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
of the requirements for the degree

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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.

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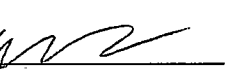
Signature 

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.

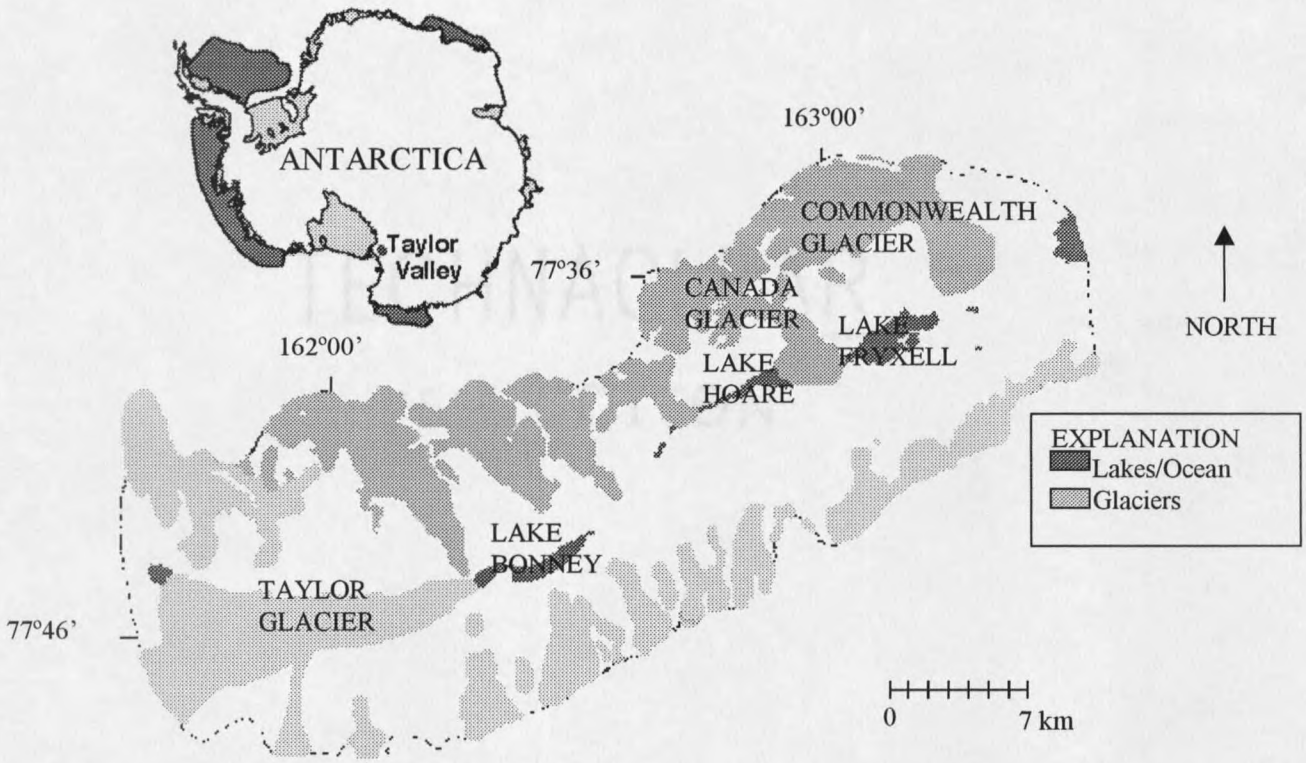

Figure 1.1. Map of Taylor Valley, McMurdo Dry Valleys, Antarctica

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

diluted by synthesis of deoxythymidine monophosphate (dTMP), but this factor is circumvented by determining the optimum concentration of thymidine, i.e. the concentration at which no further radioactivity is incorporated (Bell 1993).

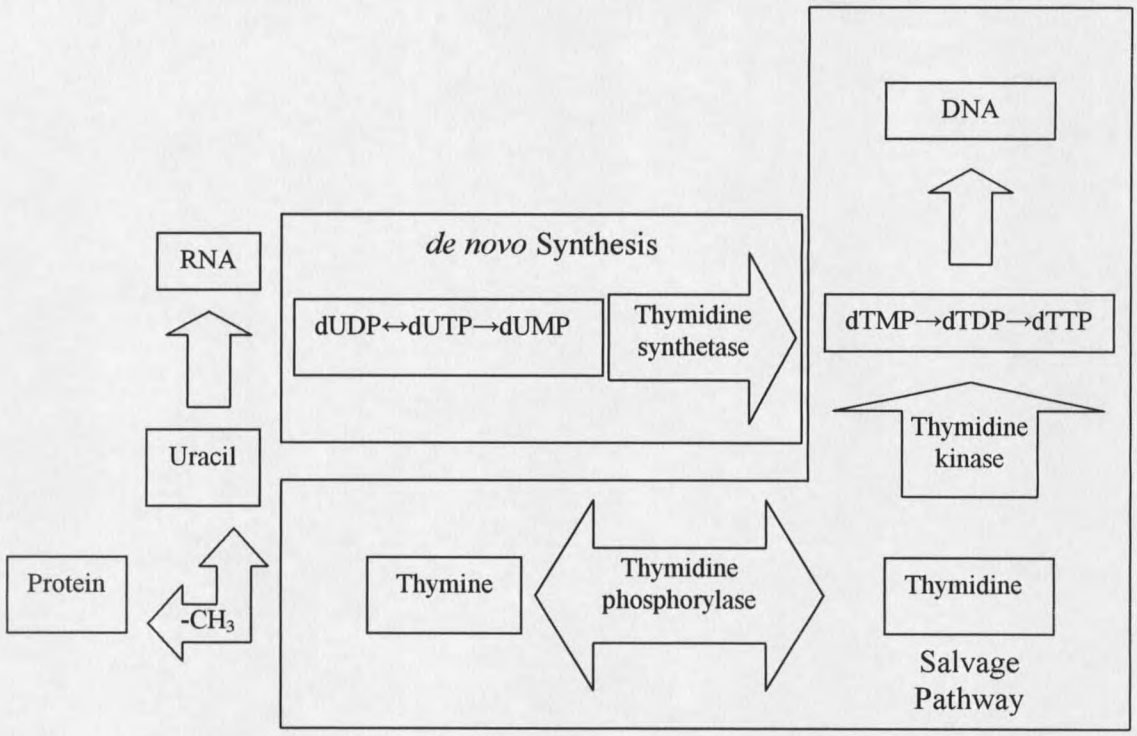

Figure 2.1. Diagram of the salvage pathway and de novo synthesis of thymidine and its incorporation into DNA. dTMP, dTDP, dTPP = deoxythymidine mono, di, and triphosphate, respectively. dUMP, dUDP, and dUTP = deoxyuridine mono, di, and triphosphate. Formation of dUMP from dUDP passes through dUTP.

Exogenously supplied thymidine may be incorporated into RNA and protein, via the salvage pathway (see Figure 2.1). Incorporation of thymidine is commonly measured in the 5% ice-cold trichloroacetic acid (TCA) extractable fraction, but this may overestimate bacterial production because this fraction represents DNA, RNA, and

proteins. Experiments from marine environments have shown that 65-80% of the total TCA insoluble material was DNA (Fuhrman and Azam 1982), and extensive fractionation studies showed that the major part of the radioactivity was found in DNA (Riemann 1984). Experiments on whole lake water indicated that the radioactivity in DNA (as a percentage of total TCA-insoluble material) decreased with incubation time, presumably because of intracellular degradation (Riemann et al. 1982). This effect may be avoided by determining an incubation period in which thymidine incorporation is linear.

Three additional assumptions of the thymidine method are 1) only microorganisms that posses a thymidine transport system and thymidine kinase are able to incorporate exogenously supplied thymidine into DNA, 2) most heterotrophic bacteria are able to incorporate exogenously supplied thymidine, and 3) algae and protozoa do not incorporate thymidine over short incubation intervals (i.e., < 1 doubling period). Microautoradiographic studies of natural bacterial assemblages (Fuhrman and Azam 1982; Riemann et al. 1984) have indicated that most bacteria do incorporate thymidine, although various cultures of *Pseudomonas* and other isolates were incapable of incorporating the label (Ramsay 1974; Pollard and Moriarty 1984). Most algae do not possess thymidine kinase (Robarts 1998) and microautoradiography studies indicated that algae took up little or no thymidine (Fuhrman and Azam 1982; Bern 1985). A study of thymidine uptake by marine diatoms and dinoflagellates showed that eukaryotic algae could incorporate thymidine, but only when thymidine was supplied at relatively high concentrations (75 nM) and during longer incubation periods (Rivkin 1986). Protozoans,

however, have been shown to possess thymidine kinase, but are not believed to be able to incorporate thymidine appreciably during the short incubation periods typically used in this technique (Riemann and Sondergaard 1986).

The most controversial aspect regarding the use of any of the methods described here is the conversion factors used to estimate bacterial production from rates of radiolabeled substrate incorporation (in carbon produced per unit volume and time). However, estimating bacterial production is essential for considering carbon flow within a system or on a global scale. Conversion factors may be determined empirically (Kirchman and Ducklow 1993), theoretically (Fuhrman and Azam 1980), or may be taken from the literature. Conversion factors reported in the literature range over more than one order of magnitude (Robarts 1998). The theoretical conversion factor, which is based on estimates of bacterial DNA content and assumes that no isotope dilution occurs, is 0.5×10^{18} cells produced per mole thymidine incorporated. However, the literature suggests that this conversion factor is at best, a conservative one because *de novo* synthesis is presumably rarely turned off (Bell 1990). A study summarizing the use of thymidine incorporation in 97 marine studies reported that conversion factors determined in these systems averaged 2×10^{18} cells produced $mole^{-1}$ thymidine incorporated (Ducklow and Carlson 1992). The literature suggests that this median value be used when an empirically determined value is not available. Although this conversion factor is based on marine estimates, it has been derived in freshwater as well (Bell et al. 1983; Smits and Riemann 1988).

Because of the many assumptions involved in all of the methods used to determine bacterial production, it is important to develop the chosen technique for the specific environment in which it will be employed. Additionally, the assumption that only bacteria incorporate the labeled substrate of interest must be validated. The objective of the research presented in this chapter was to validate methodological considerations specific to the use of thymidine incorporation to determine bacterioplankton production in Taylor Valley Lakes Fryxell, Hoare, and Bonney.

Methods

General Method

Bacterial activity was measured on 10 ml of lake water incubated for 20 h with 20 nM ^{3}H-methyl-thymidine (~2 Bq mmol^{-1}). Formalin (5% final concentration) treated replicates served as controls (kills). Incubation was terminated by the addition of 10 ml cold 10% trichloroacetic acid (TCA). Samples were filtered onto 0.45 μm polycarbonate filters and filter towers were rinsed 3 times with 2 ml cold 5% TCA. Radioactivity on the filters was assayed by standard scintillation spectroscopy. Samples were always incubated in the dark at 1 to 4°C. Thymidine incorporation rate was calculated according to the following equation:

$$\text{nM thymidine h}^{-1} = \frac{(\text{DPM}_{\text{live}} - \text{DPM}_{\text{kill}}) * [\text{TdR}]}{\mu\text{Ci added} * \text{DPM } \mu\text{Ci}^{-1} * \text{incubation period}}$$

where DPM represents the disintegrations detected per minute in the live and killed replicates, [TdR] is the final concentration of thymidine added (nM), and

DPM $\mu Ci^{-1} = 2.2x10^6$. Table 2.1 summarizes the experiments discussed in this chapter and the depths and dates on which they were performed.

Table 2.1. Depths and dates of experiments performed in the verification of the ^{3}H-thymidine method.

Experiment	Fryxell	Hoare	Bonney, east lobe	Bonney, west lobe
Thymidine Kinetics			5 and 10 m 14 Nov. 1989	
Sample Volume	5 and 9 m 30 Dec. 1996	5 and 14 m 19 Dec. 1996	5 and 13 m 26 Dec. 1996	5 and 13 m 22 Dec. 1996
Timecourse	5 and 9 m 14 Oct. 1995	5 and 14 m 10 Oct. 1995	5 and 13 m 17 Oct. 1995	5 and 13 m 19 Oct. 1995
Effect of oxygen	18 m 30 Dec. 1996	29 m 20 Dec. 1996		
Thymidine uptake into DNA and total macromolecules, and Leucine uptake into protein	5 and 9 m 28 Sept. 1995 30 Dec. 1996	5 and 14 m 25 Sept. 1995 19 Dec. 1996	5 and 13 m 6 Oct. 1995 26 Dec. 1996	5 and 13 m 4 Oct. 1995 22 Dec. 1996
Thymidine incorporation into >0.2 and >3 μm fractions			5 and 10 m 14 Nov. 1989	
Thymidine conversion factor	5 m 30 Dec. 1996	5 m 24 Dec. 1996	5 m 26 Dec. 1996	5 m 27 Dec. 1996

Thymidine Kinetics

Thymidine uptake kinetics were determined by Michael Lizotte and John Priscu in 1989. Lake water (10 ml) collected from 5 and 10 m in the east lobe of Lake Bonney was placed in 20 ml scintillation vials. Samples (1 live, 1 kill) were incubated for 15 h with 8 different final concentrations of ^{3}H-thymidine ranging from 2 to 60 nM.

Incubations were terminated with the addition of 10 ml of 10% TCA and samples were filtered and counted as described in the general method section.

The Effect of Sample Volume on Thymidine Uptake

Although thymidine incorporation is often measured in small volumes (10 ml, as suggested by Bell 1993) of whole water samples, this may lead to an overestimation of bacterial production (P. LaRock, personal communication). The increased surface area to volume ratio of a small volume may result in an increased "bottle effect", which would stimulate bacterial growth. The effect of sample volume on thymidine incorporation was determined in three different volumes (10, 125, and 500 ml incubated in 20, 250, and 1000 ml bottles, respectively) of whole lake water from Lakes Fryxell, Hoare, and Bonney. Samples were incubated in duplicate with 1 kill per treatment for 10 h with 20 nM thymidine. At the end of the incubation period, 10 ml of lake water was removed from the 125 and 500 ml treatments and placed in 20 ml scintillation vials similar to the 10 ml treatment. Ten ml of ice-cold 10% TCA was added to each of the scintillation vials and samples were filtered and counted as described in the general method section.

Thymidine Uptake Timecourse

Lake water (10 ml) collected below the ice cover (5 m) and above the chemocline (9 m in Fryxell, 14 m in Hoare, and 13 m in Bonney) was incubated (2 live, 1 kill per treatment x 6 time-points) for 24 h with 20 nM ^{3}H-thymidine. Incubations were terminated every 4 h by the addition of 10 ml 10% TCA, and samples were filtered and counted as described in the general methods section.

Effect of Oxygen on Thymidine Uptake in Samples Collected from Anoxic Zones of the Lake

Lake water from 18 m in Lake Fryxell and 29 m in Lake Hoare, which are anoxic depths, was incubated in 20 ml scintillation vials with and without headspace to determine the effect of oxygen on thymidine incorporation at these depths. Samples were incubated in triplicate (2 lives, 1 kill per treatment) with 20 nM ^{3}H-thymidine for 20 h and incubation was terminated by the addition of 10 ml of 10% ice cold TCA. Samples were filtered and counted as described in the general methods section.

Relative Uptake of Thymidine into DNA and Total Macromolecules

Lake water (125 ml) was incubated in duplicate (2 kills, 2 lives) for 20 h with 20 nM ^{3}H-thymidine in polyethylene bottles. At the end of the incubation, 10 ml aliquots from the bottles (six replicates from each live bottle and two replicates from the killed bottles) were dispensed into scintillation vials. Half of the vials (six lives, two kills) were treated with 10 ml of 10% TCA and the other half of the vials were fixed with formalin (2% final concentration). The TCA treated samples (total macromolecules) were filtered onto 0.2 μm polycarbonate filters, rinsed three times with ice cold 5% TCA, and counted by standard scintillation spectroscopy. The amount of labeled thymidine incorporated into DNA was determined in the formalin treated samples. DNA samples were alkalinized for 30 minutes with NaOH (5 N final concentration), followed by acidification with 1 ml of 100% ice cold TCA for 15 minutes. DNA samples were filtered onto 0.2 μm cellulose nitrate filters and washed with 50% (w/v) phenol-

chloroform, followed by a 5 ml rinse with ice cold 80% (v/v) ethanol. Labeled DNA collected on the filters was counted by standard liquid scintillation spectroscopy. Polycarbonate filters were subsequently found to result in DPM counts that averaged 60% higher than on cellulose nitrate filters, but the polycarbonate filters are dissolved in phenol-chloroform. Therefore, the above experiment was repeated during the 1996-1997 season, except cellulose nitrate filters were used for both extractions (total macromolecules and DNA), and there were only 2 lives and 1 kill per depth for each extraction.

Leucine Incorporation

Lake water was incubated with 10 nM of ^{3}H-leucine for 20 h. Ten ml samples (3 lives, 2 kills) were incubated in scintillation vials during 1995-1996, but duplicate 100 ml samples (2 live, 1 kill) were used during the 1996-1997 season. Ten ml were removed from each of the 100 ml samples at the end of the incubation period during the 1996-1997 experiments and pipetted into scintillation vials. Incubation was terminated during both seasons with the addition of 10 ml of 10% ice cold TCA, and samples were filtered and counted as described in the general methods section. Leucine incoporation rates were converted to bacterial production ($\mu g\ C\ l^{-1}\ h^{-1}$) using the following equation:

$$\text{Bacterial Production} = \frac{\text{Leu} * 131.2}{\%\text{Leu}} * \text{C : protein} * \text{ID}$$

where Leu is the rate of leucine uptake ($nM\ d^{-1}$), 131.2 is the formula weight of leucine, %Leu is the fraction of leucine in protein 0.073, C:protein is the ratio of cellular carbon to protein (0.86), and ID is an estimate of isotope dilution (Simon and Azam 1989).

Uptake of Thymidine by the
>0.2 μm and >3 μm Microbial Fraction

Relative thymidine uptake by different microbial fractions was determined by Michael Lizotte and John Priscu in 1989. Lake water (10 ml) collected from 5 and 10 m of the east lobe of Lake Bonney was incubated for approximately 14 h in duplicate (2 lives, 1 kill) with 30 nM ^{3}H-thymidine. Samples were filtered onto either a 0.2 μm (total microplankton) and 3 μm (primarily eukaryotic microplankton) filters to determine if algae and other eukaryotes incorporate thymidine. Radioactivity on filters was counted by standard liquid scintillation spectroscopy.

Thymidine Conversion Factor (TCF).

Lake water (1.5 liters) from just below the ice cover of the lakes (5 m) was diluted 1:1 with 0.2 μm filtered water from the same depth. Five hundred ml was aliquoted into each of six 1 liter polyethylene bottles and incubated at 4°C. Three 10 ml sub-samples were removed from duplicate bottles at the start (t=0), middle (t~10h) and end of the experiment (t~20 h), and thymidine incorporation was determined during 3 hour incubations with 20 nM ^{3}H-thymidine. Incubation was terminated by the addition of 10 ml of 10% ice cold TCA. Duplicate bacterial samples were collected at the start and end of the incubation and were fixed with formalin (2% final concentration). Bacterial cell production over the course of the experiment was determined by counting >600 cells in samples stained with acridine orange (0.1% m/v final concentration) by epifluorescent microscopy (Hobbie et al., 1977). A thymidine conversion factor was determined using both the integrative and modified derivative method (Kirchman and Ducklow, 1993). A

thymidine to cell conversion factor was calculated by the integrative method from the difference in bacterial cell numbers and the change in thymidine incorporation over the course of the experiment. The modified derivative method was used to calculate a conversion factor using the following equation:

$$TCF_{\text{modified derivative}} = \frac{\mu e^{B}}{e^{b}}$$

where μ was the growth rate determined from slope of ln(cell density) vs. time and B and b were the y-intercepts of ln(cell density) and ln(thymidine incorporation rate) vs. time.

