concentrated on Lakes Fryxell, Hoare, and the east and west lobes of Lake Bonney, which lie in the Taylor Valley (~77°37'S, ~163°00'E, Figure 1.1). Lake Fryxell, at the easternmost edge of the valley, has an approximate surface area of 7 km^2 and a maximum depth of 18 m.

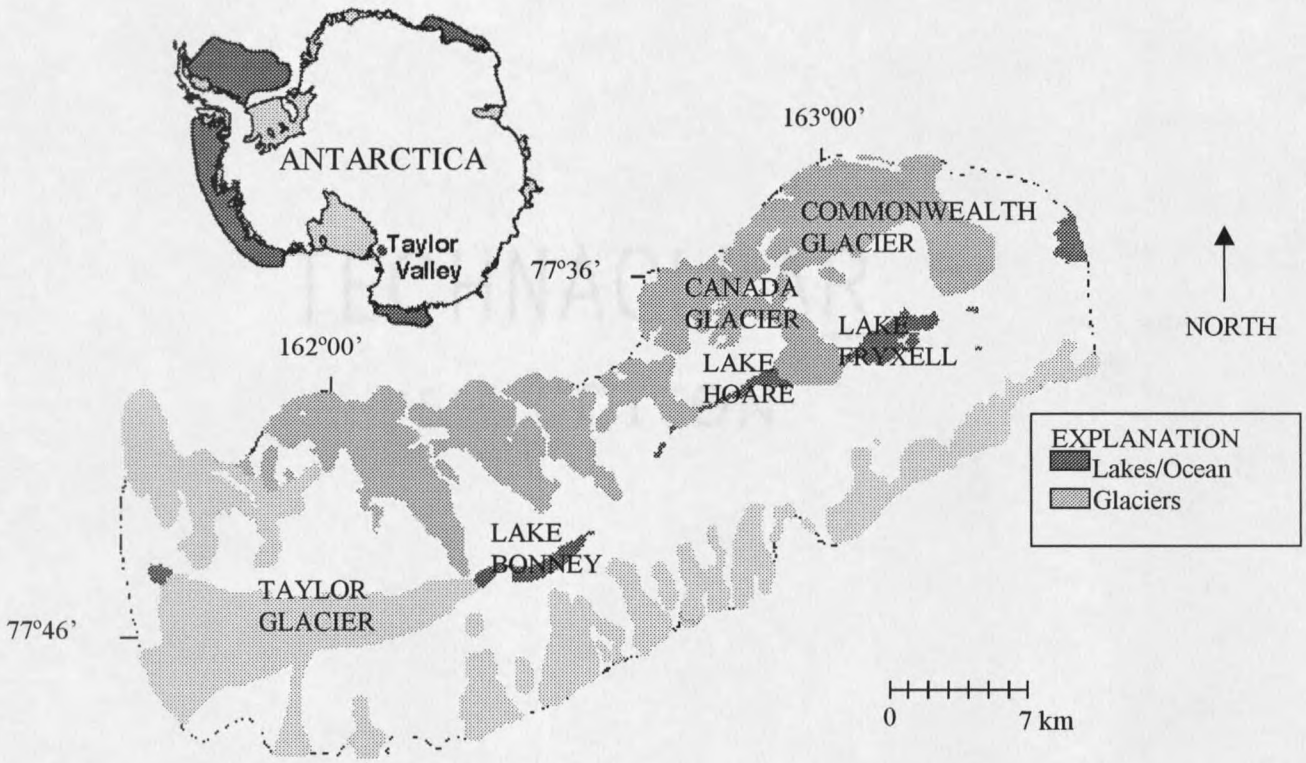

Figure 1.1. Map of Taylor Valley, McMurdo Dry Valleys, Antarctica

Lake Hoare has an approximate surface area of 2 km^2 and a maximum depth of 30 m. Lake Bonney is located at the head of the valley with a surface area of approximately 4.3 km^2 and a maximum depth of 40 m. Lake Bonney has two basins (east and west lobes), connected by a narrow (~20 m wide), shallow (12 m) sill (Angino 1964; Spigel and Priscu 1998) that prevents mixing between the two lobes below 12 m. While these lakes have varying degrees of chemical stratification, generally all contain nutrient rich deep water covered by a relatively nutrient poor trophogenic zone (Priscu 1995; Spigel and Priscu 1996). The permanent ice cover of these lakes prevents wind driven mixing, which coupled with low advective stream

input, allows vertical chemical and biological gradients to develop and persist (vertical mixing is at the molecular level throughout the water column, Spigel and Priscu 1998). Lake Bonney is the most stratified of the lakes, followed by Lake Fryxell, and then by Lake Hoare.

