

BACTERIOPLAKTON DYNAMICS IN STRATIFIED LAKES OF THE TAYLOR
VALLEY, ANTARCTICA DURING THE TRANSITION TO POLAR NIGHT

by

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ABSTRACT

Limnological research on the lakes of the McMurdo Dry Valleys (MCM), Antarctica, is typically carried out during the austral spring-summer (October-January) when logistical support is readily available; the current study marks the first sampling effort during the summer-fall transition (January-April). Sampling during the darkness of winter is logistically difficult and expensive, and my study is an important step towards understanding the year-round ecology of the dry valley lakes. Bacterial productivity, measured as protein synthesis and DNA replication, and bacterial cell numbers were measured 10-12 times between October 2007 and April 2008 in Lakes Fryxell (FRX) and the east and west lobes of Lake Bonney (ELB and WLB). Lake Fryxell was the most productive (bacterial) lake on average by an order of magnitude (average = $1.24 \text{ mg C m}^{-2} \text{ d}^{-1}$; range = 0.00 to $3.29 \text{ mg C m}^{-2} \text{ d}^{-1}$), and also contained the greatest bacterial biomass ($\sim 10^6 \text{ cells ml}^{-1}$) by 1 to 3 orders of magnitude. If bacterial production were directly linked to organic carbon supplied by photosynthetic primary production, a decrease in bacterial production would be expected during the sunset; however, no statistically significant change in bacterial production ($\alpha=0.05$) was observed during the summer-fall transition. A distinct decoupling of bacterial protein production and DNA replication was detected in FRX and ELB of the lakes as the season progressed, and was present in WLB throughout the season, indicating either a shift towards a lower growth-rate in response to decreasing light or nutrient supply, or a mechanism for dealing with the perennially low temperatures, low light, and nutrient poor conditions in the lakes. Overall, it appears that bacterial communities remain active during the darkness of winter, when the lakes enter a period of “net heterotrophy”, which cannot be sustained unless the carbon balance of the TV lakes is reset by climatic events.

INTRODUCTION

The perennially ice-covered lakes of Taylor Valley (TV), Antarctica represent an isolated, relatively pristine environment in which to study a microbial ecosystem, lacking top down influences from metazoans. The McMurdo Dry Valleys (MCM), which include TV, have a combined area of $\sim 4200 \text{ km}^2$ and comprise the largest ice-free area of the Antarctic continent ($\sim 1\%$). The region is a cold desert, with annual mean temperatures ranging from approximately -14°C to -30°C (19) and annual precipitation not exceeding 50 mm water equivalent (22). The landscape is characterized by a composite of glaciers, dry soils, ephemeral streams, and perennially ice-covered lakes. TV contains four major lakes, Fryxell, Hoare, and the East and West Lobes of Lake Bonney. My study focuses on Lakes Fryxell and Bonney owing to their strong vertical chemical stratification. The lakes provide the only year-round liquid water habitat in the TV and one of the few places where liquid water exists throughout the year on the Antarctic continent. The permanent ice cover, which ranges from approximately 3 to 5 m thick, inhibits wind-induced mixing of the water column and restricts atmospheric gas exchange (74). The lack of wind-driven turbulence, along with low advective stream flow allows stable water columns with strong physicochemical gradients to persist. These vertical gradients provide organisms with a variety of nutrient, temperature and redox gradients allowing planktonic organisms such as cyanobacteria, bacteria, flagellates, and ciliates to exist in discrete layers in the water column (67). Roberts, et al. (66) suggested that the lake ecosystems are subject to strong bottom-up control, meaning that physicochemical

properties effect greater control over microbial distribution patterns and activities than top-down controls such as predation and competition.