Results and Discussion

Most investigators incubate aquatic samples with 10 nM thymidine, though concentrations as low as 5 nM have been reported (reviewed by Riemann and Sondergaard, 1986). ^{3}H-thymidine incorporation in the east lobe of Lake Bonney was saturated at approximately 20 nM (Figure 2.2). It remains unclear at this time whether thymidine uptake is saturated at a higher concentration because of lower rates of *de novo* thymidine synthesis or a lower thymidine affinity in this microbial assemblage. Time course experiments of thymidine uptake and incorporation into DNA indicate that thymidine incorporation in all of the lakes is linearly incorporated into DNA at 20 nM thymidine (see below). Therefore, with the exception of the filter fractionation study, which was conducted at the same time that thymidine kinetics were tested, 20 nM thymidine was used in all of our research.

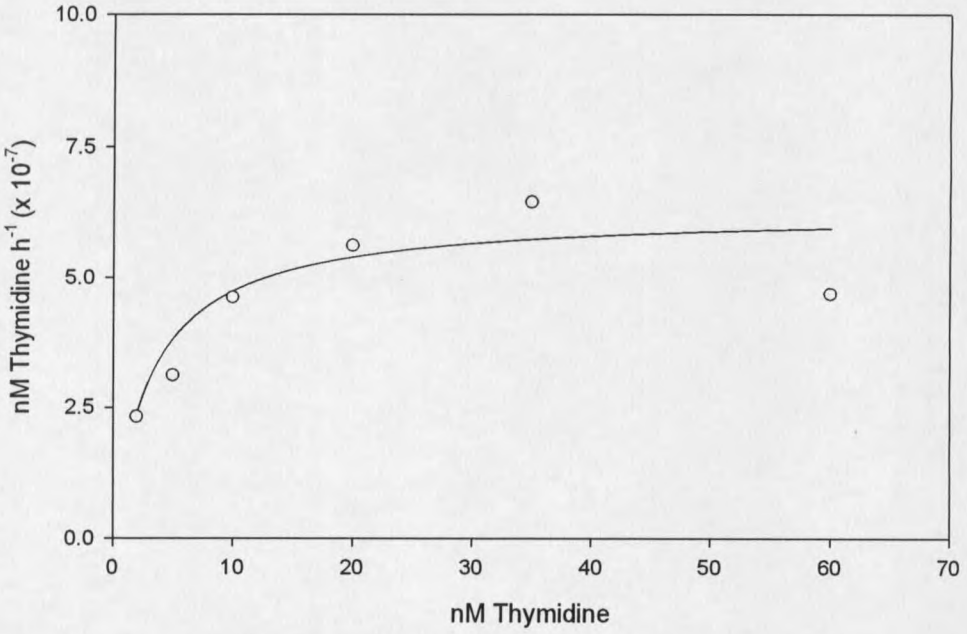

Figure 2.2. Thymidine incorporation as a function of thymidine concentration added to 10 ml samples incubated for 15 h.

"Bottle effects" result from the adsorption of nutrients and bacteria onto incubation bottles, which effectively sequesters nutrients from solution, often resulting in increased microbial activity. Increasing the volume of sample incubated can decrease bottle effects, but incubation of large volumes of lake water with ^{3}H-thymidine incorporation is not feasible for Antarctic work. Costs associated with large samples increase costs of isotopes and their disposal. One way analysis of variance indicated that thymidine incorporation rate did not differ significantly ($p=0.16$, $df=47$) among experimental volumes. Hence, a 10 ml sample size was selected.

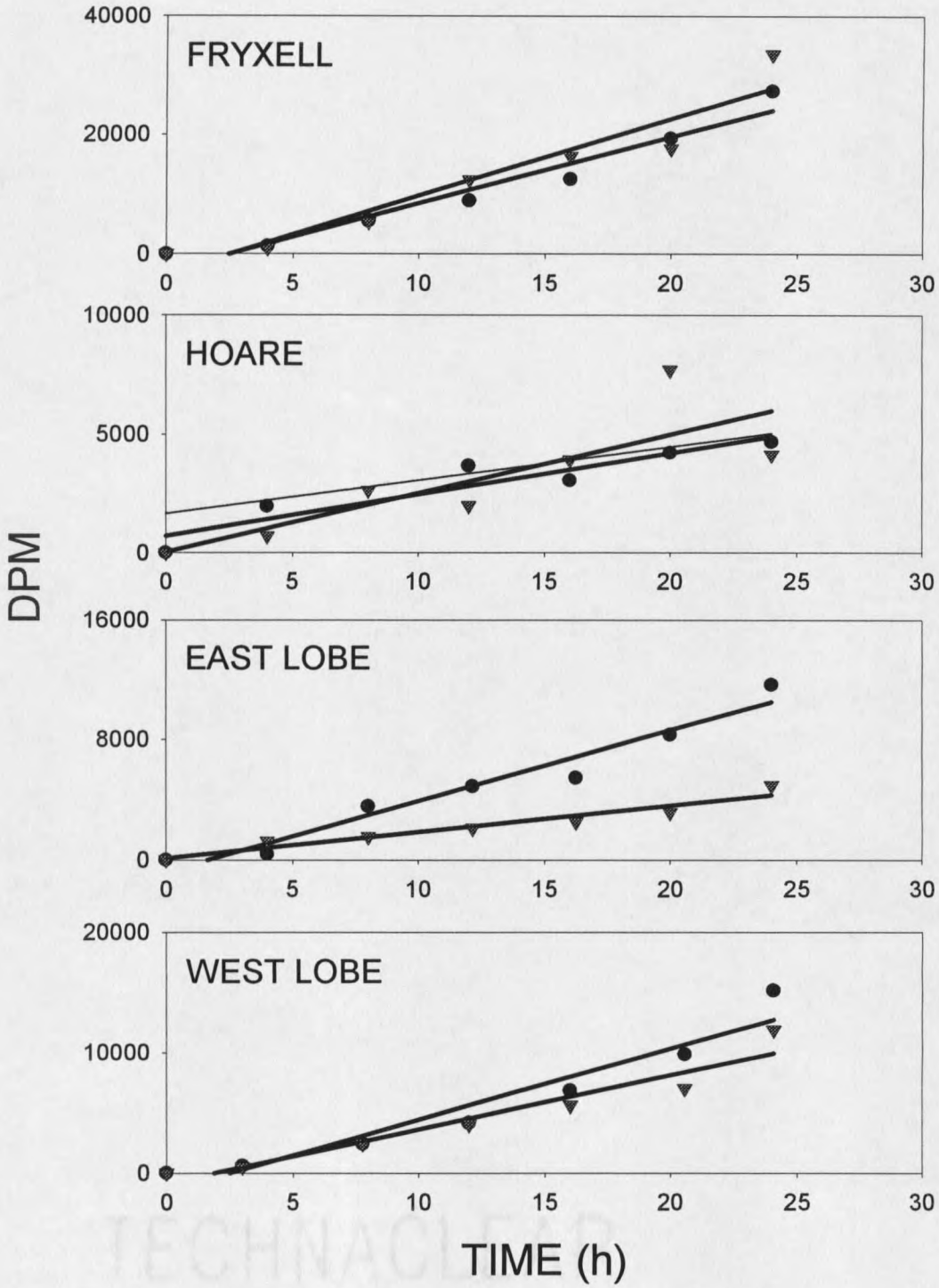

Figure 2.3. Incorporation of 3H-thymidine over a 24 h time-course. Circles represent data from 5 m and triangles represent data from 9 m in Lake Fryxell, 14 m in Lake Hoare, and 13 m in Lake Bonney.

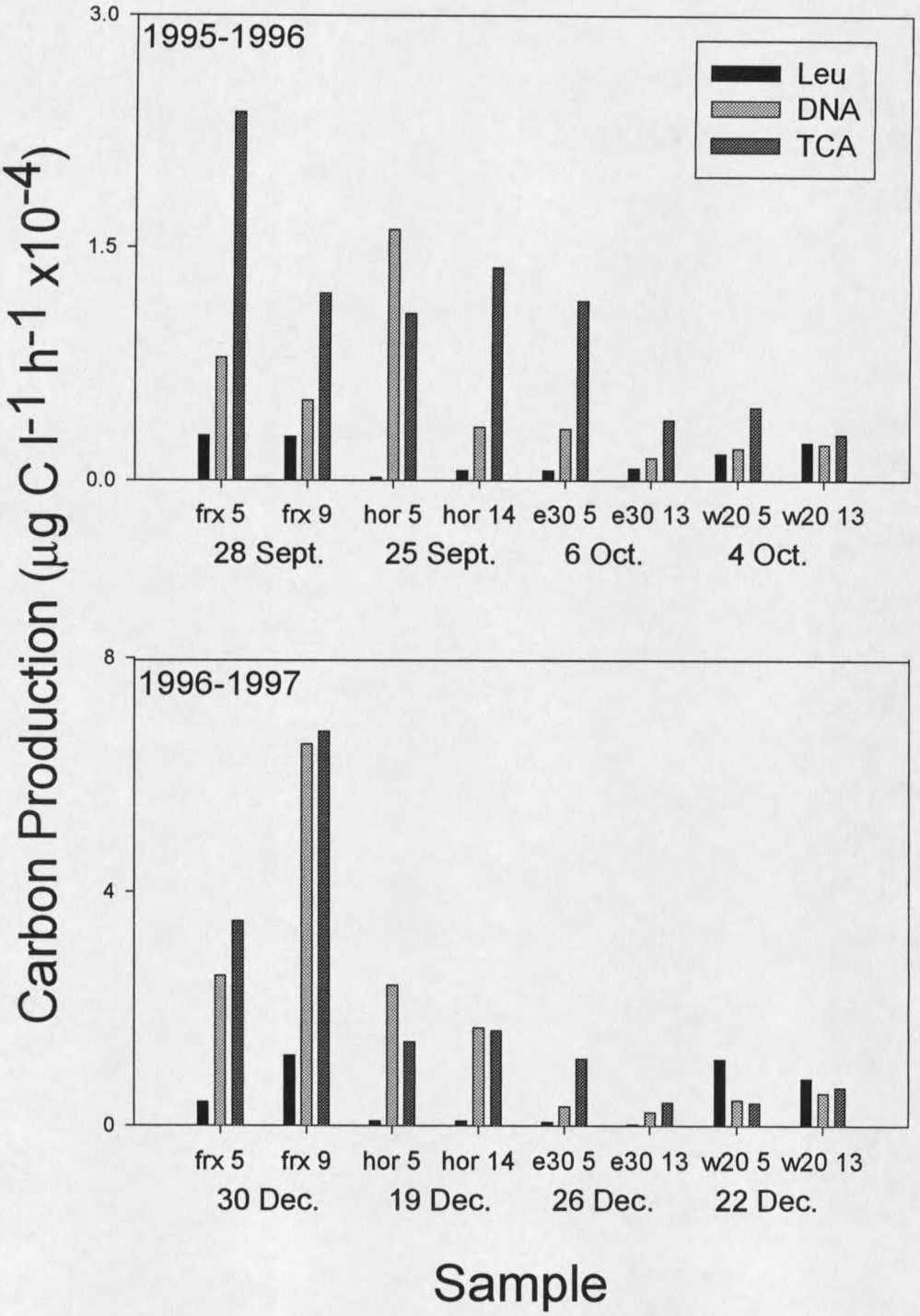

Figure 2.4. Bacterial production calculated from thymidine incorporation into DNA and total macromolecules (TCA), and from leucine (Leu) incorporation into protein during the 1995-1996 and 1996-1997 season.

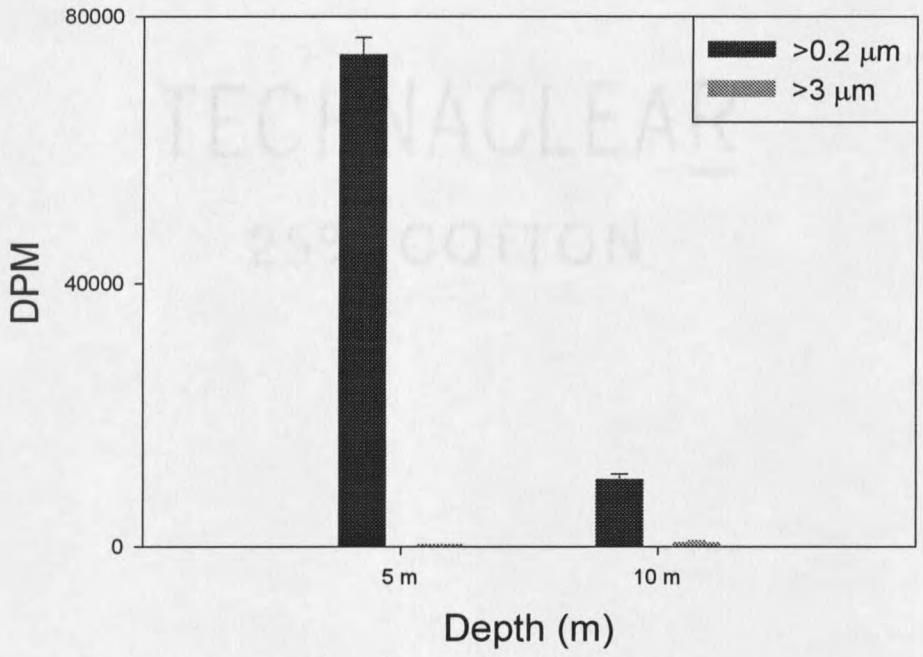

Figure 2.5. Thymidine incorporation by bacteria + phytoplankton + protozoans (>0.2 μm) and phytoplankton + protozoans (>3 μm) in 5 and 10 m water from the east lobe of Lake Bonney.

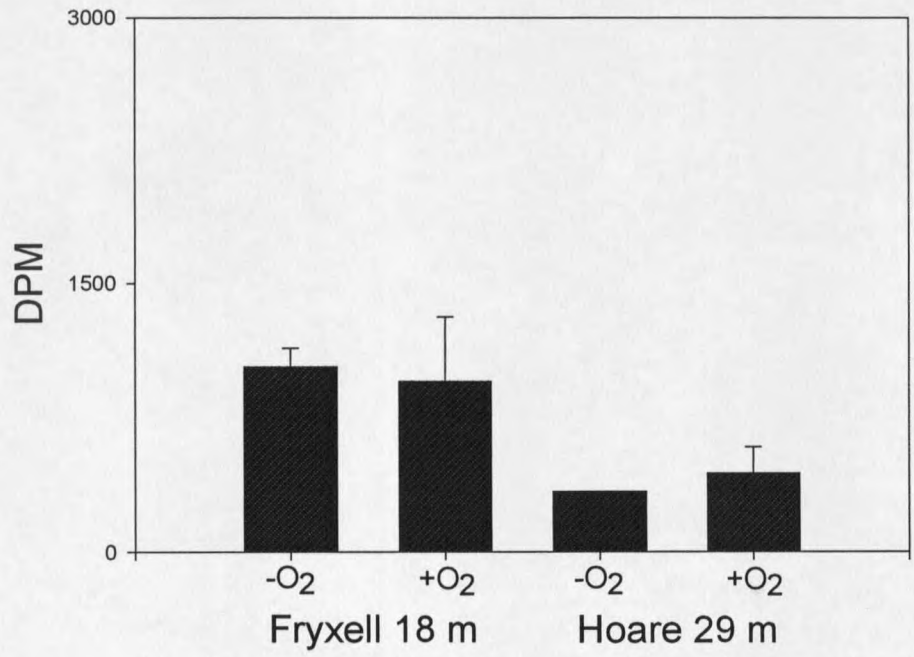

Figure 2.6. Thymidine incorporation in lake water from the anoxic zone of Lakes Fryxell and Hoare incubated with and without headspace.

^{3}H-thymidine may be metabolized by bacteria or incorporated by eukaryotes during extended incubation periods, consequently, aquatic samples are often incubated for 30 to 60 minutes only. However, because of the low bacterial activity in Antarctic lakes relative to more temperate systems, significant incorporation does not occur during such short incubations. Bell (1993) suggested that in systems where the ambient temperature is below 5°C, longer incubation periods are unavoidable and recommended that incubation time be adjusted to yield around 10^4 DPM on the filters. One test to determine whether ^{3}H-thymidine is being incorporated into DNA at a constant rate is to verify that incorporation is linear over the incubation period. Thymidine is linearly (r^2 = 0.69 to 0.95, p = 0.0002 to 0.02) incorporated in these lakes for at least 24 h (Figure 2.3). It is even more important to test whether thymidine is actually incorporated into DNA, rather than other macromolecules. Figure 2.4 shows bacterial production estimated from the incorporation of ^{3}H-thymidine into total macromolecules (TCA), DNA, and from the incorporation of ^{3}H-leucine into protein. The 1995-1996 data reflect the use of two different kinds of filters to collect the extracted molecules: a cellulose nitrate filter for DNA extraction and polycarbonate filters for the total macromolecules extraction. The polycarbonate filters are preferable because they result in higher counts compared to the cellulose nitrate filters, but they are dissolved by the phenol-chloroform step in the DNA extraction. Experiments conducted during the 1996-1997 season using cellulose nitrate filters for both extractions showed that 90% of the labeled thymidine was incorporated into DNA (Figure 2.4).

Rivkin (1986) showed that at higher thymidine concentrations and over longer incubation periods, ^{3}H-thymidine incorporation could be used to measure diatom and dinoflagellate growth. The 20 h incubation period that we use is insufficient to significantly label the algae in these lakes; 96% of ^{3}H-thymidine was incorporated by the <3.0 μm fraction, which is primarily bacteria (Figure 2.5). If algae are capable of thymidine incorporation in Taylor Valley lakes, their long doubling time (> 5 d) presumably limits their importance in thymidine incorporation rates.

Table 2.2. Thymidine conversion factors (x 10^{18} cells mol^{-1} thymidine) determined for Lakes Fryxell, Hoare, and Bonney by the integrative and modified derivative methods suggested by Kirchman and Ducklow (1993).

Lake	Integrative Method	Modified Derivative Method
Fryxell	15.6	1.8
Hoare	57.3	11.6
Bonney east lobe	24.8	5.0
Bonney, west lobe	5.2	5.7

Bacterial carbon production (μg C l^{-1} h^{-1}) measured by leucine incorporation was at least one order of magnitude lower than estimated from thymidine incorporation rates, except in the west lobe of Lake Bonney. This discrepancy is most likely a result of the conversion factors applied. The thymidine incorporation to cell conversion factors for Taylor Valley lakes (Table 2.2) were determined from one depth at the height of the growth season, and were at least two fold greater than the average reported in the literature ($2x10^{18}$ cells $mole^{-1}$ thymidine), except in Lake Fryxell. This is most apparent in Figure 2.4 where leucine and thymidine incorporation have been converted to bacterial production. The application of the thymidine to cell conversion factors resulted in

bacterial production estimates for Lake Hoare that were higher than in the west lobe of Lake Bonney. This is inconsistent with primary production, bacterial cell density, and nutrient levels in the lakes, which all indicate that the west lobe is more productive than Lake Hoare on an areal basis. This inconsistency deserves further research to determine if Taylor Valley lake thymidine to cell conversion factors are truly higher than other systems or if this result is an experimental artifact. In the meantime, the average thymidine conversion factor reported in the literature will be used at the expense of potentially underestimating bacterial production.

The ability of anaerobic bacteria to incorporate ^{3}H-thymidine is unclear (Robarts and Zohary, 1993). Reports exist indicating that, in particular, sulfate reducers and various chemolithotrophs are unable to incorporate thymidine (reviewed by Robarts 1998). Thymidine incorporation is measurable in the anoxic bottom waters of Lakes Fryxell (below 10 m) and Hoare (a meter above the sediment). Thymidine samples are incubated with a potentially oxic headspace, which might lead to an underestimation of bacterial productivity at these depths. It was therefore important to determine if the fact that we incubate samples aerobically inhibited the apparent bacterial growth in these waters. Experiments showed that thymidine uptake in samples incubated with headspace was not significantly different than in samples without headspace (Figure 2.6). This experiment does not address the potential inhibition by oxygen during sample collection and preparation, but thymidine incorporation in Lake Fryxell water anaerobically collected and handled by John Priscu (unpublished data) did not differ from water collected routinely.