The lakes are believed to be remnants of the former Lake Washburn that existed approximately 11,000 to 24,000 years before present (Denton et al. 1985). Lake levels have fluctuated over the past 6,000 years, effectively concentrating the solutes in the bottom waters of the lakes by evaporation and sublimation. An exception is Lake Hoare, which is believed to have dried out completely approximately 1,200 years ago, whereas Lakes Fryxell and Bonney are believed to have persisted (Matsubaya et al. 1979; Lyons et al. In press). Bottom water dissolved inorganic carbon ages measured by ^{14}C dating are approximately 1,200 years in Lake Hoare, and 8,000 years in Lake Bonney (Doran et al. In press). The ^{14}C age of the fulvic acid fraction of Lake Fryxell's bottom waters has been determined to be approximately 3,000 years (Aiken et al. 1996).

Previous Microbial Research on Lakes Fryxell, Hoare, and Bonney

The lakes of the Taylor Valley, Antarctica have been the subjects of limnological studies since 1961 when Angino and Armitage (1963) conducted the first scientific investigation of these waters. Goldman, et al. (1964) were the first to measure primary productivity in Lake Bonney, marking the beginning of nearly 40 years of phytoplankton studies in these lakes. Research by Goldman et al. (1964) and later by Priscu and others

(Priscu et al. 1988; Priscu et al. 1990; Lizotte and Priscu 1992) showed that the phytoplankton of these lakes have a high photosynthetic quantum yield, and are adapted to low ambient light levels. Phytoplankton nutrient deficiency has been studied extensively in these lakes. Initial studies indicated that Lake Fryxell phytoplankton were nitrogen deficient (Vincent 1981; Priscu et al. 1988), whereas Lake Hoare phytoplankton were believed to be phosphorus deficient (Parker et al. 1980). Subsequent studies by Priscu (1995) indicated that phytoplankton production in Lake Fryxell and Hoare is stimulated by the addition of both nitrogen and phosphorus, whereas phosphorus alone stimulated phytoplankton production in Lake Bonney. Decreased alkaline phosphatase activity after the addition of inorganic phosphorus in Lake Bonney further indicated phosphorus limitation in this lake (Dore and Priscu 1996).

The first report concerning dry valley bacteria was prepared by Meyer et al. (1962) who were unable to isolate any microorganisms from the neighboring Lake Vanda, and only four bacterial isolates from the littoral melt waters (moat) of Lake Bonney. A subsequent report by Goldman et al. (1967) was the first study to realize the importance of bacteria in the east lobe of Lake Bonney. Despite the fact that Goldman et al. (1967) made direct counts of bacterial cells, their data are one to three orders of magnitude lower than more recent reports (Takii et al. 1986; Takacs and Priscu 1998; Kepner et al. 1998). This discrepancy may be attributed to the use by recent researchers of direct bacterial cell counts by epifluorescent microscopy, which results in a higher efficiency of detection. Goldman et al. (1967) discussed a bacterial peak at 20 m in the east lobe of Lake Bonney, but subsequent researchers were unable to detect the same

peak by plate counts (Heywood 1984). Succeeding researchers suggested that the east lobe deep water bacterial peak was not viable (Benoit et al. 1971; Hand 1980) or was comprised of anaerobic bacteria (Takii et al. 1986). Koob and Leister (1972) found a profusion of rod-shaped organisms presumed to be cyanobacteria at the ice-water interface of Lake Bonney, but deemed them "too numerous to count"; these organisms were later considered to be heterotrophic bacteria (Heywood 1984).

Koob and Leister (1972) were the first to measure bacterial activity in dry valley lakes during their 1965-1966 summer investigation of Lake Bonney. Although absolute rates of ^{14}C-acetate uptake were not possible because ambient acetate concentrations were not measured, marked uptake of this substrate in opaque bottles was demonstrated. Uptake was greatest just below the ice cover and decreased with increasing depth. Additionally, Koob and Leister measured high dark bottle uptake of ^{14}C-bicarbonate at 15 m, which they attributed to bacterial activity. Parker et al. (1977) and Lane (1977) related bacterial biomass in the east lobe of Lake Bonney to changes in primary productivity and inflow rates of glacial melt water.