The lakes contain a complex microbial food web, which includes eukaryotic microalgae, cyanobacteria, heterotrophic bacteria, chemolithoautotrophic bacteria, members of the Archaea, mixotrophic, and heterotrophic flagellates, ciliates, and rotifers (23, 33, 43, 44, 48, 62, 70, 76). Higher organisms, such as macrozooplankton and fish, are absent from the TV lakes, making them an excellent locale for studying microbial processes and ecosystems. Limnology of the lakes has been studied since the 1957-1958 International Geophysical Year (3, 4) and integrated long-term studies have been carried out since 1993 as part of the McMurdo Long-Term Ecological Research Program (MCM LTER; <http://www.mcmlter.org/>). A majority of studies of the planktonic communities of TV lakes have focused on phytoplankton dynamics (23, 43, 44, 48, 62) or cultivation of planktonic organisms (34, 59, 69, 70), with a few focusing on bacterioplankton communities (66, 76, 78, 79). Takacs and Priscu (76) carried out a comprehensive characterization of bacterioplankton production and biomass in the TV lakes, which spanned four seasons (1994-1998) and included one winter-summer transition. Takacs and Priscu (76) used ^3H -thymidine incorporation experiments to identify vertically stratified patterns in bacterial productivity in the water columns of each lake. All of the lakes had a deep peak in bacterial productivity proximate to the chemocline, which became more pronounced later in the summer, and the highest rates of bacterial productivity occurred directly under the ice cover throughout the season. Similar patterns were noted for primary productivity and chlorophyll-*a* profiles.

The austral summer field season, when limnological research is traditionally conducted in TV, typically lasts from October to January, a period of 24-hours of daylight. Irradiance is relatively constant during these periods (the three lake average for a clear, 24 hour period is $1230 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; range 1160 – 1300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and photosynthetic primary productivity, together with stream input serve as major contributors of organic carbon to the lake ecosystems (79). With their seasonally separated light-dark cycles (4 months of light and 4 months of dark, flanked by 2 months of twilight) and corresponding bimodal differences in trophic dynamics, the MCM lakes provide an interesting intermediate between temperate ecosystems subject to a typical diel cycle, and other extreme environments such as sub-glacial systems, the deep-subsurface, deep-ocean, and hot spring environments, which function entirely without autochthonous carbon inputs from photosynthetic organisms (10, 42, 51).

Plankton responses to seasonal change in the TV lakes have been previously studied during the winter-spring transitions of 1991 and 1995. Lizotte et al. (43) and Priscu et al. (60) found that distinct phytoplankton populations (based on chlorophyll-*a* and primary productivity measurements) developed sequentially from shallow to deeper water as spring progressed towards summer, implying positive relationships between phytoplankton population growth and irradiance. McKnight et al. (48) used automated sampling devices to collect preserved phytoplankton samples from Lake Frxyell during the summers and winters of 1990 and 1991, and found that vegetative algal cells overwintered in the lake. One chlorophyte (*Stichtococcus sp.*) was present during the winter but absent during both summers, indicating heterotrophic growth during the

winter. Takacs and Priscu (76) used ^3H -thymidine incorporation assays to determine that bacterioplankton were active in TV lakes during the winter-spring transition of 1995 following 4 months of complete darkness and no phytoplankton photosynthesis. Takacs and Priscu also found cell numbers to be similar during summer and during the winter-spring transition.

Studies focusing on phytoplankton seasonal dynamics in the lakes of the Vestfold Hills in coastal Wilkes Land, Antarctica, as well as in TV, have also increased our understanding of the survival strategies employed by planktonic organisms during the winter in Antarctic lakes. Cryptophytes in lakes Fryxell and Hoare are known to graze on bacterial cells, and this strategy may allow cryptophyte populations to enter the short austral summer ready for active photosynthesis (39), which may be critical to their success (60). Bell and Laybourn-Parry (7) showed that planktonic organisms that enter the more productive summer months with populations that are already actively growing may be more successful during the summer. Cyst-forming phytoflagellates have been detected in Ace Lake, a seasonally ice-covered saline lake in the Vestfold Hills, and cyst-forming capability may be important for the persistence of phytoflagellate communities through the winter months (7). Bacterial communities in Crooked Lake, a large, oligotrophic, freshwater lake in the Vestfold Hills were found to actively incorporate ^3H -thymidine and ^{14}C -leucine throughout the winter, although growth was unbalanced and incorporation rates were depressed relative to summer (40).

My study presents the first data on bacterioplankton dynamics collected from the permanently ice-covered lakes of the Taylor Valley during the transition to the 24 hours

of darkness of the polar night. The overarching hypothesis of my study is that bacterial productivity decreases during the sunset as primary productivity ceases. My alternative hypothesis is that bacterial productivity does not cease in concert with primary productivity; rather, the heterotrophic bacterial communities adapt, through changes in physiology, to the loss of photosynthetic carbon. To test my primary hypothesis, I experimentally measured rates of bacterial and primary productivity throughout the season using radioactive tracers and concurrent physical and chemical characteristics of the lakes during the polar night transition.