Thymidine incorporation is the most commonly accepted and applied method for estimating bacterial production (Robarts 1998), allowing bacterial production data to be compared among systems. My results show that this method is specific for bacteria in dry valley lakes, and that ^{3}H-thymidine is incorporated primarily into DNA. Additionally, this method works in both the aerobic and anaerobic regions of Lakes Fryxell and Hoare, and uptake is linear during the comparatively long incubation periods needed for these lakes. Most importantly, the ^{3}H-thymidine method has been used in Antarctic dry valley lakes for ten years and provided consistent, reproducible data, enabling the seasonal and inter-annual bacterioplankton production dynamics of these lakes to be elucidated.

CHAPTER 3

BACTERIOPLANKTON DYNAMICS IN TAYLOR VALLEY LAKES: PRODUCTION AND BIOMASS LOSS OVER FOUR SEASONS

Introduction

The lakes of the Taylor Valley, Antarctica offer a unique opportunity to study microbial ecology in an extreme environment. These lakes, located at approximately 77° S 168° E in the McMurdo Dry Valleys, are characterized by permanent 3 to 5 m thick ice covers and strong vertical conductivity, nutrient, and oxygen gradients. The ice covers, physical and chemical gradients, and low stream input create distinct layers in which the plankton live (Angino et al. 1964). Eukaryotic phototrophs, heterotrophic flagellates, and bacteria dominate the plankton community; macrozooplankton or fish are not present. The physically stable water column (Spigel et al. 1990) and lack of a complex food web provide a relatively simple system to examine microbial processes in a pristine environment with little anthropogenic influence.

Rapid growth rates of aquatic heterotrophic bacteria in other aquatic systems show that production of bacterial biomass represents an important link among detritus, dissolved organic matter (DOM), and higher trophic levels (Fuhrman and Azam 1980). Bacteria have also been shown to be important in scavenging and transforming DOM, degrading substrates that might otherwise accumulate, and producing new biomass which

may be passed up the food web (Hollibaugh 1994). A revised concept of pelagic food web structure (Pomeroy 1974) has been instrumental in determining that bacteria may be a source of organic carbon to higher trophic levels through grazing by microheterotrophic ciliates and flagellates (Azam et al. 1983; Ducklow 1983; Pace 1988; Sherr and Sherr 1987).

The microbial ecology of the Taylor Valley lakes has been studied since 1961 (e.g. Goldman 1964; Parker et al. 1977; Simmons et al. 1981; Green et al. 1989; McKnight et al. 1991; Priscu 1995). Much of this work has concentrated on photoautrophs; relatively little is known about the bacterioplankton. Historically, grazing in these lakes has either been ignored or believed absent. Though no experimental data showing phagotrophy in these lakes has been reported, several genera of phytoplankton and protozoans present in Taylor Valley lakes have been shown to graze bacteria in other systems (Laybourn-Parry et al. 1997; McKnight et al. Submitted).

The perennially ice-covered lakes of the McMurdo Dry Valleys, Antarctica have been used extensively as analogues for the study of exobiology because they pose one of the most extreme environments on our planet that harbors life. Despite the importance of these lakes as an "end-member" in the spectrum of environments on earth, there are no focused reports of the bacterioplankton that exist within them. Previous studies were conducted during a period when colony forming units was still the standard bacterial enumeration method (Koob and Leister 1972) or concentrated on a particular bacterial adaptation or process within usually only one of these lakes (Mikell et al. 1986; Voytek et al. 1998; Ward and Priscu 1997). The objectives of this chapter were to define the spatial

and seasonal distribution of bacterial biomass and production in three major lakes in the McMurdo Dry Valleys and to numerically model bacterial losses.

Materials and Methods

Study Site

The McMurdo Dry Valleys is a 4800 km^2 area of Antarctica's southern Victoria Land that has been ice free for approximately the last 3.5 million years. Studies were conducted on Lakes Fryxell, Hoare, and the east and west lobes of Lake Bonney. The lakes, which lie in the Taylor Valley, are remnants of Lake Washburn, a larger lake which existed 10,000 to 24,000 years ago (Lawrence and Hendy 1989; Denton et al. 1989). Lake Fryxell, at the easternmost edge of the valley has a surface area of 7 km^2, with a maximum depth of 18 m. Lake Hoare has a surface area of 3 km^2 and a maximum depth of 30 m. Lake Bonney is situated at the head of the valley with a surface area of approximately 4 km^2 and a maximum depth of 40 m. Lake Bonney has two basins (lobes), connected by a narrow (~20 m wide), shallow (12 m) channel (Angino et al. 1964; Spigel et al. 1990; Spigel and Priscu 1996). While these lakes have varying degrees of chemical stratification, generally all contain nutrient rich deep water overlaid by a relatively nutrient poor epilimnion. The permanent ice cover of these lakes prevents wind driven mixing, which coupled with low advective stream input, allows vertical gradients to develop and persist. Lake Bonney is the most strongly stratified of the lakes, followed by Lakes Fryxell, and Hoare. Detailed descriptions of these lakes may be found in Green and Friedmann (1993) and Priscu (1998).

Sampling Procedure

Sampling holes were made in the ice over the deepest portion of the lake. Each lake was sampled 3-5 times during the austral spring and summer (October to January) of 1993-1997, or approximately every 20 to 30 days. Each lake was sampled approximately every 10 days during the winter to spring transition (September to October) of 1995. This period marks the transition from complete darkness to continuous sunlight. Samples were collected at selected depths using a Niskin bottle with Teflon coated components. All sampling depths were measured from the piezometric water level (water level within the sampling hole). The sampling bottle was gently inverted several times before decanting to eliminate possible gradients within the Niskin bottle. Lake water was collected in acid washed polyethylene bottles following 3 rinses with sample water and stored at 1 to 5°C until processed in a lakeside laboratory.

Bacterial Cell Counts

Bacterial samples (10 ml) were preserved with formalin (5% final concentration) at 4°C until counted. Sample vials were vortexed before staining cells with acridine orange [0.01% (w/v) final concentration] for two minutes and filtered onto black 0.2 μm polycarbonate filters. Non-autofluorescing rods and cocci were counted by epifluorescent microscopy (Hobbie et al. 1977) at 1000X magnification. At least 200 cells were counted on each prepared filter. Acridine orange stained cells were found to be more stable than DAPI stained cells in the wide range of salinity (0 to 120 ppt, Spigel and Priscu 1996) found in these lakes. All samples were counted 6 months after

collection with the exception of the 1993-1994 samples, which were counted 16 months after collection. We adjusted bacterial cell count data by fitting an exponential decay equation to cell counts of Lake Bonney samples mounted between zero and 365 days after collection (Spinrad et al. 1989). Cell count decay rates were determined for samples collected from the spectrum of salinities found in these lakes.

Heterotrophic Bacterial Activity

Bacterial activity was measured on 10 ml of lake water incubated in the dark for 20 h with 20 nM ^{3}H-methyl-thymidine (2 Bq mmol^{-1}) at 1 to 4°C. Three replicates and two kills (formalin at 5% final concentration) were incubated for each sample analyzed. Incubation was ended by the addition of 10 ml cold 10% trichloroacetic acid (TCA). Samples were stored 1 to 4 weeks before filtering. This storage interval may be a source of minor variability in thymidine uptake rates (TdR). Wicks and Robarts (1987) found that 91% and 74% of ^{3}H-DNA was recovered in NaOH treated samples after being stored at 4°C for 2 and 50 days, respectively. Storage of samples at a low pH, such as when treated with TCA, would allow for metals to cleave DNA or form DNA dimers, but should not interfere with the recovery of the ^{3}H-labelled extract any more than NaOH would. Samples were filtered onto 0.45 µm polycarbonate filters and filter towers were rinsed 3 times with 2 ml cold 5% TCA. Radioactivity on the filters was assayed by standard scintillation spectroscopy. Thymidine uptake rates have been shown to be linear for up to 24 h using this protocol (see chapter 2). Thymidine uptake rates (corrected for formalin kills) from laboratory incubations were converted to rates of *in situ* temperature using an energy of activation of 12,600 Kcal mol^{-1}. This energy of activation was

determined from temperature experiments on the east lobe of Lake Bonney. Thymidine uptake was converted to bacterial production based on the published value of $2x10^{18}$ cells $mole^{-1}$ thymidine. We empirically estimated a thymidine conversion factor (TCF) for each of these lakes by the method of Kirchman and Ducklow (1993). An average TCF of $2.57x10^{19}$ cell $mole^{-1}$ thymidine was computed by the integration method and $4.75x10^{18}$ cells $mole^{-1}$ thymidine by the modified derivative method. These results are based upon limited data during one month (December) only and probably overestimate the TCF for these lakes. We therefore used the published value which is based upon 97 marine studies, and is commonly derived in fresh water as well (Bell 1993). A value of 11 fg C $cell^{-1}$ was used to calculate bacterial carbon and was empirically determined for the lakes by Kepner et al. (1998) from the biovolume of 400 cells. Carbon production was divided by cell counts to determine specific activity (μg C $cell^{-1}$ day^{-1}). One-way analysis of variance was used to determine if water column integrated bacterial production varied among years (data were normally distributed). Differences between years were determined using Tukey's Test.

Relative Contribution of Bacterial Carbon to Microplankton Carbon and Particulate Organic Carbon

The fraction of organic carbon attributed to bacteria and phytoplankton, with respect to total microplankton carbon (phytoplankton + bacterial carbon) and total particulate organic carbon (POC) was computed as volume-weighted concentrations integrated throughout the trophogenic zone (18 m in Lake Hoare and the east lobe of Lake Bonney, 17 m in the west lobe of Lake Bonney, and 9 m in Lake Fryxell) and

the entire water column of the lakes. Bacterial cell density was converted to bacterial carbon using a conversion factor of 11 fg C $cell^{-1}$ (see above). A phytoplankton carbon to chlorophyll (CHL) ratio (25:1, g:g) was determined for Lake Hoare, which is relatively detritus free compared to Lakes Fryxell and Bonney (Lizotte and Priscu, 1992; Sharp, 1993), and applied to all of the lakes. Briefly, POC:CHL ratios were plotted versus CHL to determine asymptotic values of POC:CHL, which indicate that water column POC was most closely associated with phytoplankton (Ducklow et al., 1993). Chlorophyll-*a* (CHL) was measured fluorometrically (Holm-Hansen et al., 1965) as described previously (Lizotte and Priscu, 1998; Priscu, 1995). Particulate organic carbon (POC) was determined by flash-combustion gas chromatography (Carlo Erba model 1500 elemental analyzer, Priscu, 1995).

Bacterial Cell Loss Rates

Bacterial cell loss rate (cells m^{-2} day^{-1}) during the interval between successive samplings was computed over the trophogenic zone (9 m in Lake Fryxell, 14 m in Lake Hoare, 13 m in Lake Bonney) and over the entire water column of the lakes using the general relationship:

$$\text{Loss rate} = BP - dB/dt,$$

where BP is the average bacterial production over the time step (cells m^{-2} day^{-1}), B is cell density in cells m^{-2}, and t is the number of days over the time step (Jassby and Goldman 1974). The trophogenic zone was defined as the water column above the chemocline in each lake and loss rates were computed separately here because this is where a majority

of bacterial grazing is believed to occur (James et al. 1998; Roberts and Laybourn-Parry, personal communication). Loss rates were not determined for intervals between sampling seasons because little is known about the winter period. Loss rate is positive in this model and therefore biomass gains are negative. BP was determined as

$$BP = 0.5(BP_t + BP_{t+1}),$$

where BP_{t+1} and BP_t are the cell production rates on successive sampling days integrated over the trophogenic zone and the entire water column of each lake. dB/dt was determined by

$$dB/dt = (B_{t+1} - B_t)/t,$$

Where B_t and B_{t+1} are the integrated cell counts over the trophogenic zone and entire water column on successive samplings (excluding winter). Loss rates throughout the entire water column were also computed to exclude vertical transport as a means of cell loss. We determined that bacterial cell sedimentation rate is less than 0.9% of loss rate and negligible in this model by computing flux of cells to the sediment according to the relationship:

$$J_{cells} = V_s * [cells]$$

Where J_{cells} is flux of bacterial cells to the sediment, V_s is sedimentation velocity, and [cells] is cell density just above the sediment. Turnover rate (day^{-1}) for each interval was estimated by dividing the loss rate by the average B for the corresponding interval. A theoretical grazing rate was calculated by dividing trophogenic zone and water column cell loss by the depth (m) of the trophogenic zone or of the entire lake to estimate average cell loss rates (cells m^{-3} day^{-1}).

Results

Seasonal and Annual Variations in Bacterial Production and Biomass

The influence of sample storage time on bacterial cell counts was consistent throughout the water column of the east and west lobe of Lake Bonney (which covers the salinity range of all the Taylor Valley lakes). A time course experiment (0 to 365 days) of sample storage produced data that could be fitted with an exponential decay function ($r=0.548$; $n=60$; $p<0.01$) of the form:

$$N_t=N_o e^{-0.0037t}$$

where N_t is the number of cells counted at time t, time t is the time elapsed in days, and N_o is the number of cells in the sample at time zero. All bacterial cell counts were corrected for storage time using this relationship.

Bacterial production, distribution, and cell specific activity in Lakes Fryxell, Hoare and Bonney are summarized in figures 3.1 through 3.4. Generally, production, cell numbers, and specific activity showed the same trends in all three lakes, but differed in magnitude. Production was greatest just below the ice cover, with a smaller, less pronounced peak at or near the chemocline (9 m in Lake Fryxell, 14 m in Lake Hoare, 13 m in Lake Bonney) in the beginning of each season. Both maxima increased as the season progressed, with the deeper peak becoming more pronounced by December to January. Bacterial distributions generally followed production profiles from November to December. Exceptions were a third peak that formed in Lake Fryxell at 15 m and high cell numbers in the east lobe of Lake Bonney between 20 and 30 m; both of these

maxima were below the trophogenic zone. Specific activity was greatest just below the ice cover and at the chemocline, but remained relatively unchanged throughout the season each year.

Biomass typically decreased by up to 88% in mid-December. Following this decrease in cell numbers, biomass began to increase again, often decreasing by January (see Figure 3.5). Bacterioplankton in the trophogenic zone and entire water column in the east lobe of Lake Bonney typically comprised 30 to 50% of total microplankton biomass (bacteria + phytoplankton), whereas only 20 to 30% of microplankton carbon consisted of bacterioplankton biomass in the west lobe of Lake Bonney and Lake Hoare. Bacterioplankton biomass in Lake Fryxell was only approximately 20% of trophogenic zone microplankton carbon, but comprised a larger proportion (40 to 50%) of total water column microplankton carbon (Figure 3.5). Bacteria comprised only 0.2 to 19% of total POC in the lakes. The phytoplankton comprised 6 to 65% of the POC in Lakes Hoare and Fryxell, but only 1 to 20% of POC in both lobes of Lake Bonney. Total microplankton carbon (bacteria+phytoplankton) comprised the largest proportion of total POC in Lake Hoare (16 to 80%), followed by Lake Fryxell (6 to 57%), the west lobe of Lake Bonney (8 to 23%), and the east lobe of Lake Bonney (3 to 18%, Figure 3.6).

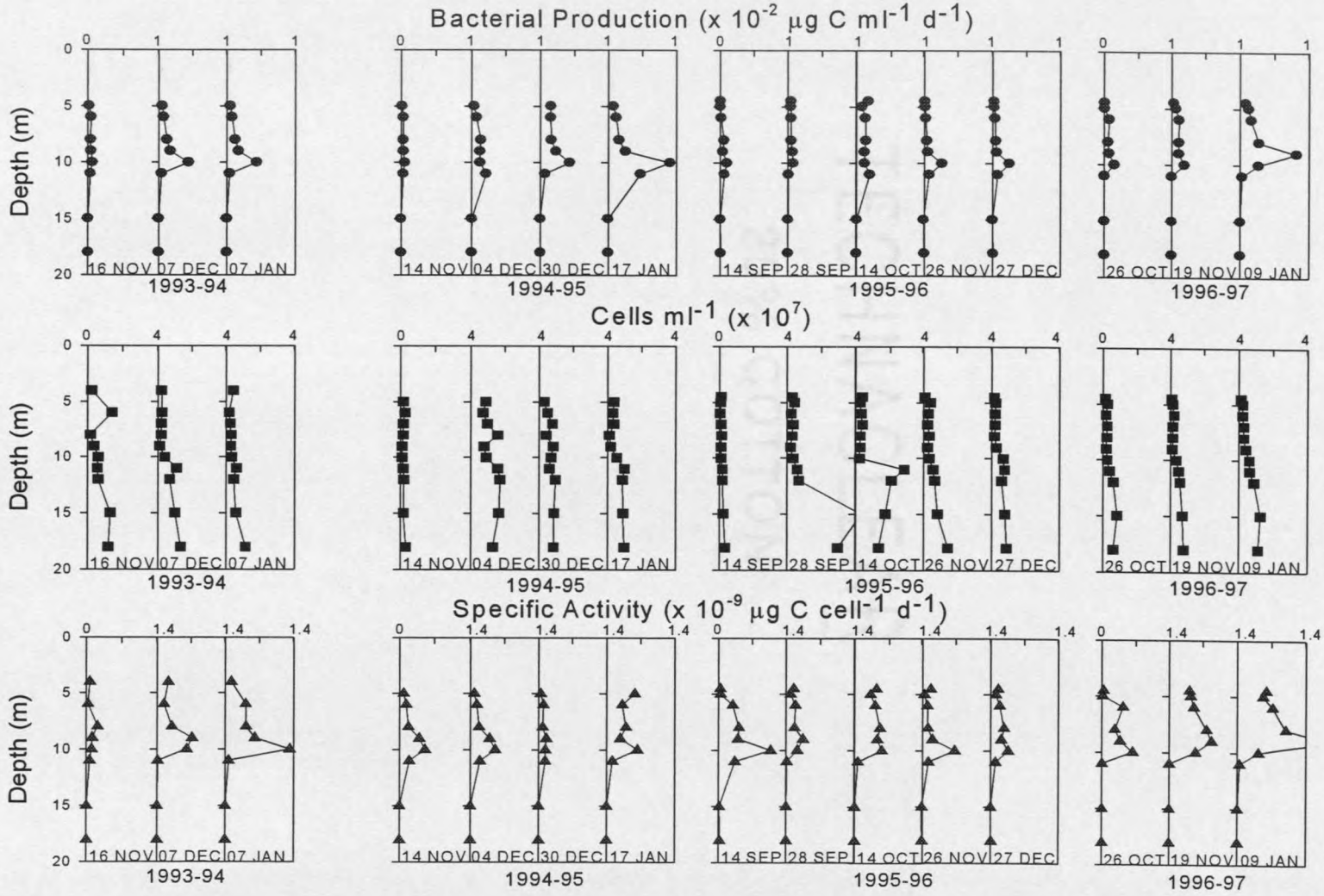

Fig. 3.1. Lake Fryxell bacterial production (x 10^{-2} µg C ml^{-1} d^{-1}), cell numbers (x10^7 cells ml^{-1}), and specific activity (x10^{-9} µg C $cell^{-1}$ d^{-1}) during the 1993-1997 sampling seasons.