Lakes Fryxell, Hoare and Bonney are similar in that they all have supersaturated dissolved oxygen concentrations above the chemocline relative to the atmospheric saturation (Lake Hoare is supersaturated throughout the water column, except for the bottom meter of water). Experiments have shown that ^{14}C-glucose assimilation and respiration in whole lake water were unaffected or stimulated by high dissolved oxygen concentrations, however, bacteria grown on nutrient rich agar plates at 12°C were inhibited at high dissolved oxygen concentrations (Mikell et al. 1984). A subsequent

study (Mikell et al. 1986) performed under a variety of nutrient concentrations showed that Lake Hoare bacterial isolates grew more optimally at high dissolved oxygen concentrations as nutrient concentrations were decreased. Additionally, carotenoid containing isolates were found to be more resistant to high dissolved oxygen concentrations, whereas a carotenoid-negative mutant showed a decreased growth rate relative to the parent strain under high dissolved oxygen concentrations. In addition to providing some of the first bacterial cell density profiles in Lake Hoare, albeit by the colony forming unit method, Mikell et al. (1986) isolated 32 different bacterial strains from various depths of this lake. The bacteria were gram-negative rods, motile, oxidase positive, catalase positive, superoxide-dismutase positive, and contained carotenoids.

A number of studies on the bacteria of Lake Fryxell were reported in the 1970s and early 1980s (Waguri 1976; Matsumoto and Hanya 1977; Wharton et al. 1983; Vincent 1981; Parker et al. 1983). However, the first study that concentrated on Lake Fryxell bacterioplankton was not published until 1985 (Harfoot, 1985). Harfoot (1985) provided a preliminary, but comprehensive discussion about carbon and sulfur cycling that described the prevalence of oxygenic photosynthesis above the chemocline of Lake Fryxell, and anoxygenic photosynthesis in the anaerobic bottom waters. Bacterial distributions, and carbon and sulfur cycling rates clearly pointed to the importance of bacteria in Lake Fryxell. Unfortunately, Harfoot did not publish any other reports about Lake Fryxell and subsequent reports failed to focus as intently on the bacterial fraction of the microplankton in this lake. Priscu et al. (1987) studied Lake Fryxell primary production, and reported the occurrence of bacterial anoxic photosynthesis below the

chlorophyll-*a* maximum. Carbon cycling was studied by Smith and Howes (1990), who performed direct counts of bacterial cells by epifluorescent microscopy and measured heterotrophic activity by ^{14}C-acetate and glucose uptake. Bacterial biomass and productivity was shown to increase with depth to a maximum at 10.5 m, and bacterial biomass was 2 to 3 times greater in the anoxic 12 to 18 m region than in the upper aerobic water column. Uptake of ^{14}C-glucose and acetate was greatest in the 9.5 to 10 m interval of the lake. Bacteria in the 9.5 to 10 m biomass and activity maximum were characteristically larger than bacterial assemblages from the rest of the lake. This biomass and activity peak corresponded with an adenylate energy charge peak, indicating that the bacteria in this region were actively growing. Despite the bacterial biomass and adenylate energy charge peak at the chemocline of this lake, an apparent "gap" in carbon mineralization was detected (Howes et al. 1992). This region of the water column coincided with dissolved iron and manganese maxima, and the authors proposed that these metals served as alternative electron acceptors in this region. Despite the bacterial biomass peak in the anoxic bottom waters of Lake Fryxell, Howes et al. (1992) concluded that aerobic decomposition accounted for the majority of carbon cycling in the lake.

An extensive study of nitrogen dynamics in Lake Bonney by Priscu and co-workers was conducted in which the role of water column nitrification and denitrification was explored (Voytek and Ward 1995; Ward and Priscu 1997; Priscu et al. 1996 & 1997). Lake Bonney's west lobe showed inorganic nitrogen distributions typical of an oxygen stratified system: the surface layer was nitrogen depleted and the deep anoxic layer had high ammonium concentrations, but nitrate was present at very low

concentrations. Denitrification was detected in the anoxic layer of the west lobe by the acetylene block method (Priscu et al. 1996 & 1997). Inorganic nitrogen was low in the upper oxic layer of the east lobe also, but below the chemocline, ammonium, as well as nitrate and nitrite concentrations were high. Denitrification was not detectable in the east lobe of Lake Bonney, but nitrous oxide was present at very high levels at the oxic/anoxic interface (Ward and Priscu 1997). Polyclonal antisera were prepared against two denitrifying isolates from Lake Bonney to determine the distribution of denitrifiers, which indicated that potential denitrifiers were scarce in the deep waters of the east lobe, but were more numerous in the west lobe. Denitrification was proposed to be absent in the east lobe because of inhibition by salts, temperature, or possibly some other chemical limiting or inhibiting factor (Ward and Priscu 1997).