METHODS

Samples were collected and analyzed according to procedures established by the Priscu Research Group as part of the McMurdo Long-Term Ecological Research Program (MCM LTER), which can be found at: http://mcmlter.org/queries/lakes/lakes_home.jsp. Procedures are summarized briefly below.

Study Site

The McMurdo Dry Valleys (MCM) are located below the Antarctic Circle, between $76^{\circ}30'$ – $78^{\circ}00'S$ and $160^{\circ}00'$ – $165^{\circ}00'E$. For this study, I sampled three lake basins in the TV: Fryxell (FRX), East Lobe Bonney (ELB), and West Lobe Bonney (WLB) (Figure 1). A sill at 13 m divides Lake Bonney into east and west lobes (74).

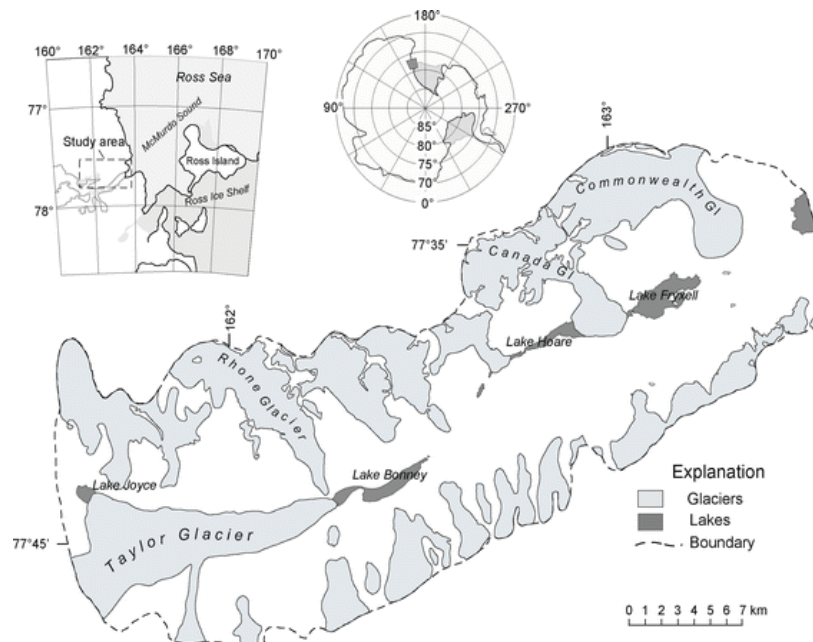


Figure 1. Map of the Taylor Valley (TV) showing the locations of Lake Fryxell and Lake Bonney. Map by Green and Lyons (27).

There is exchange between ELB and WLB in the fresher waters above the sill; however, beneath the depth of the sill, the two lobes are geochemically distinct, and are therefore discussed as separate lakes (74).

I partitioned the water columns of all three lakes into an epilimnion, metalimnion, and hypolimnion based on oxygen concentrations, where each lake has an O₂ saturated or supersaturated epilimnion (equilibrium atmospheric saturation of O₂ at 0°C is 14.1 mg L⁻¹; (87)), an oxycline (metalimnion), and an anoxic or sub-oxic hypolimnion (Table 1; seasonal O₂ profiles in Appendix B.3). There are also layers between these where there are changes in temperature and conductivity that may also be physiologically relevant (74).

Table 1. Physical parameters relevant to each lake. Depth, oxygen, conductivity, and temperature are given as averaged ranges over the depths of the epilimnia, metalimnia and hypolimnia.