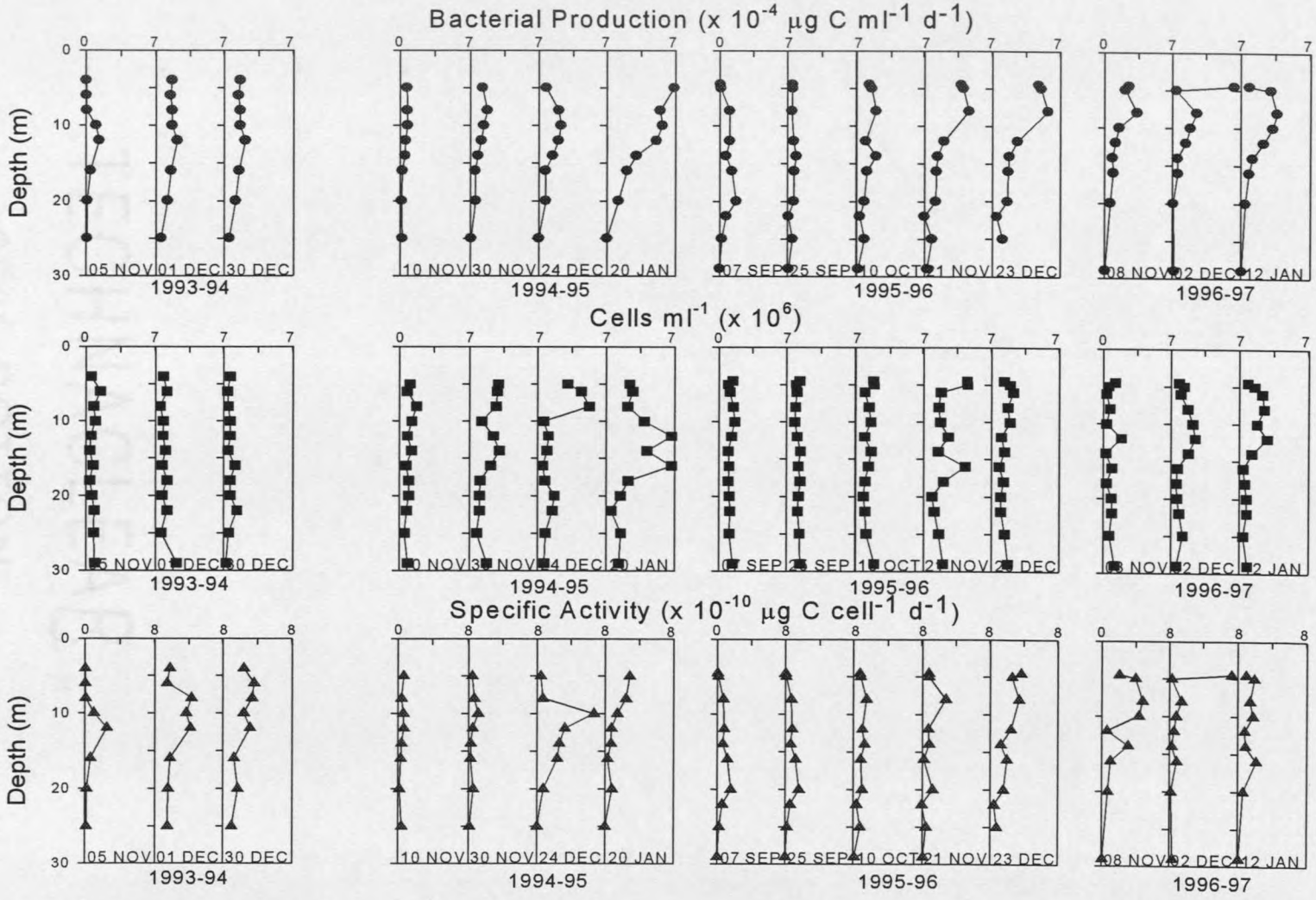

Fig. 3.2. Lake Hoare bacterial production (x 10^{-4} µg C ml^{-1} d^{-1}), cell numbers (x10^6 cells ml^{-1}), and specific activity (x10^{-10} µg C $cell^{-1}$ d^{-1}) during the 1993-1997 sampling seasons.

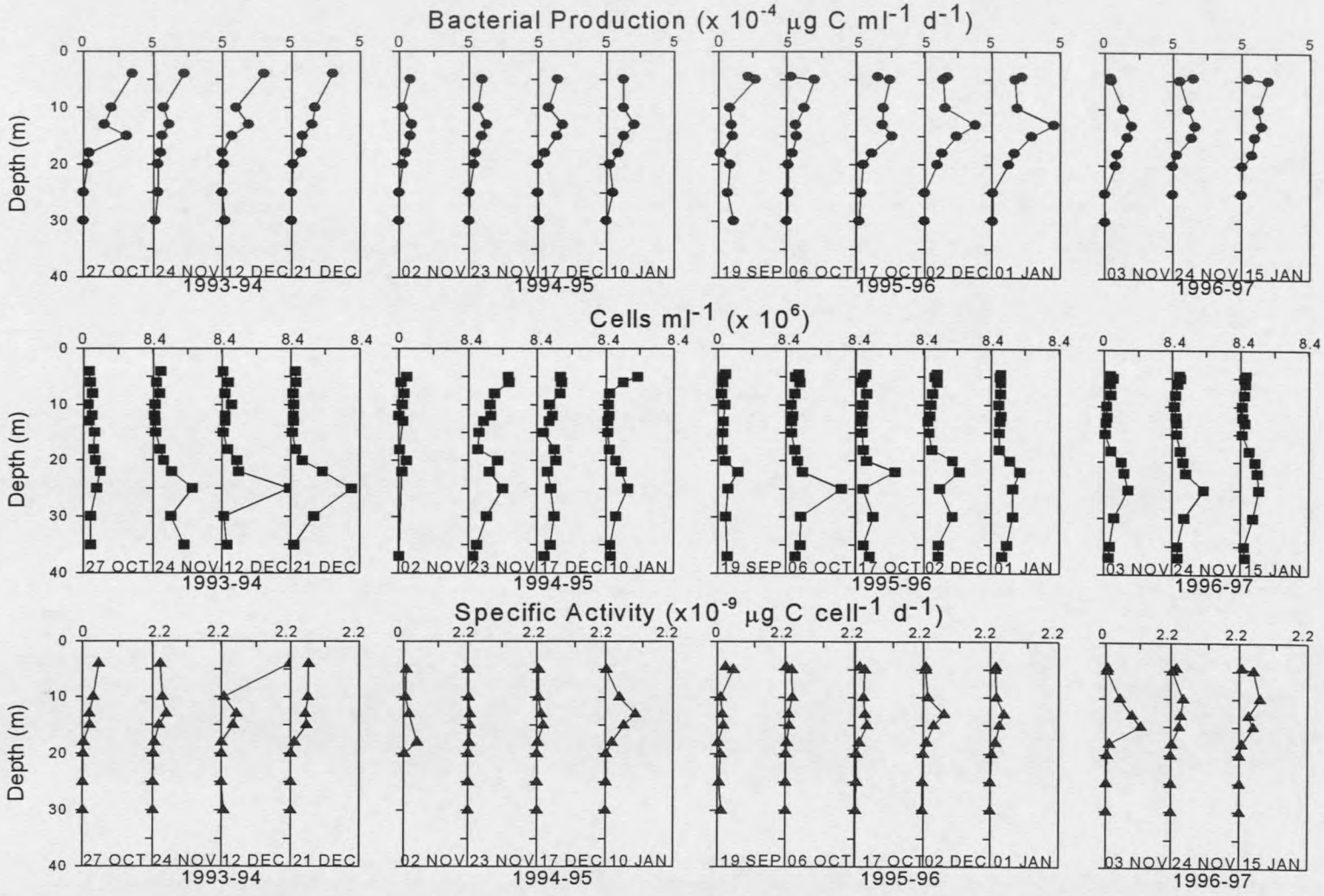

Fig. 3.3. East lobe, Lake Bonney bacterial production (x 10^{-4} µg C ml^{-1} d^{-1}), cell numbers (x10^6 cells ml^{-1}), and specific activity (x10^{-9} µg C $cell^{-1}$ d^{-1}) during the 1993-1997 sampling seasons.

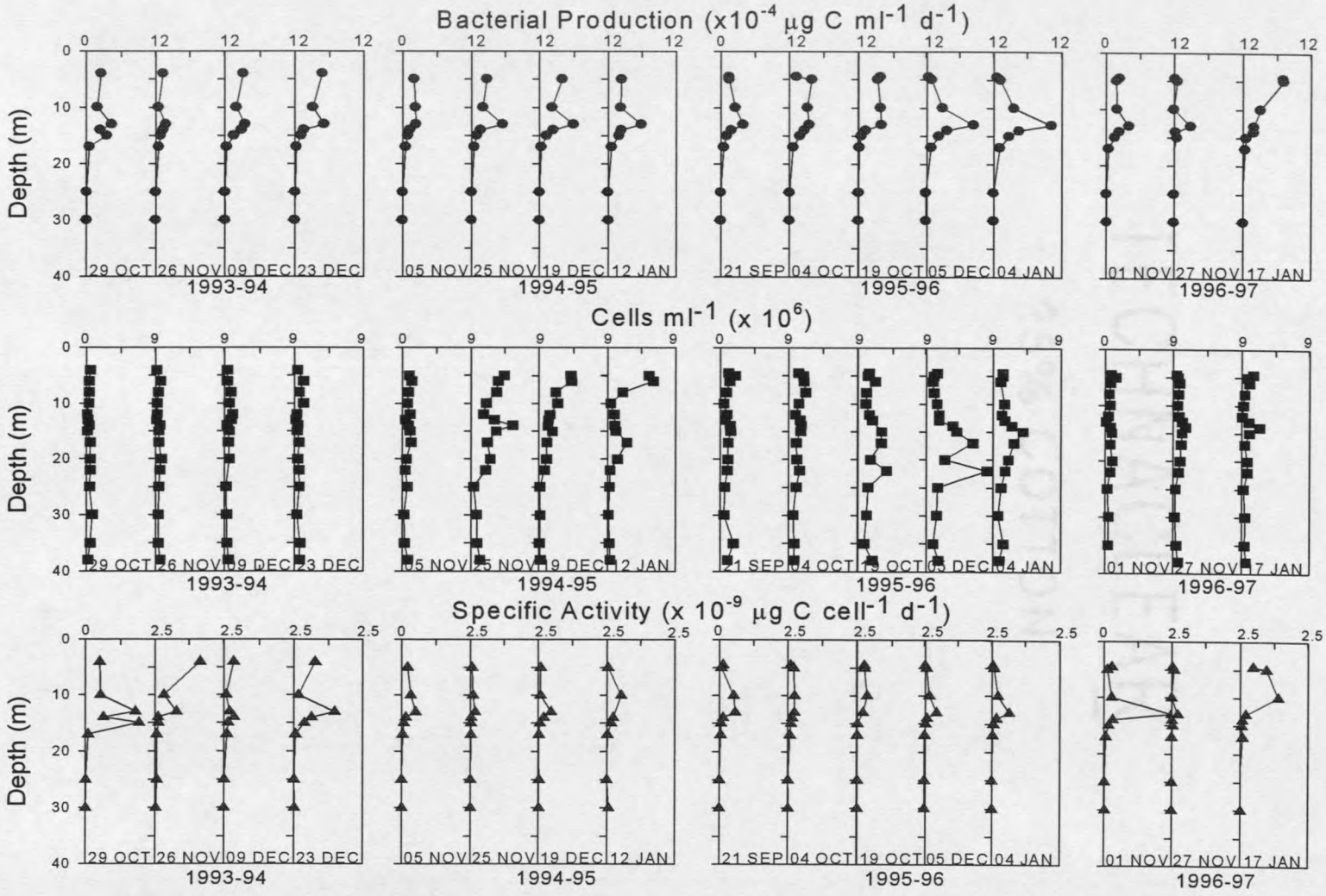

Fig. 3.4. West lobe, Lake Bonney bacterial production (x 10^{-4} µg C ml^{-1} d^{-1}), cell numbers (x10^6 cells ml^{-1}), and specific activity (x10^{-9} µg C $cell^{-1}$ d^{-1}) during the 1993-1997 sampling seasons.

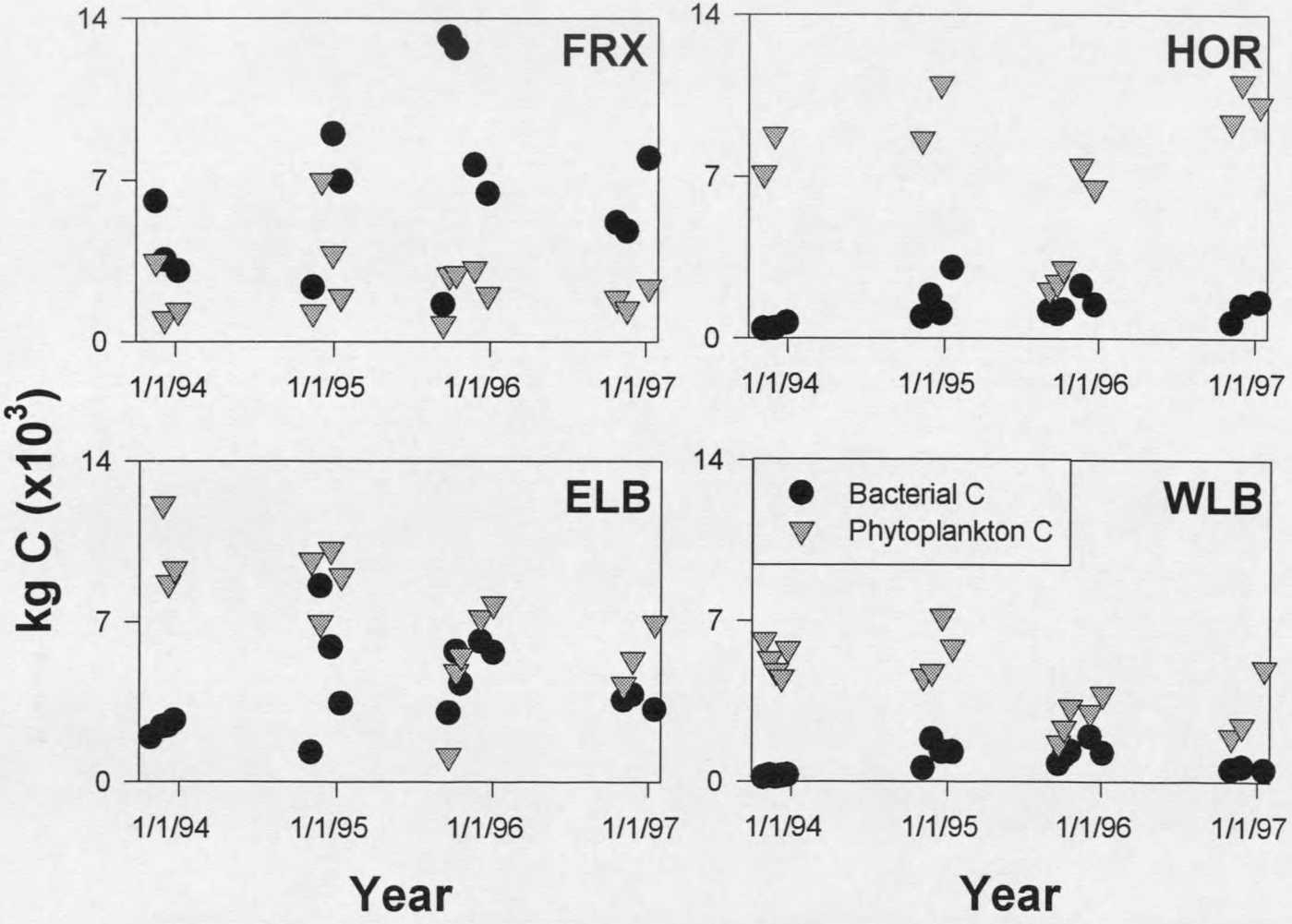

Figure 3.5. Volume weighted and depth integrated bacterial and phytoplankton carbon during 1993 to 1997 in the water columns of Lakes Fryxell (FRX), Hoare (HOR), and the east (ELB) and west (WLB) lobe of Lake Bonney.

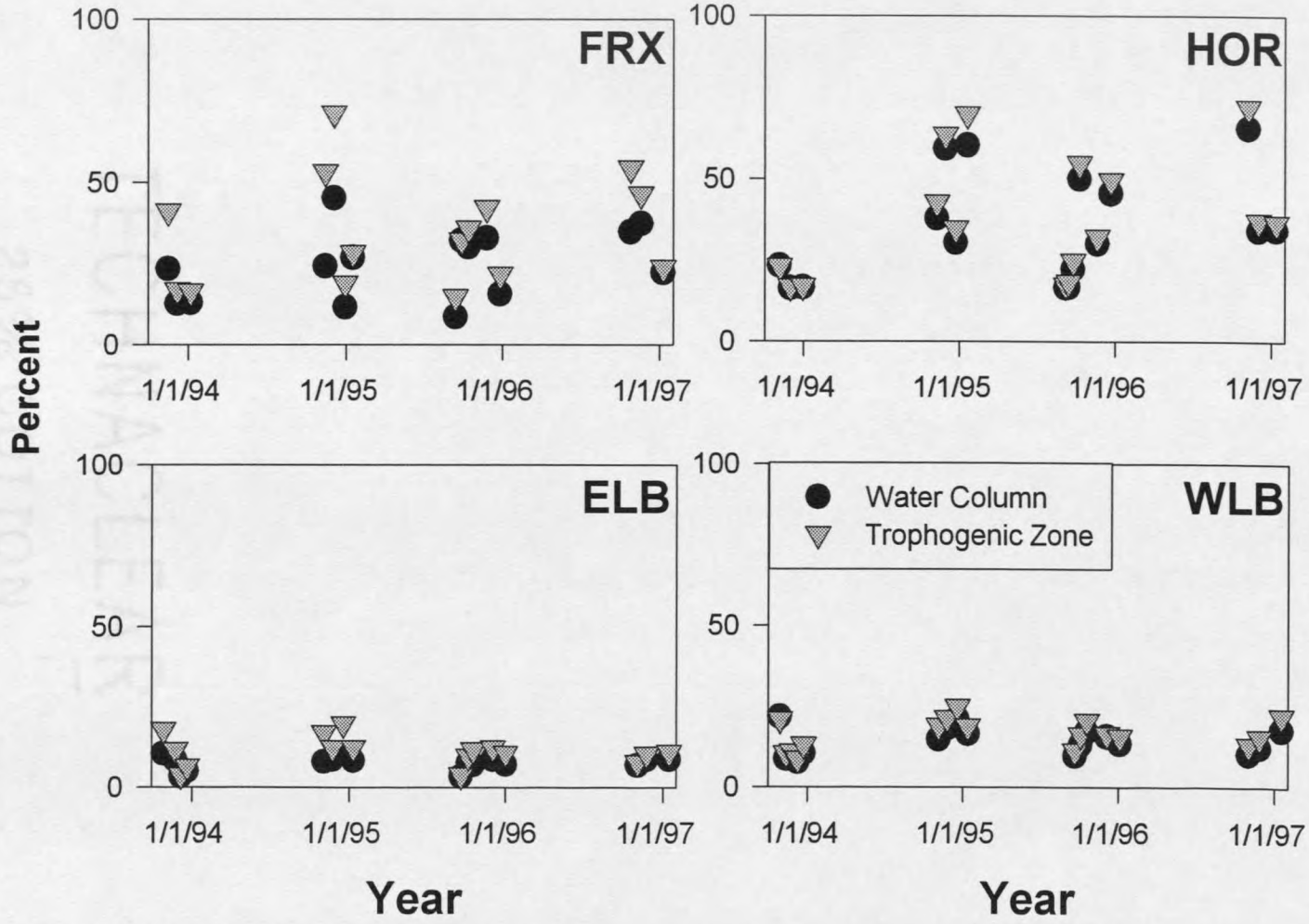

Figure 3.6. Relative contribution of microplankton carbon (bacterial + phytoplankton carbon) to particulate organic carbon in the water column and trophogenic zones of Lakes Fryxell (FRX), Hoare (HOR), and the east (ELB) and west (WLB) lobes of Lake Bonney.