The role of nitrifying bacteria in various dry valley lakes was determined by the development of a polymerase chain reaction assay for the detection of ammonium oxidizers (Voytek and Ward 1995; Voytek et al. 1998). Ammonium oxidizers of the beta subclass were present in all lakes tested, whereas members of the gamma sub-class, which is represented primarily by marine organisms, were detected in the saline Lakes Fryxell and Bonney, but not in Hoare (Voytek 1996). Ammonium oxidizers were most abundant above the chemocline of the lakes and were associated with lower concentrations of ammonium and higher concentrations of nitrate and nitrite. Lake Bonney's east lobe was shown to have the highest level of nitrous oxide yet reported for an aquatic system, but neither classic denitrification, nor nitrifier denitrification were detectable in the region of the deep water nitrous oxide peak region. This anomaly led to

the suggestion that the east lobe nitrous oxide peak and other chemical gradients in Dry Valley lakes represent remnants of microbial activity that existed during an earlier period of the lake's history. Long mixing times of the waters in these lakes would allow such relic gradients to persist (Priscu 1997).

The lakes of the Taylor Valley are often hailed as unique systems in which to study microbial ecology because of various reasons relating to the simplicity of the foodweb. The most often quoted reason has been the essential lack of grazing owing to the paucity of potential grazers (Parker and Simmons 1985). However, increasing knowledge of the lakes has indicated that this perception is unfounded, at least in Lakes Fryxell and Hoare. Potential protozoan grazers have been recorded in Lakes Fryxell and Hoare (Laybourn-Parry et al. 1997; James et al. 1998), and the first potential grazing rates were recently reported (Roberts and Laybourn-Parry In press). During winter, cryptophyte densities in Lake Fryxell were shown to increase, and were hypothesized to remain active during this time by grazing upon bacteria (McKnight et al. Submitted). Nevertheless, the lakes may still be considered simple systems, but a new role for bacteria as a source of carbon in these systems has been elucidated, indicating that the food webs in these lakes are more complex than previously anticipated.

Although various bacterial biomass and activity measurements for Lakes Fryxell, Hoare and Bonney exist, measurements have not been made from consistent depths within the lakes, nor have the lakes been sampled routinely within and among seasons. Consequently, it has been difficult to determine intra- and interannual changes in bacterial biomass and production, and thus determine the factors affecting bacterial

biomass and productivity distribution and dynamics within these lakes. The first long-term limnological data set of these lakes was initiated upon the east lobe of Lake Bonney in 1989, followed by the inclusion of routine sampling on the west lobe of Lake Bonney in 1991 by Priscu and co-workers. Subsequently, a National Science Foundation funded Long-Term Ecological Research (LTER) Project was established in 1993, which includes Lakes Fryxell, Hoare, and Bonney as its limnological focus, and promises at least a 12 year data set for these lakes. The LTER has undertaken a routine limnological sampling regime that includes more than 15 different physical, chemical, and biological measurements collected at least three times during each Austral summer. Although the LTER is a multidisciplinary project, one objective of the limnological component is to monitor the lakes for physical, chemical, or biological changes, and determine if this change is caused by climatic change or increased anthropogenic impact in the Dry Valley region. However, many more basic questions concerning the factors affecting bacterioplankton biomass and productivity in the lakes remain unanswered.

Hypotheses and Objectives

Aquatic bacteria are now known to be important in transforming organic matter, regenerating nutrients, and supplying carbon for higher trophic levels. Although the lakes of the Taylor Valley, Antarctica have been extensively studied since the 1960s, the importance of the bacterioplankton in these lakes, and the factors affecting their biomass and production distributions remain unknown. This study was designed to test the following hypotheses:

1. Bacterial biomass is an important component of the total microplankton of these lakes.
2. Bacterial biomass losses to grazing are significant in these lakes.
3. Summer bacterial dissolved organic carbon (DOC) demand is greater than new DOC supply and bacteria are dependent upon alternate sources of organic carbon for production.
4. Inorganic nutrients do not limit bacterial production in these lakes, whereas temperature and salinity do.

The following objectives were undertaken to test these hypotheses:

1. Measure physical, chemical, and biological variables (including bacterial biomass and activity) in the lakes from consistent depths during four Austral summers.
2. Construct a forward difference model of trophogenic zone and water column bacterial biomass losses.
3. Construct a DOC budget for the trophogenic zone and water columns of the lakes.
4. Measure bacterial responses to temperature, salinity, and nutrient amendment.
5. Correlate bacterial parameters with ambient variables to identify factors that are most closely related to bacterial production.