FRX (19 m)*				
	Depth (m)	O₂ (mg L⁻¹) (x 10⁵)	Conductivity (mS cm⁻¹)	Temperature (°C)
Epilimnion	5 – 9	7.5 – 9.9	0.7 – 3.2	1.8 – 2.6
Metalimnion	10 – 12	2.1 – 3.5	4.2 – 5.6	2.2 – 2.5
Hypolimnion	below 12	BD	6.7 – 8.5	2.1 – 2.2
ELB (39.2 m)*				
Epilimnion	4 – 13	3.1 – 3.7	0.9 – 15.2	1.5 – 4.6
Metalimnion	14 – 22	1.4 – 1.9	59.4 – 65.1	4.5 – 4.8
Hypolimnion	below 22	0.002 – 0.07 **	108.0 – 109.0	-4.9 – (-2.0)
WLB (41.5 m)*				
Epilimnion	4 – 14	2.7 – 5.0	1.0 – 31.7	0.6 – 1.2
Metalimnion	15 – 17	0.1 – 0.4	59.4 – 65.1	-0.7 – 0.03
Hypolimnion	below 17	BD	66.7 – 79.6	-4.4 – (-1.0)

* Lake (average depth of water column below sampling hole).

** mg O₂ L⁻¹ x 10¹⁰ instead of x 10⁵.

BD = below detection

The oxycline was chosen as the dividing point of the water column because the depth of the oxycline (relative to the piezometric water level in the sampling hole) did not change during the course of the season, and oxygen concentration has a role in determining the diversity and metabolic function within the lakes. Biological and physical processes both control the oxygen profiles within these lakes (16, 86). Studies in other aquatic and soil systems (18, 45, 68), as well as in FRX (Mikucki et al., unpublished), showed that distinct shifts in microbial community structures are correlated with gradients in oxygen concentrations.

Sampling Procedures

Briefly, 30-50 cm diameter sampling holes were made in the ice cover of each lake over the deepest portion of the lake using a combination of drilling and melting. Lake depths were measured from the piezometric water level in the sampling hole to the bottom of the lake. Sample depths were also measured from the piezometric water level. Each lake was sampled four times between Nov 2007-February 2008 (summer) and then every 1-2 weeks during March and April 2008 (fall). Routine sampling parameters and depths for each lake are shown in Appendix A.2. For specific sampling dates and depths, see Appendix A.1.

Samples were collected using a DI water and lake water rinsed Niskin bottle with teflon-coated components and decanted through silicon tubing into acid-washed and sample-rinsed amber HDPE sample bottles. Samples were stored near 4°C in insulated containers until assays were performed at lakeside laboratories (less than 4 hours).

Physical and Chemical Measurements

Physical (light and temperature) and chemical (conductivity, macronutrients, dissolved organic carbon, and dissolved oxygen) parameters were monitored over the water column of each lake by the MCM limnology group according to the schedule mentioned above.

Temperature and Conductivity

Temperature and conductivity were logged at approximately 10 cm intervals using a SBE 25 Sealogger CTD (Seabird Electronics, Bellevue, WA, USA) equipped with a SBE 03-01/F temperature sensor (-5 to +35°C detection limits) and a SBE 04C conductivity sensor (0 to 7 S m⁻¹ detection limits). The instrument was calibrated at Seabird Electronics before use.

Photosynthetically Active Radiation (PAR)

Underwater PAR was monitored during PPR incubations using a LI-COR LI-193SA spherical quantum sensor, and logged with a LI-COR Model 1000 data logger (LI-COR Biosciences, Lincoln, NB). The sensor was placed 10 m below the surface water depth in the PPR incubation hole in ELB and WLB, and at 7 m in FRX.

Chemistry

Macronutrients, dissolved organic carbon (DOC), and dissolved oxygen (DO), were measured at selected depths during each sampling period. Macronutrient and DOC sample analyses were carried by the MCM LTER analytical group. DO was measured in the field by MCM LTER limnology field team members.

Samples for inorganic macronutrients (NO_3^- -N, NO_2^- -N, NH_4^+ -N, and soluble reactive phosphorous (SRP)) were filtered through combusted and acidified 25mm GF/F filters into acid washed, 125ml HDPE bottles and stored at -20°C until analysis.

Macronutrients were analyzed in the Crary Laboratory at McMurdo Station using a Lachat QuikChem AE Automated Ion Analyzer (Lachat Instruments, Hach Company, Loveland, CO). Method detection limits for each of the analytes are: NO_3^- -N = $0.4 \mu\text{g L}^{-1}$; NO_2^- -N = $0.3 \mu\text{g L}^{-1}$; NH_4^+ -N = $0.7 \mu\text{g L}^{-1}$; SRP = $0.3 \mu\text{g L}^{-1}$.