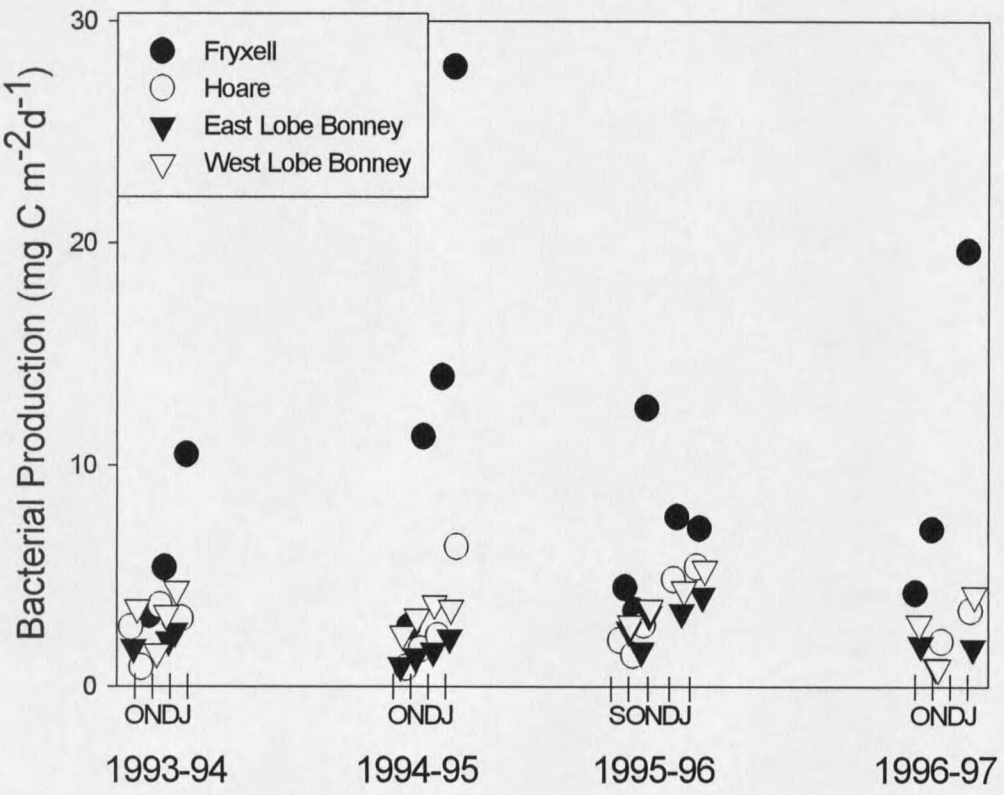

Figure 3.7. Water column integrated bacterial production (mg C m^{-2} d^{-1}) in Lakes Fryxell, Hoare, and Bonney during 1993-1997.

Areally integrated bacterial production was greatest in Lake Fryxell, followed by the west lobe of Lake Bonney, Lake Hoare, and the east lobe of Lake Bonney (Figure 3.7). One-way analysis of variance of areally integrated bacterial production (mg C m^{-2} d^{-1}) showed no significant differences, except in the east lobe Lake Bonney between the 1994-1995 and 1995-1996 sampling seasons ($p<0.048$, $n=16$). Lake Fryxell production was higher in 1994-1995, whereas production in Lake Hoare remained relatively unchanged. Seasonal and annual bacterial production varied similarly in the east lobe and west lobes of Lake Bonney, except that production was higher in the west lobe than in the east lobe. Lake Fryxell was the most productive lake based on total lake volume, followed by the east lobe of Lake Bonney, Lake Hoare, and the west lobe of Lake Bonney (Table 3.1). Lake Bonney was more productive than Lake Hoare, though, when the two lobes were considered together.

Table 3.1. Average (min-max) bacterial production in Lakes Fryxell, Hoare, and Bonney (integrated volumetrically) listed in order of decreasing productivity.

Lake	Volume (m^3 x 10^5)	Average Bacterial Production (x 10^5 mg C d^{-1})
Fryxell	53.56	41.2 (11.1 – 115.1)
Bonney, East lobe	51.67	3.43 (1.34 – 6.42)
Hoare	24.34	2.83 (0.89 – 6.66)
Bonney, West lobe	27.82	2.71 (0.70 – 4.34)

Bacterial Gains and Losses

Bacterial cell loss rates and turnover rates in the trophogenic zone and throughout the entire water column of Lakes Fryxell, Hoare and Bonney are summarized in Table 3.2. Lake Fryxell's trophogenic zone showed the greatest biomass loss, whereas loss rates computed in Lakes Bonney and Hoare were lower and varied within the same range. The higher loss rate computed in Fryxell may be due to the increased stream input this lake received, especially in the 1993-1994 season, compared to other years (Conovitz et al. 1998; McKnight, unpublished data). The relatively fresh stream input has been observed to mix with the upper 2 m of lake water (Welch and Lyons, unpublished data) and this would result in cell dilution. Generally, water column loss rates were highest in Fryxell, followed by Lake Bonney's east and west lobe, and Lake Hoare. Loss rates increased mid-season, then decreased, and rose again in January during 1996. Loss rates and turnover rates varied the most and were greatest in Lake Fryxell.

Theoretical grazing rates calculated from trophogenic zone loss rates ranged from 1.03×10^{9} to 4.82×10^{13} cells m^{-3} d^{-1}. Lake Fryxell had the greatest trophogenic cell loss, followed by Lake Hoare, and Bonney's east lobe and west lobe. Theoretical grazing rates calculated from water column loss rates ranged from 5.02×10^{8} to 3.20×10^{11} cells m^{-3} d^{-1} and were greatest in Lake Fryxell, followed by the east lobe of lake Bonney, the west lobe of lake Bonney, and Lake Hoare.

Table 3.2 Average bacterial cell loss rates and turnover time (min-max) in the trophogenic zone and throughout the entire water column of Taylor Valley Lakes Fryxell, Hoare, and Bonney.

Lake	Bacterial Cell Loss Rate (x 10^{11} cells m^{-2} d^{-1})	Turnover Rate (d^{-1})
Fryxell		
TZ[a]	2775 (3.19-6271)	18.3 (0.05-37.9)
WC[b]	22 (8.84-41.6)	0.02 (0.001-0.03)
Hoare		
TZ	2.47 (0.24-5.19)	0.02 (0.001-0.03)
WC	4.59 (0.52-10.3)	0.01 (0.002-0.03)
Bonney East lobe		
TZ	2.49 (0.52-7.10)	0.03 (0.006-0.09)
WC	16.7 (2.4-64.8)	0.03 (0.004-0.14)
Bonney West lobe		
TZ	2.73 (0.78-4.26)	0.03(0.01-0.05)
WC	6.33 (0.17-17.7)	0.02 (0.009-0.03)

[a]TZ, trophogenic zone
[b]WC, entire water column

Discussion

Summer bacterial production in the lakes of the McMurdo Dry Valleys is comparable to other high latitude lakes in winter, whereas bacterial numbers are comparable to more productive systems (Table 3.3). These lakes present a paradox in that bacterial numbers are relatively high, yet bacterial production is comparatively lower. The following explanations are offered to interpret this paradox. Firstly, low temperature and salinity restrict bacterial production. Secondly, cells accumulate and persist due to the low ambient temperature and long water column turnover time of these lakes. Thirdly, though bacterial production is low for most of the year, low grazing rates and viral infection maintain bacterial numbers near 10^6 cells ml^{-1}.

Table 3.3. Cross system comparison of bacterial production and cell numbers. Aquatic systems are listed in order of decreasing productivity.

Aquatic system	nM thymidine d^{-1}	Cells ml^{-1} ($x10^6$)	Reference
Lake SØbygard			Jeppesen et al. 1997
Denmark			
Winter	0.12-0.36	3-8	
Summer	7.2-10.8	15-30	
Hartbeespoort Dam, South Africa			Robarts and Wicks 1990
Surface	0.024-6.0	2.45-32.20	
Bottom	0-0.64	4-8	
Amazon River System			Benner et al. 1995
Mainstem	0.11-0.36	0.9-1.26	
Tributaries	0.08-0.63	0.46-2.59	
Upton Lake, NY	~0.06-0.6	4-6	Pace et al. 1990
Lake Fryxell	**0-0.453**	**0.53-43.6**	**Present study**
	NR[a]	**1-8**	**Smith and Howes 1990**
Ross Sea Polynya	~0.03-0.56	1-4	Ducklow et al. 1995
McMurdo Sound Antarctica	0.00006-0.26	0.06-1	Fuhrman and Azam 1980
West lobe, Bonney	**0-0.05**	**0.1-8.23**	**Present study**
Bottom	**NR[a]**	**0.3**	**Takii et al. 1986**
Lake Hoare	**0-0.03**	**0.26-6.68**	**Present study**
	NR[a]	**0.1-4**	**Mikell et al. 1986**
East lobe, Bonney	**0-0.02**	**0.032-8.18**	**Present study**
	NR[a]	**0-0.7**	**Koob and Leister 1972**
Bottom	**NR[a]**	**0.1**	**Takii et al. 1986**
Caribbean Sea	0-0.0144	0.1-0.6	Rivken and Anderson 1997
Gulf Stream	0.0024-0.0132	0.09-0.9	Rivken and Anderson 1997
Sargasso Sea	0.0036-0.011	0.15-0.55	Rivken and Anderson 1997

[a]NR, not reported

The magnitude of bacterial production and cell numbers differs among the three lakes studied, whereas seasonal and annual trends are similar. The increase in bacterial production throughout the sampling season corresponds with an increase in primary production (Lizotte et al. 1996; Priscu, unpublished data] indicating a close coupling between these two trophic levels. This is most likely due to the release of dissolved

organic carbon by phytoplankton, which has been estimated to be between 5 and 23% of primary productivity in these lakes (Sharp 1993). Inter-lake differences may be attributed to the distinct nutrient and primary productivity profiles of each lake (Priscu 1995). Lake Fryxell's trophogenic zone is more nutrient rich, compared to the other lakes, and is the most productive (primary and secondary) lake on a volumetric basis. Lake Hoare, the least productive lake, has the lowest nutrient levels and primary productivity. Phytoplankton have been shown to be primarily phosphorus deficient in these lakes (Priscu 1995), but repeated bacterial nutrient bioassay experiments have shown little stimulation by inorganic nitrogen, phosphorus, or glucose (Takacs and Priscu 1995). However, laboratory studies have revealed that the bacterial populations of these lakes are growing well below their optimum temperature and above their optimum salinity. Ward and Priscu (1997) estimated optimal salt concentrations and temperatures for growth of three denitrifiers isolated from Lake Bonney. They found that under *in situ* conditions, the bacteria were growing at only 42% of their maximum rate. Experiments conducted on unidentified isolates from all three lakes have shown that optimal salt concentration for growth was 0 to 5% and temperature optima ranged from 12 to 20°C (see chapter 5). These data show that the low temperature (-5 to 7°C) and high conductivity below the chemocline (0.11 to 14 0 mS m^{-1}) of the lakes (Spigel and Priscu 1998) are two factors limiting growth of bacteria *in situ*. Presumably, the difference in magnitude of bacterial activity among the lakes is due to the magnitude of primary productivity.

Though bacterial numbers are relatively high, with respect to productivity, the percentage of live cells is unknown, with the exception of Lake Bonney. Respiring cells measured by the tetrazolium salt reduction method were estimated to be 10 to 35% of total cell counts in the east lobe and 45 to 73% in the west lobe (Smith and Priscu 1993). A significant fraction of bacterial cells are most likely not respiring in these lakes and specific activity is underestimated. Dead cells may persist at depth for extended periods due to slow decomposition and sinking rates at the low temperatures (-5 to 7°C) and high salinities of these lakes (0-120 ppt). Additionally, water column mixing times in these lakes have been estimated to exceed 20,000 years because molecular diffusion is the dominant mixing mechanism (Spigel and Priscu 1996).

Bacterioplankton biomass typically dominates microplankton biomass in oligotrophic aquatic systems, while phytoplankton often dominate in more productive systems (Schwaerter et al., 1988; Kristiansen et al., 1992; Cho and Azam, 1990). However, no clear trend that relates bacterial dominance to the productivity of these lakes is apparent. One explanation for the predominance of bacteria in oligotrophic systems is that bacterioplankton compete better than phytoplankton for inorganic nutrients (Currie and Kalff, 1984). While this may also be true of Taylor Valley lake bacterioplankton, we believe that phytoplankton dominate the trophogenic zones in Lakes Fryxell and Hoare because of selective grazing. Phytoplankton are not significantly grazed in these two lakes, while bacteria are highly grazed by both mixotrophic and heterotrophic flagellates and ciliates (Roberts and Laybourn-Parry, personal communication). Compared to Lakes Hoare and

Fryxell, grazing upon bacteria in Lake Bonney is low, which enables bacterial biomass to comprise a larger proportion of microplankton biomass.

Phototrophic nanoflagellates (PNAN), heterotrophic nanoflagellates (HNAN), ciliates and rotifers capable of bacterivory have been detected in these lakes (Laybourn-Parry et al. 1997), and populations of PNAN, HNAN, and ciliates are associated with bacterial peaks (James et al. 1998). Roberts and Laybourn-Parry recently initiated experimental studies to determine ingestion rates in Lakes Hoare and Fryxell. Preliminary results show that phototrophic cryptophytes, present at concentrations up to 6000 cells ml^{-1} in Lake Fryxell and 1600 cells ml^{-1} in Lake Hoare, have been observed to ingest bacteria at a rate of 0.6 to 2.2 bacteria $cryptophyte^{-1}$ h^{-1} (Roberts and Laybourn-Parry, unpublished data). Based on these preliminary experimental results, grazing by cryptophytes alone may range from 8.64×10^{11} to 31.68×10^{11} cells m^3 d^{-1} in Lake Fryxell and agree with our theoretical grazing rates. Grazing by HNAN may be as high as 6.5×10^{10} cells m^3 d^{-1} in Lake Fryxell, based on preliminary results, and abundance ranges from 0 to 694 HNAN ml^{-1} (Roberts and Laybourn-Parry, unpublished data). Grazing rates by ciliates in these lakes are presently not available. Ciliates and HNAN have been reported to remove up to 9.7% of bacterial production in Crooked Lake, Antarctica, but ciliates were not found to be an important component of the plankton community in Lake Bonney (Laybourn-Parry et al. 1995; James et al. 1998). Removal of bacterial water column biomass averaged 3% d^{-1} (range = 0.01 to 7% d^{-1}), excluding a single rate of 15% d^{-1} on 24 October 1993 in the east lobe of Bonney. Rotifer feeding experiments have thus far been inconclusive (Shultz, Kepner, and Wharton, unpublished

data), but rotifers are not believed to be significant in bacterioplankton losses because they are primarily confined to the littoral zone of these lakes (James et al. 1998).

Another possibility for the cause of bacterial cell loss may be viruses. Planktonic, extracellular, icosahedral viruses have been observed in Lakes Bonney and Hoare by transmission electron microscopy, and production potential by viruses in Lake Hoare was found to be as much as 58% higher than measured in coastal seawater mesocosms by the same method (Kepner et al. 1998). Though bacterial cells infected by viruses have yet to be observed, bacteria have been observed by TEM to be surrounded by viruses. Additionally, water column virus like particle (VLP) profiles enumerated by epifluorescent microscopy were found to be significantly correlated with bacterial numbers. VLP profiles were found to increase throughout the season and a mid-summer peak was observed in December (Kepner et al. 1998). Virally mediated mortality may be a factor regulating bacterial biomass and may play a role in the regeneration of nutrients and organic carbon. Additional sources of bacterial loss may be Myxobacteria, Bdellovibrio spp., or other parasitic bacteria, but have not been investigated specifically.

Our study is the first to rigorously sample bacterioplankton in Lakes Fryxell, Hoare, and Bonney during the winter–spring transition (September-October). Though chlorophyll-*a* and primary productivity during this period were low to not detectable, (Lizotte et al. 1996; Priscu, unpublished data), bacterial populations were active and cells numbers were high in September, especially just below the ice cover. These data show that bacterial populations remain active and are not grazed to extinction during the winter. The limited sampling season, due to logistical constraints, has left the autumn

and winter period largely unstudied. Phytoplankton data do exist for Lake Fryxell during two winters where automated sampling devices were used to collect preserved samples (McKnight et al. Submitted). Though biomass decreased through winter, algal abundance was greatest in April and populations of two cryptophytes (*Cryptomonas* and *Rhodomonas*) were found to increase during winter. Mixotrophy, as a means of survival, has been proposed for these populations (Laybourn-Parry et al. 1997). No longer can the "long, dark winter" be considered insignificant; winter microbial dynamics must be studied in the future to gain a more complete understanding of these lakes.

The data set presented here represents the start of an accumulation of long term data in the McMurdo Dry Valleys, Antarctica. Lake Fryxell is the most dynamic of these lakes with respect to integrated bacterial production, cell numbers, and loss rates, followed by west lobe Lake Bonney, east lobe Lake Bonney, and Lake Hoare. Changes in physical, chemical, and biological profiles over time may be an indication of local or global climate change, or increased anthropogenic impact in the area. One-way analysis of variance did not show an increase in lake bacterial production during this study, but four years is not a sufficient period in which to detect "long-term" changes. Increased intra-annual sampling and a longer data set would be a more rigorous test of change. Changes in bacterial production and biomass are significant in the carbon budgets of these lakes and may ultimately prove to be important to the overall food web.

CHAPTER 4

BACTERIAL DISSOLVED ORGANIC CARBON DEMAND IN ANTARCTIC DRY VALLEY LAKES

Introduction

The accumulation of dissolved organic carbon (DOC) in the photic zone of the ocean has previously been explained to result from the recalcitrant nature of the DOC pool (Aminot et al. 1990; Carlson and Ducklow 1996). Explanations of DOC accumulation based on low degradability implied that aquatic bacterial production is carbon limited (Thingstad et al. 1997). However, recent studies have indicated that a large proportion of lake and marine surface water DOC is biologically degradable (Servais et al. 1987; Kirchman et al. 1991; Sondergaard and Middleboe 1995; Wetzel 1995). In contrast to the hypothesis of low DOC degradability, Thingstad et al. (1997) proposed that inorganic nutrient limitation better explains the observed uncoupling of aquatic bacterial-phytoplankton production, which is consistent with data indicating the labile nature of surface water DOC (Servais et al. 1987; Kirchman et al 1991) and bacterial P limitation (Zweifel et al. 1993; Morris and Lewis 1992). Studies concerning the production and fate of phytoplankton in ice covered lakes during the winter suggested that surplus DOC accumulated during the summer is stored in a lake, and used during winter by mixotrophic algae (Rodhe 1955). Thingstad et al. (1997) reapplied this concept to bacteria, proposing a functional process where accumulated DOC is stored in a system,

and used later to sustain heterotrophic bacterial growth during dark or unproductive seasons.

The Taylor Valley lakes (Lakes Fryxell, Hoare, and Bonney) of the McMurdo Dry Valleys, Antarctica, provide a unique system in which to study the temporal uncoupling of phytoplankton and bacterioplankton production. Photosynthesis is limited to approximately five months each year due to the darkness of the Austral winter, whereas bacterial activity occurs throughout the year (Takacs and Priscu 1998; Priscu et al. In press). In addition to the annual variation in photosynthesis, the Taylor Valley lakes provide a relatively simple setting in which to study bacterial-phytoplankton interactions. The plankton of these lakes are comprised primarily of eukaryotic phytoplankton, bacteria, and protozoans, and the lakes are closed systems owing to the permanent 4 to 5 m ice cover, lack of outflow, and relatively low inflow. The streams in the valley, which travel over a barren desert landscape, only flow for 4 to 8 weeks each year (Conovitz et al. 1998). Phytoplankton extracellular release (ECR) and upward diffusion from the nutrient rich hypolimnia are believed to be the two dominant mechanisms that supply new DOC to the photic zone of the lakes (Howes et al. 1992; Priscu 1995). McKnight et al. (1991 & 1993) determined that approximately 20% of the DOC pool in Lakes Fryxell and Hoare is comprised of low molecular weight fulvic acid that is microbial in origin. The remainder of the bulk DOC pool of these lakes is relatively high molecular weight DOC that is presumably recalcitrant.

Few reports exist concerning the bacterioplankton of Taylor Valley lakes; a majority of the microbial research has concentrated on either specific bacterial genera

(Ward and Priscu 1997; Voytek et al. 1998) or the phytoplankton (e.g., Priscu 1995; Lizotte and Priscu 1992; Vincent 1981; Goldman 1964). We constructed a carbon budget to determine the potential role of DOC supplied from ECR, stream input, and diffusion in the regulation of bacterial productivity during the Austral summer. The results of our budgets are discussed with respect to the annual light/dark cycle in Taylor Valley lakes and alternative sources of DOC.