Organization of the Report

The remainder of this thesis consists of a compilation of my research on Lakes Fryxell, Hoare, and Bonney in the form of a chapter that substantiates the ^{3}H-thymidine method used in this research (Chapter 2), manuscripts that have either been published (Chapter 3) or submitted for publication (Chapter 4), or are in preparation (Chapter 5) to be submitted in April 1999. Chapter 3 describes seasonal and annual bacterioplankton biomass and production dynamics in the three lakes and uses a forward difference model to estimate bacterial biomass losses in the lakes. A DOC budget was constructed in Chapter 4 for both the trophogenic zone and the water columns of the lakes to determine potential bacterial DOC deficiency. Chapter 5 uses both observational and experimental data to determine the role of physical, chemical, and biological factors regulating bacterial activity in the lakes. The final chapter summarizes my major conclusions and makes recommendations for future research.

CHAPTER 2

THE MEASUREMENT OF BACTERIAL ACTIVITY

Introduction

The realization of the importance of heterotrophic bacteria in the environment, and the further understanding of their role in an ecosystem has been dependent upon the development of accurate methods of estimating bacterial biomass and production. The enumeration and measurement of fluorochrome (e.g., acridine orange and DAPI) stained bacterial cells by epifluorescent microscopy (Hobbie et al. 1977; Porter and Feig 1980), despite minor drawbacks, is now widely accepted as an accurate method to estimate bacterial biomass. However, the search for a universal substrate to measure bacterial production easily and accurately has been unsuccessful, resulting in differing views of the most appropriate method (Robarts 1998).

During the 1960s and 1970s, research focused on specific heterotrophic processes, and ^{14}C and ^{3}H labeled substrates were employed to measure bacterial dissolved organic matter transformation rates. It was not until the 1980s that radioisotopic methods evolved to measure bacterial production. Of the numerous methods that exist, most commonly, the ^{3}H-thymidine incorporation method is applied to measure DNA synthesis, followed by ^{3}H-leucine to measure protein synthesis, ^{3}H-adenine to measure RNA and DNA synthesis, and $H_3{}^{32}PO_4$ incorporation as a measure of phospholipid synthesis. The

application of these methods relies on various assumptions. For example, the thymidine and adenine method both assume that all microorganisms incorporate exogenously supplied thymidine or adenine by a common and predictable pathway, and that there is a uniform metabolic response by the various organisms within an assemblage. However, although adenine incorporation is often applied to measure bacterial production, algae, yeast, and fungi also have been shown to incorporate this purine. The adenine and thymidine methods have the potential for specifically measuring bacterial growth rate because rates of DNA synthesis are measured, which occurs only during growth. However, an additional assumption of the adenine method is that exogenously incorporated adenine does not affect the ATP cell quota, ATP turnover rate, or the rate of microbial RNA and DNA synthesis (Robarts 1998). This assumption requires that the specific activity of the radioactively labeled precursor pool be measured because otherwise it is impossible to extrapolate incorporation measurements into rates of nucleic acid synthesis accurately. Even comparisons of relative incorporation are not believed to be justified because different rates of DNA and RNA labeling may result from either variations in the specific activity of the precursor pools or different rates of RNA and DNA synthesis (Karl 1993).

The leucine method may not specifically measure growth at times because bacteria have been shown to incorporate leucine into protein even though net protein synthesis and cell production are not occurring (Kirchman et al. 1986). Kirchman et al. (1986) concluded that non-growth related protein synthesis is not significant in the natural environment. However, leucine incorporation has been shown to remain high in

batch cultures of *Vibrio* species during stationary phase, whereas thymidine incorporation simultaneously fell to zero (Snyder et al. 1994).

All of the methods that are currently used to measure bacterial production have drawbacks, and no particular method is perfect. The methods all suffer from the fact that a conversion factor must be used to convert substrate incorporation into biomass production. Additionally, when applying these techniques, whole levels of biological diversity are ignored because of methodological limitations. The treatment of bacteria as a "black box" by aquatic microbial ecologists is a common criticism of this field. Nevertheless, this is an approach that has led to many new insights about bacteria in the natural environment and generated many questions regarding aquatic ecosystems. The research presented in this thesis is largely concerned with bacterial production. The thymidine method was applied in these studies because this is the most widely applied method, which enables comparisons to be made between dry valley lakes and other aquatic systems.

Thymidine Incorporation

Thymidine is a unique nucleotide precursor because it is incorporated during DNA synthesis primarily, which occurs only in growing cells. The major assumption of this assay is that only growing cells take up exogenously supplied thymidine and thus non-growing cells should not be labeled above background with ^{3}H-thymidine. Nucleotides are synthesized in cells by two pathways: *de novo* synthesis and the salvage pathway (Figure 2.1). The incorporation rate of exogenously supplied thymidine may be