Samples for dissolved organic carbon (DOC) were filtered through combusted and acidified 25mm GF/F filters into acid washed and combusted 125ml amber borosilicate glass bottles with Teflon lined caps, acidified to $\sim\text{pH } 2$ with 6N HCl, and stored at 4°C until analysis. DOC was analyzed using a Shimadzu TOC-V Series (Shimadzu Scientific Instruments, Columbia, MD).

Samples for DO analysis were decanted directly from the Niskin bottle tubing into 20ml scintillation vials, fixed with Mn^{2+} and alkaline-iodide-azide, and capped with no headspace. Care was taken to fill each vial from the bottom to avoid gas entrainment. DO was detected using the azide modification of the mini-Winkler titration (limit of detection = $0.09 \text{ mg O}_2/\text{L}$).

Biological Parameters

Biological parameters were monitored over the course of the season at the time points shown in Appendix A.1. Specific parameters measured were: light dependant primary productivity, chlorophyll-*a*, heterotrophic bacterial productivity, and bacterial

cell numbers. Heterotrophic bacterial productivity methods validations were conducted over the 2009-2010 sampling season.

Primary Productivity (PPR)

Primary productivity was measured by incubating samples collected from discrete photic depths *in situ* with ^{14}C -labelled bicarbonate for 24 hours, in 150 ml clear (2) and darkened (1) polycarbonate bottles. Previous experiments in the TV lakes have shown that cellular lipid, polysaccharide, and protein macromolecules are equilibrium labeled after 24 hours of incubation, so PPR refers approximately to net primary productivity (60, 63). Following incubation, samples were transported in the dark to lakeside laboratories, and incubations were terminated by filtration onto pre-combusted GF/F filters (Whatman Ltd.) in the dark. Unincorporated ^{14}C -labelled bicarbonate was removed by acidification of filters, which were then dried in a fume hood at 60-80°C. Radioactive label retained in the cellular residue on the filters was counted on a calibrated Beckman LS6000 scintillation counter within 3 months. Disintegrations per minute (DPM) from dark bottles were subtracted from the average DPM from the light bottles to correct for chemosynthetic ^{14}C -bicarbonate incorporation, and incorporation into phytoplankton from anapleurotic reactions.

In-situ Spectral Fluorescence

A submersible spectrophotometer (bbe Moldaenke Fluoroprobe®) was used to provide high density (~10 cm) vertical profiles of total chl-*a* ($\mu\text{g chl-}a \text{ L}^{-1}$) and the chl-*a* concentration of 5 specific algal groups based on the excitation wavelength of accessory pigments.

The instrument uses light emitting diodes (LEDs) at 450nm, 525nm, 570nm, 590nm, and 610nm to detect *in-situ* fluorescence (680nm) and also detects pressure (converted to depth) and water temperature. Fluoroprobe® software uses empirically defined algorithms to estimate the proportions of the algal groups *Chlorophyta* (450 nm), *Cyanobacteria* (610 nm), *Cryptophyta* (570 nm), and *Chrysophyta* (525 nm), based on the excitation spectra that generally describe the accessory pigments relevant to each group. A 370nm LED excites “yellow matter” and is used to correct for background fluorescence of DOC. Total *in-situ* spectral fluorescence minus background fluorescence was used to profile the concentration of total chl-*a* during routine limnological sampling runs throughout the season.

Heterotrophic Bacterial Productivity (BP)

Heterotrophic bacterial productivity was measured using [³H]*methyl*-thymidine incorporation into DNA (24) and [³H]leucine incorporation into protein (36). Thymidine is primarily incorporated only during DNA synthesis; thus thymidine incorporation assays measure growing cells. Cells have been shown to incorporate leucine into protein during growth and during stationary phase. Samples were incubated with 20 nM of radio labeled thymidine (1.32×10^6 disintegrations per minute (DPM)) or leucine (5.54×10^6 DPM) at 2-4°C in the dark for 20 hours. Incubations were terminated by the addition of 100 µl of 100% cold trichloroacetic acid (TCA; 5% final), washed with cold 5% TCA and cold 80% ethanol, and dried overnight before being placed at 4°C for storage. Processed samples were stored for no more than 9 months before the addition of CytoScint ES Scintillation Cocktail (MP Biomedicals, OH) and counting on a calibrated

Beckman LS6000 scintillation counter. Disintegrations per minute (DPM) were corrected for decay as needed, using the half-life correction for tritium.