Site Description

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). Our 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). Lake Fryxell, at the eastern edge of the valley, has an approximate surface area of 7 km^2 and a maximum depth of 18 m. 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 of waters 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 photic zone (Priscu 1995; Spigel and Priscu 1996). The photic zone is defined as the layer where oxygenic photosynthesis is measurable: to 18 m in Lake Hoare and the east lobe of

Lake Bonney, to 17 m in the west lobe of Lake Bonney, and to 9 m in Lake Fryxell. 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 strongly stratified of the lakes, followed by Lake Fryxell, and then by Lake Hoare. Dissolved organic carbon in these lakes increases with depth, and in the highly stratified Lakes Fryxell and Bonney, the gradients are extreme (Figure 4.1). DOC concentrations range from 0.16 mg liter^{-1} in the surface waters of the lakes to 34 mg liter^{-1} in the hypolimnia. Photic zone and water column DOC concentrations (volume weighted and depth integrated) are most variable in Lake Bonney's east lobe and Lake Fryxell during the Austral summers of 1993 to 1997, whereas DOC concentrations in the west lobe of Lake Bonney and Lake Hoare are less variable (Figure 4.2). Although DOC varies over the season, a clear temporal trend is not apparent. Phytoplankton and bacterioplankton production (Figure 4.3) are greatest just below the ice cover (5m) in the spring, but production peaks at the chemocline become more pronounced than the 5 m peaks as the summer progresses (Lizotte et al. 1996, Priscu 1995, Takacs and Priscu 1998).

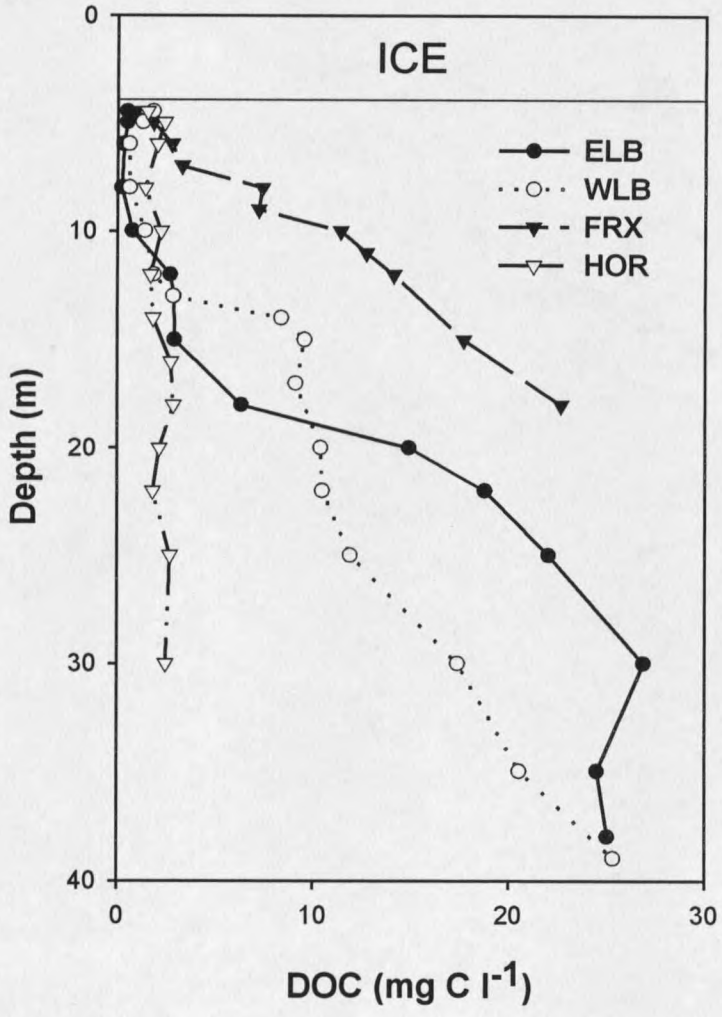

Figure 4.1. Dissolved organic carbon (DOC, mg C liter^{-1}) profiles in Lakes Fryxell (FRX), Hoare (HOR), east lobe Bonney (ELB), and west lobe Bonney (WLB) during early December, 1995.

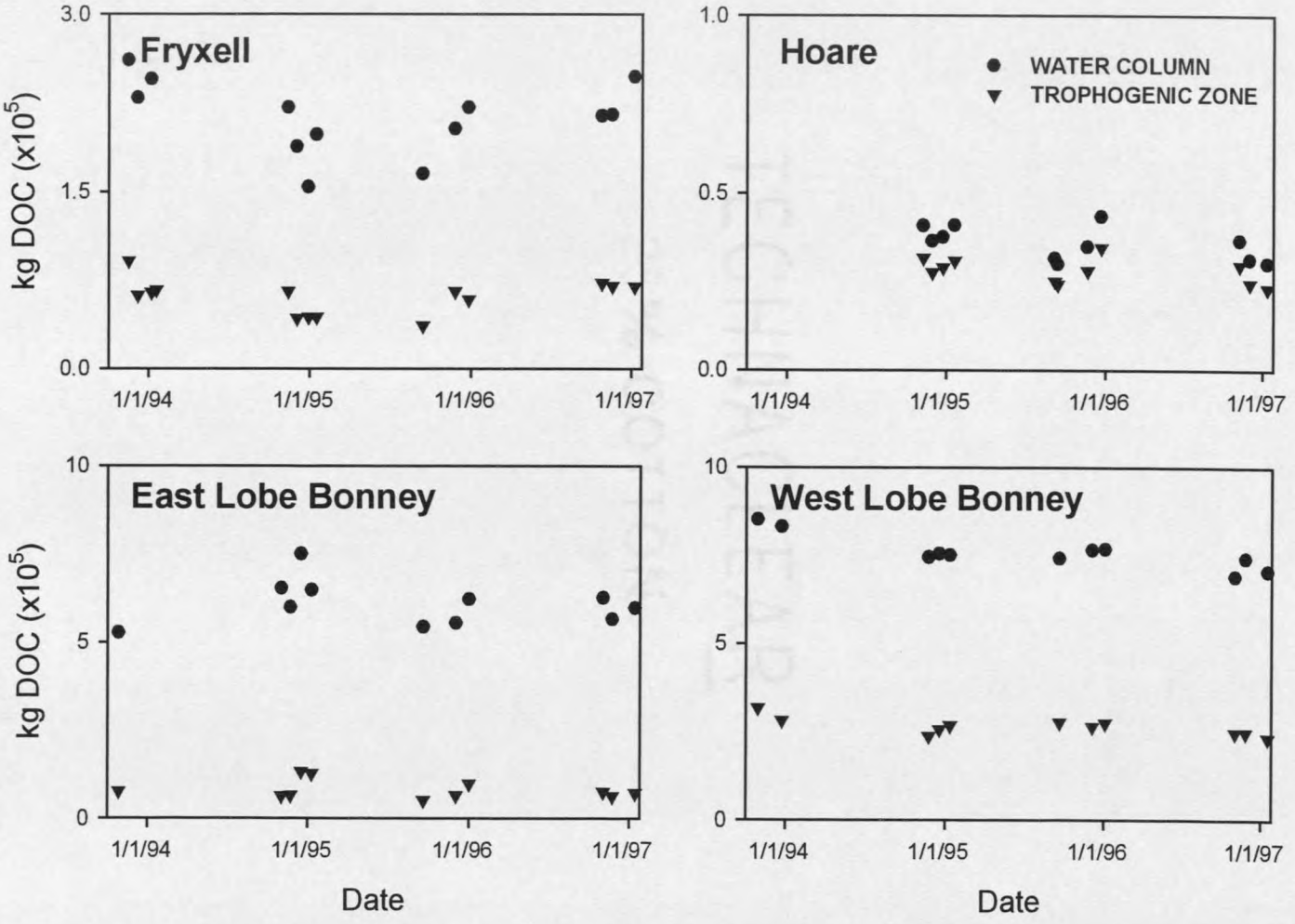

Figure 4.2. Volume weighted dissolved organic carbon (DOC, kg C x 10^5) integrated throughout the photic zone and the entire water column of Taylor Valley lakes during the Austral summers of 1993-1997. Note that DOC was not measured during the 1993-1994 sampling season in Lake Hoare.

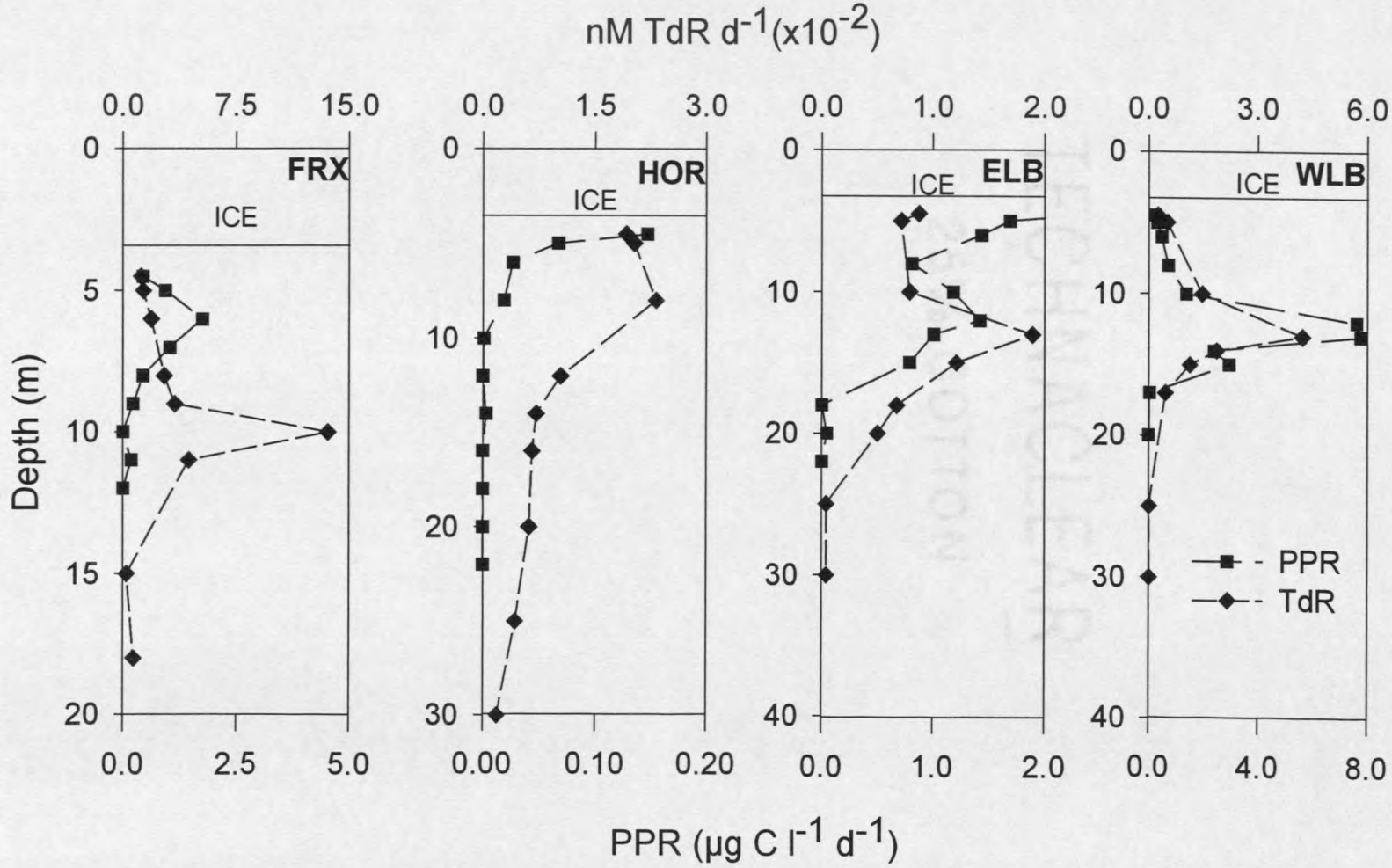

Figure 4.3. Primary productivity (PPR) and thymidine incorporation rate (TdR x 10^{-2}) in Lakes Fryxell (FRX), Hoare (HOR), east lobe Bonney (ELB), and west lobe Bonney (WLB) during early December, 1995. Note the different axes scales for the lakes.

The lakes are believed to be remnants of a former Lake Washburn that existed approximately 11,000 to 24,000 years before present (Denton et al. 1985). Lake levels have risen and fallen over the past 6,000 years, effectively concentrating the solutes in the bottom waters of the lakes. 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 and corrected for the reservoir effect are approximately 1200 years in Lake Hoare, and 8000 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 3000 years (Aiken et al. 1996).

The streams of the valley are fed by glacial meltwater when 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 highly labile (Vincent et al. 1993; Aiken et al. 1996) 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).

DOC Budgets

DOC budgets were calculated for each sampling season (October to January and September to January in 1995-1996) in the photic zone and water column of each of the lakes. Separate budgets were constructed for the east and west lobes of

Lake Bonney because of the lack of deep-water interchange between the two lobes. Limnological sampling was conducted at least once a month at 2 to 3 m intervals throughout the water column during the sampling season; all depths are reported from the piezometric water level (water level within the sampling hole). Discharge was computed for streams with continuous flow recorders as the sum of mean daily flows at the site recorded. Estimates for non-gauged streams were computed by comparing periodic discharge measurements at non-recording sites to discharge simultaneously occurring at neighboring gauged streams, and computing a ratio of discharges at the respective streams (Conovitz et al. 1998).

Seasonal bacterial DOC demand was estimated as the sum of volume weighted, depth integrated bacterial production and respiration during each sampling season. Bacterioplankton productivity was assayed by ^{3}H-thymidine incorporation (20 nM) on whole lake water in the dark at 1 to 4°C as described in detail previously (Takacs and Priscu 1998). The thymidine incorporation to cell conversion factor determined for these lakes during peak bacterial growth is at least two times greater than published values. Because we did not want to overestimate bacterial production in our budget, we used the more conservative value of 2.0×10^{18} cells mole^{-1} thymidine, which is the average of 97 studies (Ducklow and Carlson 1992). A carbon conversion factor of 11 fg C cell^{-1} was applied, which was determined from bacterial biovolume measurements in these lakes (Kepner et al. 1998; Takacs and Priscu 1998). We estimated bacterial respiration (BR) from the following relationship (del Giorgio et al. 1997), rearranged to solve for BR (mg C m^{-3} d^{-1}):

$$BGE = \frac{BP}{BP + BR}$$

where BGE represents bacterial growth efficiency, and BP is bacterial production (mg C m^{-3} d^{-1}). Bacterial growth efficiency was determined experimentally by measuring the amount of biomass produced compared to the amount of DOC consumed by bacteria grown on lake water during December, 1996. Opaque bottles were filled with lake water (130 ml) from 5 m and 9 m in Lake Fryxell, 5 m and 13 m in Lake Bonney, and 5 m and 14 m in Lake Hoare, and incubated *in situ* for approximately 14 days. Acridine orange stained bacterial cell were counted (>600 cells per sample, Hobbie et al. 1977; Takacs and Priscu 1998) and DOC was measured before and after incubation; BGE was calculated as the ratio of bacterial biomass produced to DOC consumed. Growth efficiency of lake bacterial assemblages averaged 0.07 (range = 0.01 to 0.11). Bacterial adhesion to the incubation bottles was not determined during these growth experiments, but has been reported to result in a 10% underestimation of bacterial growth efficiency (Kristiansen et al. 1992). Our BGE is within the range found in a comparison of freshwater and marine systems reported by del Giorgio et al. (1997). Bacterial respiration rates were compared to potential rates of community respiration (Priscu et al. In press) estimated from measuring respiratory electron system (ETS) activity (Packard et al. 1971 as modified by Kenner and Ahmed 1975). Bacterial respiration averaged 42, 46, 40, and 44% of community respiration in Lakes Fryxell, Hoare, east lobe Lake Bonney, and west lobe Lake Bonney, respectively, which is consistent

with estimates from other aquatic systems during both phytoplankton blooms and unproductive periods (30 to 70%, del Giorgio et al. 1997; Schwaerter et al. 1988).

DOC supply to the photic zone of the lakes was calculated as:

$$\text{DOC} = (\text{ECR} + \text{stream DOC input} + \text{upward diffusion})$$

where ECR represents phytoplankton extracellular release, and diffusion represents upward DOC flux across the chemocline into the photic zone during the sampling period. ECR was estimated to be 5% of volume weighted primary productivity (Sharp 1993) during the sampling season. Primary production was determined by measuring light-mediated uptake of ^{14}C-bicarbonate into particulate matter during 24 h *in situ* incubations (Lizotte et al. 1996). Dissolved inorganic carbon concentration, required for productivity rate calculations, was determined by infrared gas analysis of sparged lake water.

DOC diffused upward across the 18 m plane (9 m in Lake Fryxell) into the photic zone during each sampling season (kg C) was computed according to the following equation:

$$\text{DOC}_{\text{diffused}} = \left(D \frac{\partial C}{\partial z} \right) A * t$$

where D is the vertical diffusion coefficient ($m^2\ d^{-1}$) estimated for the lakes, $\delta C/\delta z$ is an average DOC gradient (kg C m^{-3} m^{-1}) across the bottom of the photic zone of each lake, A is the area (m^2) of the lake at the bottom of the photic zone, and t is the length of the sampling season (d). The vertical diffusion coefficient is assumed to be

near $1.8x10^{-4}$ m^2 d^{-1} in these lakes (Priscu 1996; Spigel and Priscu, 1998), however, this diffusion coefficient would overestimate DOC flux because of the molecular mass of DOC in these lakes (463-468 Daltons, Aiken et al. 1991). We estimated a vertical diffusion coefficient of $1.6x10^{-5}$ m^2 d^{-1} for DOC based on the Wilke-Chang correlation (Bird et al. 1960), which accounts for the molecular mass of the solute.

Stream DOC load (kg C) to the lakes was computed during each season by multiplying the average DOC concentration of each stream by its respective discharge during the sampling season. Stream DOC is highest during the beginning of the summer when streams first begin to flow (Aiken et al. 1996); hence, stream DOC load has been overestimated in this budget.

DOC supply to the entire water column of the lakes was calculated as for the photic zone, but DOC flux from the sediments was substituted for flux from the hypolimnion. Lake Fryxell sediment DOC is of a different composition than deep water DOC, and is a significant source of DOC to these lakes (Aiken et al. 1991; McKnight et al. 1993). DOC flux from the sediment was calculated as from the hypolimnion, but tortuosity and bioturbation were ignored because little is known about the composition of the sediments of these lakes, and their benthic fauna are primarily microbial. The diffusion coefficient ($1.6x10^{-5}$ m^2 d^{-1}) that we used for DOC is on the same order of magnitude applied in other pore waters (Jones and Bowser 1978). We estimated the DOC gradient at the bottom of the lakes from a second order polynomial function fitted to DOC concentration plotted against depth

(z) from the sediment (r^2=0.82-0.99, Figure 4.4). The first derivative of this function was evaluated at z=0 to estimate the DOC gradient at the bottom of each lake.

Photic zone DOC supply and bacterial demand during 1993 to 1997 is shown in Figure 4.5. Bacterial DOC demand was two to nine times greater than total DOC supply during the sampling season in Lakes Fryxell and Hoare, except during 1993-1994 when stream flow to these lakes was greater than in subsequent years. Bacterial carbon demand in Lake Bonney was 30 to 150% of DOC supply. Water column DOC demand and supply relationships were similar to photic zone results (data not shown), but differed in the relative contribution of the various sources of supply. Phytoplankton ECR and stream input provided the majority of DOC supplied to the photic zone of Lake Bonney, while stream input alone was most important in Lakes Fryxell and Hoare (Table 4.1). Diffusion from the hypolimnia and the sediments contributed 1 to 3% or 1 to 8% of DOC supply to the photic zone and the entire water column of the lakes, respectively. An exception was in Lake Fryxell, where DOC flux from the sediments contributed 51% of DOC supplied to the entire water column.

Table 4.1. Percent of total DOC supply contributed by phytoplankton extracellular release (ECR), stream input, and upward diffusion across the chemocline in the photic zone of Taylor Valley lakes. The data represent averages for all seasons considered (1993-1997). Data for the entire water column are included in parentheses.

Lake	ECR	Streams	Diffusion
Fryxell	27 (14)	70 (35)	3 (51)
Hoare	35 (35)	64 (64)	1 (1)
East Lobe, Bonney	57 (54)	40 (38)	2 (8)
West Lobe, Bonney	55 (55)	44 (44)	1 (1)

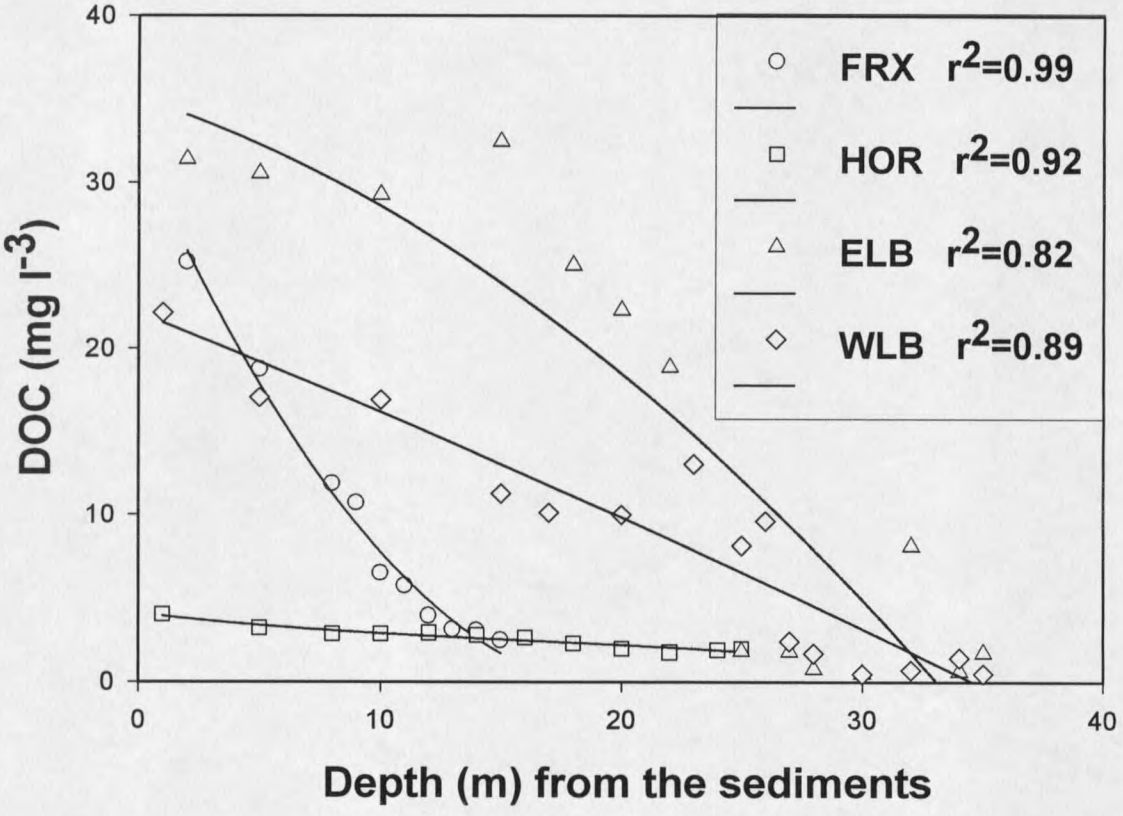

Figure 4.4. Dissolved organic carbon (DOC, mg l^{-3}) plotted against depth (m) from the bottom of Lakes Fryxell (FRX), Hoare, (HOR), and the east (ELB) and west (WLB) lobes of Lake Bonney. A second order polynomial was fitted to each lake's data to estimate the DOC gradient across the sediment-lake water interface.

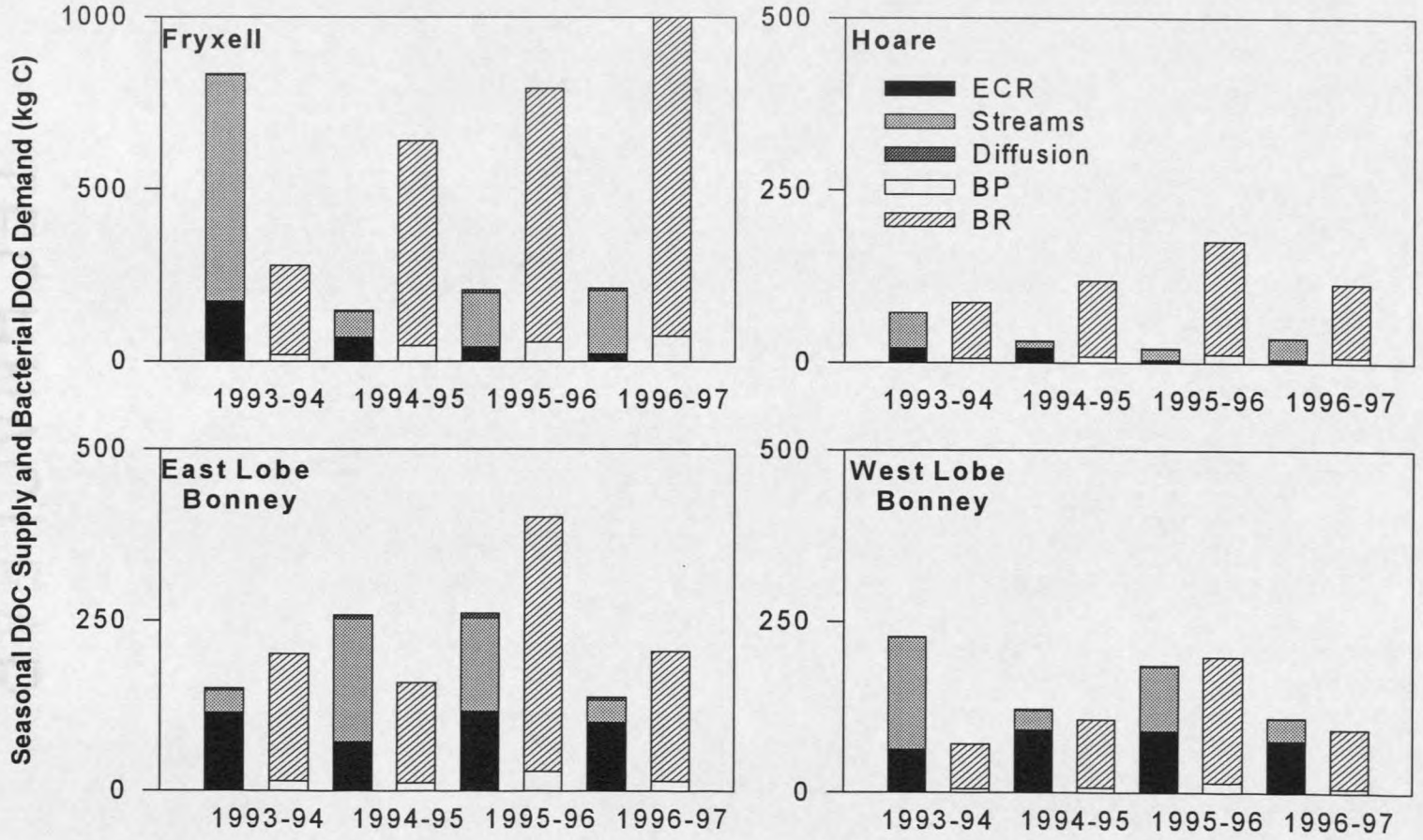

Figure 4.5. Photic zone DOC budget for Lakes Fryxell, Hoare, and Bonney during the 1993-1997 sampling seasons. DOC supply was estimated as the sum of phytoplankton extracellular release (ECR=5% of primary productivity), DOC input by streams during the sampling period (streams), and DOC diffused from the hypolimnia during the sampling season. DOC demand was estimated as the sum of net bacterial production (BP), and bacterial respiration (BR assumes that bacterial growth efficiency=7%) during the sampling season.

Discussion

Bacterial DOC demand in Lakes Fryxell and Hoare virtually always exceeded DOC supply, whereas DOC demand/supply relationships were more variable in Lake Bonney, alternating among seasons between net DOC demand and supply. This result occurred despite the potential problems (discussed above) that may underestimate DOC demand and overestimate DOC supply. Sensitivity analysis indicated that increasing supply or decreasing demand in the photic zone of the lakes by as much as 50% did not affect the general results of this budget. The budgets were affected most by a change in stream supply in Lakes Fryxell and Hoare, and a change in DOC flux in the east lobe of Lake Bonney. The west lobe of Lake Bonney budget was most sensitive to a change in ECR or stream input. An imposed 50% decrease in bacterial DOC demand was the only change that had any overall effect on the water column budgets. Our DOC budget clearly indicates that seasonal bacterial carbon demand is not balanced by DOC supplied from ECR, streams, and diffusion in Lakes Fryxell and Hoare, but is approximately balanced in Lake Bonney. Consequently, bacterial production in Lakes Fryxell and Hoare must be dependent upon alternate sources of DOC during the Austral summer, while Lake Bonney bacteria may primarily use newly supplied DOC.

Additional sources of organic carbon for the bacterioplankton include the bulk DOC pool, particulate organic matter, and DOC provided from the lysis of cells by grazers and viruses. Fulvic acids in Lakes Fryxell and Hoare were determined to constitute approximately 20% of the total DOC pool, and in Lake Fryxell were

determined to be low molecular mass throughout the water column (McKnight et al. 1991 & 1993). The low molecular weight fraction of the DOC pool is presumed to be relatively labile and important to bacterial production in these lakes. The turnover time of water column DOC ranged from 924 to 8159 years or 185-1632 years if only 20% of the DOC was assumed to be utilizable (Table 4.2). Additionally, the remaining fraction of the DOC pool, which is of a relatively higher molecular mass, is presumed to be less labile, but would offer an additional source of DOC to the bacteria. Although bacteria grow more efficiently on labile carbon, they are capable of growing on the less labile carbon pool at a slower rate (Wetzel 1995). Despite low decomposition rates of phytoplankton in Lake Bonney (Priscu 1992), bacteria can also supplement their DOC supply from decomposition of non-phytoplankton carbon, and potentially from DOC provided from cells lysed by viruses and bacterial predators.

Table 4.2. Average turnover times (years) of photic zone and water column DOC pools in Lakes Fryxell, Hoare, and Bonney. Values in parentheses represent turnover time (years) when only 20% of the DOC pool is assumed to be labile.

Lake	Photic Zone	Water Column
Fryxell	566 (113)	924 (185)
Hoare	751 (150)	946 (189)
East Lobe, Bonney	1627 (326)	8159 (1632)
West Lobe, Bonney	772 (154)	2210 (442)

Our budget is based solely on measurements from the sampling season because, owing to logistical constraints, we could not collect data from autumn and winter. Autumn is potentially a period of rapid phytoplankton growth because of increased light penetration through the ice cover (Fritsen and Priscu 1999). Based on

light driven primary production models, autumn phytoplankton growth can provide a considerable pulse of DOC to the bacterioplankton (Priscu et al. in press; Fritsen et al. In press), which would be important in an annual budget.

In addition to the lack of autumn-winter bacterioplankton data, we are presently unable to quantify biologically available DOC supplied from the bulk DOC pool, cell lysis, and annual decomposition. Hence, it is not clear whether bacterioplankton DOC demand is balanced by supply on an annual basis. However, alternate carbon sources would have to provide as much as 75% of total DOC supplied for bacterioplankton growth to balance summer DOC demand in Lakes Fryxell and Hoare. Conversely, Lake Bonney bacterioplankton production is apparently not carbon deficient during the period in which we sampled, and may continue to grow at summer rates into the autumn or winter on surplus DOC supplied during the summer. One scenario that would explain the differences in summer DOC budgets among lakes is bacterial inorganic nutrient limitation in Lake Bonney. Although phosphorus addition did not significantly stimulate bacterial production in this lake (Takacs and Priscu 1995), phosphorus addition did decrease alkaline phosphatase activity in the 0.2 to 2.0 μm fraction (Dore and Priscu 1996). Summer phosphorus limitation in Lake Bonney may prevent bacterioplankton DOC demand from exceeding DOC supply. Based on the bacterial DOC demand and summer DOC supply budgets presented here, there is no significant surplus of DOC supplied to Lakes Fryxell and Hoare during the sampling season that would enable bacterioplankton to sustain summer production rates into the winter. Winter

bacterioplankton growth must be dependent upon decomposition of particulate organic matter, the bulk DOC pool, and DOC released by lysed cells. Annual data collection will allow the ecological significance of the seasonal uncoupling of phytoplankton and bacterioplankton production in these lakes to be determined.

CHAPTER 5

BACTERIAL RESPONSES TO INORGANIC NUTRIENTS, TEMPERATURE, AND SALINITY: IMPLICATIONS FOR REGULATION OF BACTERIAL PRODUCTIVITY

Introduction

Heterotrophic bacteria provide a major link between primary producers and eukaryotic heterotrophs in aquatic systems. Bacteria regenerate inorganic nutrients and carbon by the decomposition of organic matter, making ammonium, phosphorus, and carbon dioxide available to primary producers (Fuhrman 1992). Additionally, bacterial biomass is a major carbon source for microzooplankton such as heterotrophic flagellates and ciliates (Azam et al. 1983). Microzooplankton and algae are consumed by the larger zooplankton, which are a food source for macroinvertebrates in freshwater and marine systems. Because of the significance of bacteria in aquatic systems, it is important to understand the factors regulating their productivity.

Aquatic bacterial production was previously believed to be carbon limited because of the perceived recalcitrance of oceanic and limnetic surface water dissolved organic carbon (DOC, Carlson and Ducklow 1996) and high bacterial affinity for inorganic nutrients (Currie and Kalff 1984). In systems that do not receive major inputs of allochthonous carbon (e.g., the open ocean), bacterial production is dependent upon phytoplankton production as a source of organic carbon (Meyer-Reil 1979; Fuhrman et al. 1980; Bird and Kalff 1984). However, the discovery of uncoupled phytoplankton and

bacterial production (Findlay et al. 1991; Billen 1990), and the ability of bacteria to use less labile DOC has directed research toward alternative factors that may regulate bacterial production (Zweifel 1993; Thingstad 1997).

Bacterial inorganic nutrient limitation has been invoked to explain the accumulation of relatively labile DOC in the surface waters of the ocean during periods of high primary productivity and low bacterial activity (Zwiefel et al. 1993; Cotner et al. 1997; Thingstad et al. 1998). Inorganic nutrient limitation was believed to primarily affect phytoplankton because bacteria have higher affinities than phytoplankton for inorganic nutrients and are known to sequester a large amount of nitrogen and phosphorus compared to other planktonic organisms (Bird and Kalff 1984). However, neither high nutrient affinity, nor the rapid turnover times of inorganic nutrients indicate conclusively that bacteria grow under nutrient sufficiency. Bacterial inorganic nutrient limitation has received increased attention during the past ten years and bacterial phosphorus deficiency has been shown in both freshwater and marine systems (Lewis and Morris 1992; Thingstad et al. 1998).

Seasonal variations in bacterial production are largely effected by temperature, but may be a constant constraint in consistently cold environments, such as the deep sea, the Southern Ocean, and polar lakes. Temperature regulates bacterial growth in any system because of its obvious effect on biochemical reaction rates, but is especially important in polar systems because bacterial N and P affinity has been shown to decrease with temperature (Nedwell and Rutter 1994). Bacteria have been isolated from cold regions that are either obligate psychrophiles (have optimum growth rates below 15°C,

but do not grow above 20°C) or simply cold tolerant strains, psychrotrophs (able to grow below 5°C, but optimal temperature is 20°C or higher, Morita, 1975). Despite the ability of psychrophiles to thrive in cold environments, psychrotrophs tend to dominate in these systems (Upton and Nedwell 1989; Zucconi et al. 1996).

Temperature in the water columns of Taylor Valley lakes range from -6 to 6°C. Low temperature and high salinity below the chemoclines of the lakes (Figure 5.1) presumably have an important role in restricting bacterial production. Owing to the high N:P ratios and low nutrient concentrations in the upper water columns of these lakes (Figure 5.2), inorganic nutrient dynamics may also be important in regulating bacterial production. This chapter presents information on how inorganic nutrients, temperature, and salinity affect bacterial growth in Lakes Fryxell, Hoare, and Bonney. Bacterial responses to DOC, inorganic nitrogen and phosphorus addition, temperature, and salinity were determined using lake water and bacterial strains isolated from the lakes. Additionally, relationships were explored among bacterial production and biological, chemical, and physical limnological variables measured in the lakes from 1993 to 1997 by correlation analysis.

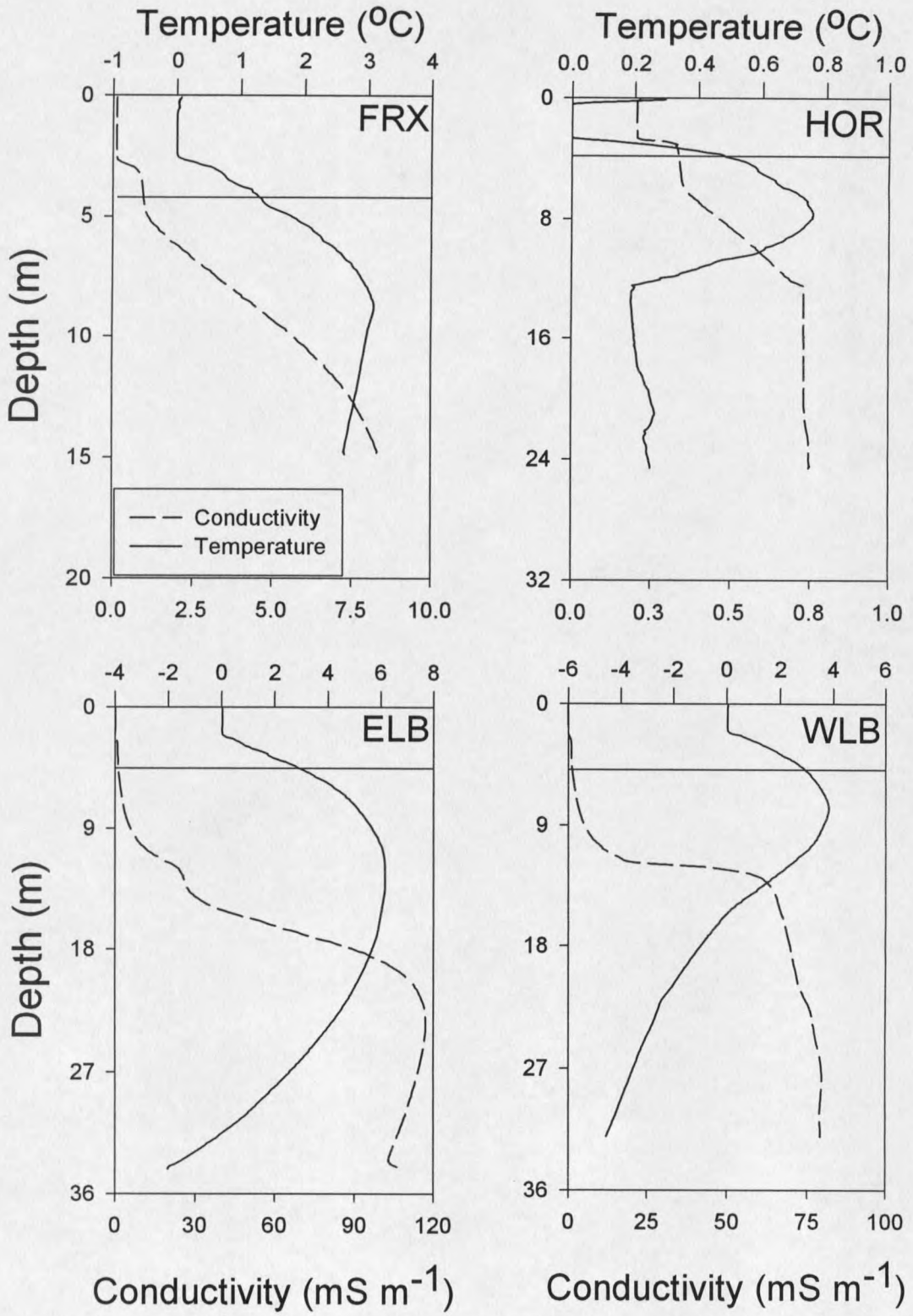

Figure 5.1. Conductivity and temperature profiles of Taylor Valley lakes during January 1995. Note the different axes scales among the lakes.

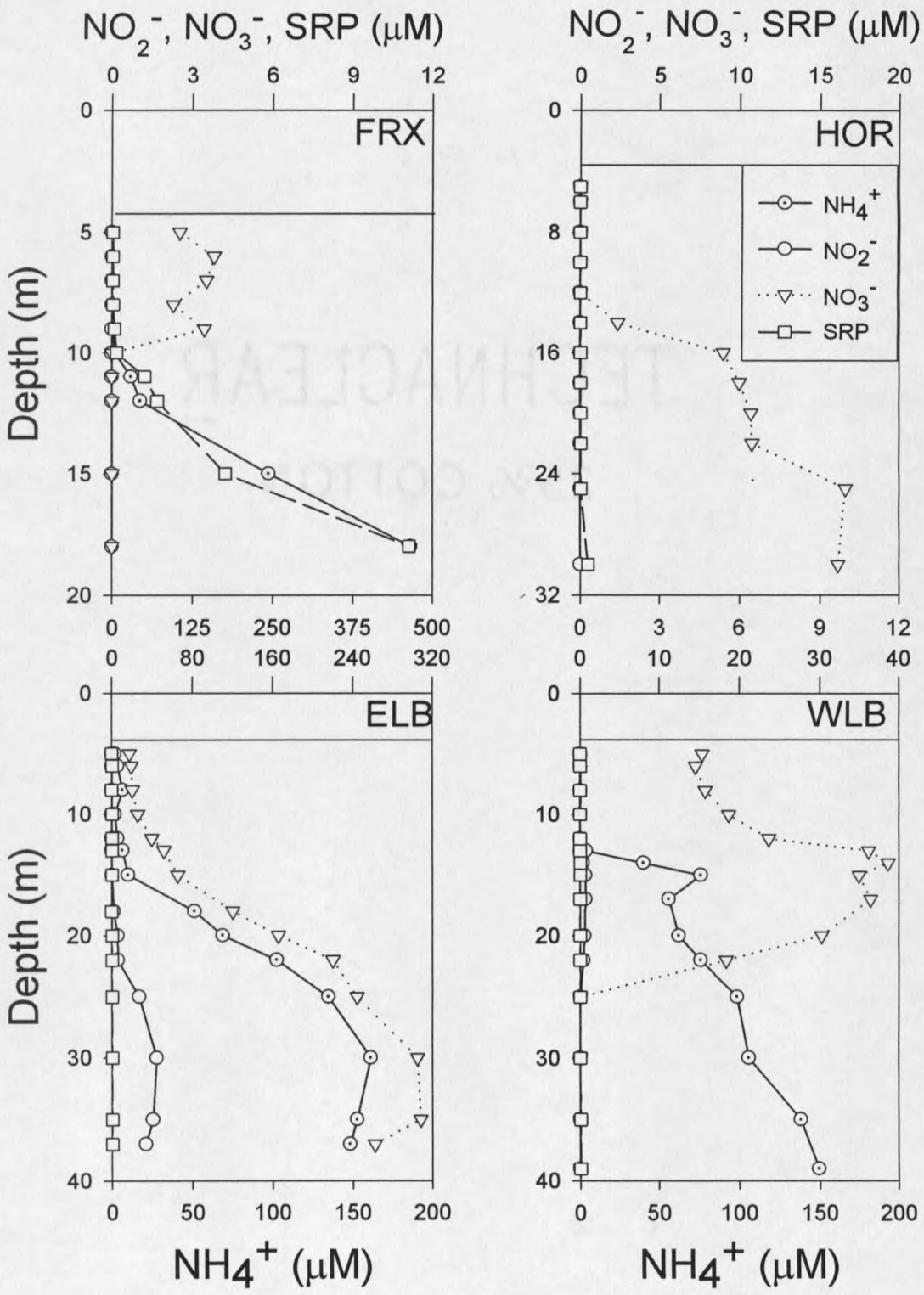

Figure 5.2. Nutrient profiles of Taylor Valley lakes during December 1994. Note the different axes scales among the lakes. SRP = soluble reactive phosphorus.

Methods

Nutrients

Nutrient Bioassays. The response of bacterial production to inorganic nutrient and organic carbon addition was tested on lake water from the primary productivity maxima of Lakes Fryxell (5 and 9 m), Hoare (5 and 14 m), and the east and west lobes of Lake Bonney (5 and 13 m) during November to December of 1994. Lake water (10 liters) was collected using a Niskin bottle through a hole drilled in the lake ice and decanted into an acid washed and lake water rinsed 20 liter polyethylene bottle. Samples were transported to a lakeside laboratory where 500 ml was aliquoted into each of 15 acid washed and lake water rinsed polycarbonate incubation bottles. Nutrients were added to the incubation bottles at the following final concentrations: 2 μM NH_4Cl, 20 μM Na_2HPO_4, 2 μM NH_4Cl + 20 μM Na_2HPO_4 and 200 μM glucose. Nutrient amendments were performed in triplicate and three unamended bottles served as controls. ^{3}H-thymidine was added to each incubation bottle at a final concentration of 10 nM. Bottles were incubated at 4°C in the dark and were subsampled every 24 h for 4 d, beginning with an initial sample immediately after thymidine addition. Two 10 ml samples from each bottle were pipetted into each of two 20 ml scintillation vials. Ten ml of 10% trichloroacetic acid was added to each subsample and stored at 4°C for two weeks before filtration. TCA treated samples were filtered onto 0.45 μm polycarbonate filters and rinsed three times with 5 ml 5% ice cold TCA. Filters were placed in clean scintillation vials with 20 ml of scintillation cocktail and counted by standard liquid scintillation spectroscopy.

Similar nutrient bioassays were performed during December of 1996 with the following modifications. Lake water was diluted 1:1 with 0.2 μm filtered water from the same depth to reduce the density of potential grazers. Treatments were incubated in duplicate, as opposed to triplicate, and ^{3}H-thymidine was not added directly to each bottle. Rather, three 10 ml aliquots (2 lives and 1 kill) were removed from each treatment bottle, and thymidine incorporation was measured separately during a 4 h incubation. This modification reduced the amount of isotope used and volume of radioactive waste generated in these experiments. Bottles were incubated for 24 h only to reduce the potential bottle effects that were evident at the end of the 1994 experiment. The bottles were subsampled at the beginning of the experiment, and after 12 and 24 h. At each time-point an incubation bottle was randomly selected for each treatment and discarded after subsamples were removed. This served to reduce the multicollinearity that results from repeatedly sampling the same population over time. Finally, in addition to the ammonium, phosphorus, and glucose additions that were tested in 1994, a peptone addition (1% final concentration) was included. Results from the 1994 experiment were analyzed by comparing the relative growth rates of the treatments to the control using a t-test (Steele and Torrie 1980). The 1996 data were analyzed by 2-way analysis-of-variance to determine if there were differences among the treatments and among the treatments at different timepoints over the course of the experiment.

Inorganic Nitrogen and Phosphorus Supply and Bacterial Demand. Nitrate, nitrite, ammonium, and soluble reactive phosphorus (SRP) were measured at least 3 times a year during the austral summers of 1993 to 1997 in lake water (100 ml) filtered through

precombusted Whatman GF/F filters. Samples were frozen in acid washed polyethylene bottles until analyzed with a LACHAT autoanalyzer using standard methods. Dissolved inorganic nitrogen was measured by the method of Parsons et al. (1984), except that it was necessary to dilute samples from depths where salinity exceeded seawater with deionized water to seawater levels or lower. Recovery of internal nutrient standards ranged 90 to 110%. SRP was determined by the molybdate/potassium antimonyl tartate method (Downes 1978), except the reduction step to prevent arsenate interference was omitted (Sharp 1993). Bacterial inorganic nitrogen and phosphorus demand were estimated using a molar C:N:P ratio of 45:9:1 determined in both batch cultures and marine bacteria (Goldman et al. 1987; Fukuda et al. 1998). Phytoplankton N and P demand were determined using primary productivity measurements in the lakes (see below) and the molar Redfield ratio (106:16:1, Redfield 1958).

Bacterial Responses to Temperature and Salinity

Bacterial Community Response to Temperature. Lake water (10 ml) from the primary productivity maxima of the lakes was incubated in the dark with 20 nM ^{3}H thymidine for 20 h at four temperatures ranging from 4 to 20 °C during September 1995. However, there was only one incubator available at the field camp, so three temperatures (1, 10, and 20°C) were effected by incubating samples in various places around the laboratory; temperatures varied +/- 5° during the 10 and 20°C incubations. The experiments were repeated during 1996 using incubators and water baths for 2 and 30°C. Samples were acclimated to their respective incubation temperatures for two hours before adding 20 nM

^{3}H-thymidine and incubating for 2 h. Ten ml of 10% TCA was added to all samples at the end of incubation. Samples were filtered and radioactivity on the filters was quantified as described above.

Temperature and Salinity Optima of Bacterial Strains Isolated from the Lakes. Bacteria were isolated from Lakes Fryxell, Hoare, and Bonney during 1995 and 1997, however two different approaches were taken between seasons. During 1995, 0.5 to 1 ml of lake water from depths throughout the water columns of the lakes was spread onto nutrient agar plates and incubated at room temperature, which varied from 15 to 20°C. Colonies were picked, streaked to purity, and frozen in nutrient broth with 15% glycerol. Elevated incubation temperatures used in 1995, relative to *in situ*, may have selected for psychrotrophic (as opposed to psychrophilic) organisms, if not mesophillic allochthonous strains. Therefore, in 1997 bacteria were isolated at 4°C, and all media, plates, and instruments were chilled before use, with the exception of the spreader.

Bacterial isolates from the primary productivity maxima of the lakes were randomly chosen from the 1995 and 1997 collections, and grown in duplicate in nutrient broth at four temperatures ranging from 4 to 37°C. Growth was determined in the cultures by periodically measuring optical absorbance (660 nm) and growth rates were calculated as the slope of log transformed optical absorbance data during exponential growth of the cultures. Salinity optima were determined for bacterial isolates chosen from various depths and salinities of the Lakes Fryxell and Bonney. Isolates were incubated at 10°C in nutrient broth prepared with various concentrations of NaCl ranging from 0 to 15%, which are approximately representative of the range of salt concentrations

in the lakes (Spigel and Priscu 1996). Growth rates of the isolates were determined as described for the temperature experiments.

Statistical Analyses

Model Development. As part of the limnological component of the LTER, approximately 21 different limnological variables are measured in Lakes Fryxell, Hoare, and Bonney at least three times during the austral summer. These data may be explored by multiple linear regression anlyses to determine if bacterial production dynamics in the lakes are related to temporal and vertical variations in biological, physical, and chemical variables. Data were analyzed for the trophogenic zones (the depth interval that extends from just below the ice cover to 9 m in Lake Fryxell, 14 m in Lake Hoare, and 13 m in Lake Bonney), the hypolimnia (the depth interval that extends from the bottom of the lakes to the bottom of each lake's trophogenic zones), and at the primary productivity maxima of the lakes. Two statistical approaches were used in these analyses. The first approach developed biologically intuitive models that were tested in the trophogenic zones and hypolimnia of the lakes to determine how well bacterial production could be explained by relevant independent variables. Three different models were tested and are shown in Table 5.1. Note that primary productivity (PPR) and chlorophyll-*a* were not incorporated into the same models because they are correlated ($r = 0.38\text{-}0.70$, $p < 0.0001$). The second approach used stepwise regression, which is an automated regression procedure that statistically determines the best model to describe variation of a dependent variable (bacterial production) using a selection of independent variables.

However, stepwise regression does not correct for multicollinearity among independent variables.

Limnological Sampling. Limnological sampling was conducted at least once a month during the austral summer (October to January) of 1993-1997, including the winter-spring transition (September to October) during 1995-1996. Water was collected using a Niskin bottle at 2 to 3 m intervals throughout the water column, as measured from the piezometric water level (water level within the sampling hole).

Bacterial production was measured by ^{3}H-thymidine incorporation as described above and in previous chapters. Primary production was determined by measuring uptake of ^{14}C-bicarbonate into particulate matter during 24 h *in situ* incubations (Lizotte et al. 1996). Dissolved inorganic carbon concentration, required for productivity rate calculations, was determined by infrared gas analysis of sparged lake water. Chlorophyll-*a* (CHL) was measured fluorometrically (Holm-Hansen et al. 1965) in lake water filtered onto Whatman GF/F glass fiber filters extracted in 90% acetone overnight at 4°C (Lizotte and Priscu 1998; Priscu 1995).

Particulate organic carbon and nitrogen (POC and PON) were determined in samples filtered onto pre-combusted Whatman GF/F filters at low vacuum (<0.3 atm) by flash-combustion gas chromatography (Carlo Erba model 1500 elemental analyzer). Dissolved inorganic nitrogen and phosphorus were determined as described in the Nutrient section above. DOC was measured in filtered lake water using an Oceanographic International model 700 carbon analyzer (McKnight et al.,

1991 & 1993). Dissolved oxygen and temperature were measured *in situ* using a YSI Model 58 system.

Results

Bacterial production was not stimulated significantly ($p > 0.05$) compared to the control by the addition of nutrients in any of the lakes during 1994 and 1996. Figure 5.3 shows the two types of growth curves that were measured during 1994 and demonstrates the lack of response to the four nutrient amendments that were tested. However, nitrogen and phosphorus supply demand relationships (Figure 5.4 and 5.5) indicate that there is a potential for N and P deficiency in these lakes when bacterial and phytoplankton (Total) inorganic nutrient demand are summed. Nitrogen deficiency was especially evident when ammonium supply, which has been shown to be the preferred source of nitrogen by phytoplankton in Lake Bonney (Woolston 1994), was considered alone.

Response to temperature of bacterial communities from the primary productivity maxima as measured by ^{3}H-thymidine incorporation in whole lake water indicated that bacterial growth was depressed at *in situ* temperatures relative to incorporation at the temperature optima. Thymidine incorporation at 1°C was reduced 20 to 67% compared to samples incubated at apparent temperature optima between 10 to 20°C (Figure 5.6). When the experiment was repeated in 1996 under more controlled conditions, the samples for 9 m in Lake Fryxell showed a psychrophilic response to temperature. With the exception of two strains, growth experiments of bacterial isolates grown at different temperatures showed a psychrotrophic response to temperature; that is, the strains were

all capable of growth below 15°C, but had optimal growth temperatures above 20°C (Figure 5.7). The prevalence of psychrotolerant strains of bacteria over obligate psychrophiles has been reported by other researchers (DeLille and Perret 1989; Ferroni and Kaminski 1980; Upton and Nedwell 1989; Zucconi et al. 1996). Surprisingly, the two true psychrophiles were isolated at room temperature, however, the isolates tested represent only 1% of the total number of strains isolated at 4°C. Bacterial isolates grew optimally at NaCl concentrations ranging from 0 to 5% (Figure 5.7), but no attempt was made to culture these organisms at ambient salinities. Nutrient broth and agar has a NaCl concentration of 0.5%. However, Ward and Priscu (1997) reported similar salinity optima (2 to 5%) for three denitrifiers isolated from Lake Bonney on media containing *in situ* salt concentrations.

Three models that used combinations of PPR, CHL, DOC, inorganic nutrients, and temperature to describe bacterial production were developed for these lakes (Table 5.1). The models were most useful in explaining Lake Fryxell bacterial production, and were suitable in Lakes Hoare and Bonney to a lesser extent. The best fit model differed among the lakes and within the lakes depending on the depth interval tested. Best fit model parameters for the water column of each of the lakes are shown in Table 5.2. Standardized parameters indicate that PPR or CHL were the most important variable in these models, except in Lake Hoare where SRP was most important. Bacterial production in all the lakes was negatively related to DOC, N, and P, except in Lake Hoare where bacterial production was positively related to SRP. The least important

variable in the models was either SRP (Lake Fryxell and the west lobe of Lake Bonney) or NH_4^+ (Lake Hoare and the east lobe of Lake Bonney).

Stepwise regression analysis resulted in different models to explain bacterial production in the lakes, but in general, PPR, CHL, NH_4^+, NO_3^-, SRP, and Temperature accounted for a higher degree of variation in bacterial production (Table 5.3). Note that correlated variables such as PPR and CHL or NO_3^- and $N0_2^-$ were selected in the final model because the automated stepwise procedure does not account for autocollinearity.

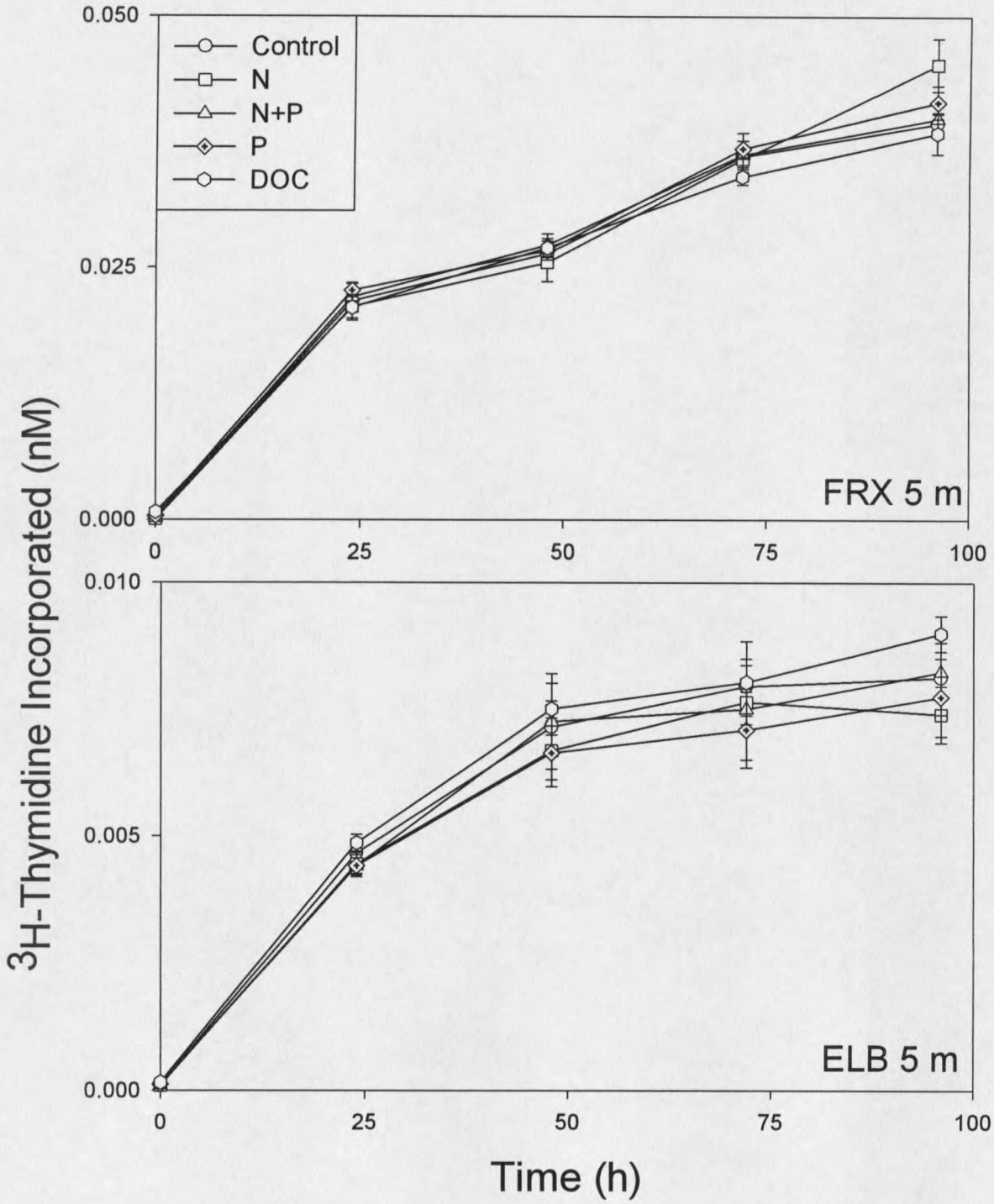

Figure 5.3. ^{3}H-thymidine incorporation (nM) in 5 m water from Lake Fryxell (FRX) and the east lobe of Lake Bonney (ELB) enriched with 20 μM NH_4Cl, 20 μM NH_4Cl and 2 μM Na_2HPO_4, 2 μM Na_2HPO_4, and 200 μM glucose.