Thymidine and leucine incorporation rates ($\text{nM TdR or Leu d}^{-1}$) at 4°C were converted to rates at *in situ* temperatures using energy of activation of $12,600 \text{ kcal mol}^{-1}$, which was determined from temperature experiments on ELB (76). Rates were converted to bacterial carbon production using conversion factors of $2 \times 10^{18} \text{ cells mol}^{-1}$ thymidine (76) and $1.42 \times 10^{17} \text{ cells mol}^{-1}$ leucine (13), respectively. These rates were then converted to carbon using a cellular carbon content of $11 \text{ fg C cell}^{-1}$ (76). Cell specific activity (SA) was calculated as bacterial productivity in terms of carbon divided bacterial cell counts in terms of carbon.

Takacs and Priscu (76) empirically determined thymidine conversion factors (TCF) for each of the TV lakes, and found the TCFs to range from $2.57 \times 10^{19} \text{ cell mol}^{-1}$ thymidine and $4.75 \times 10^{18} \text{ cell mol}^{-1}$ thymidine. However, the calculated TCFs were based on a limited dataset from the month of December only, and the authors concluded that they likely overestimated the true TCFs for the TV lakes. The factor used here, and by Takacs and Priscu (76) was determined based upon studies of 97 environments, and has consistently been used by the MCM LTER to determine bacterial production. Because leucine conversion factors (LCF) have not been empirically determined for the TV lakes, I used the value determined by Chin-Leo and Kirchman (1988) based on experiments in the Mid-Atlantic Bight and Chesapeake Bay. This value was representative of a range found in the literature.

Thymidine and Leucine Method Validation Experiments

Several assumptions are integral to the TdR and Leu methods:

1. Organisms other than heterotrophic bacteria do not take up thymidine or leucine, and most heterotrophic bacteria are able to take up exogenously supplied thymidine or leucine.
2. The specific activity of incorporated thymidine or leucine is not impacted by internal or external isotope dilution.
3. Thymidine and leucine are not incorporated into macromolecules other than DNA or protein in significant amounts.
4. Bottle effects may lead to overestimation of bacterial productivity in assays performed with small sample volumes and extended incubation times.

Takacs (77) performed experiments with TdR in ELB to examine these assumptions. Results showed that the 0.2 to 3 μm size fraction (bacteria, phytoplankton and protozoan) was responsible for significantly greater TdR incorporation in ELB than the >3 μm size fraction (phytoplankton and protozoan), that 90% of the labeled TdR was incorporated into DNA (rather than other macromolecules), and that TdR incorporation into DNA was linear for up to 24 hours and for TdR concentrations from 5 to 20 nM. These results indicate that bacteria, rather than phytoplankton or protozoa, are responsible for a majority of TdR incorporation in ELB, that TdR incorporation measures DNA synthesis rather than incorporation into macromolecules, that incubation times of up to 24 hours do not bias resultant thymidine incorporation rates, and that between 5 and 20 nM TdR is sufficient to account for isotope dilution.

Takacs (77) also tested for bottle effects by running parallel thymidine incorporation experiments in sample volumes ranging from 10 to 500 ml and found no significant difference ($\alpha=0.05$) among experimental volumes. The Priscu Research Group tested 10 vs. 1.5 ml volumes, and found no significant difference ($\alpha=0.05$). These results indicate that the 1.5 ml sample volumes used in this study do not affect bacterial productivity measurements.

Recently, it has been shown that freshwater bacterial populations may contrast in their abilities to take up thymidine and leucine (58). Pérez et al. (58) used microautoradiography (MAR) to show that 70-80% of bacterial cells were positive for leucine uptake, while only 15-43% were positive for thymidine. The ability to incorporate thymidine varied among phyla. In particular, some members of the *Betaproteobacteria* were unable to take up thymidine. Members of the *Actinobacteria* and *Cytophaga*-like cells were found to readily incorporate thymidine and accounted for up to 80% of thymidine-positive cells (58). The TV lakes are dominated by members of the *Cytophaga-Flavobacteria-Bacteroides* (22.8% relative abundance), *Proteobacteria* (36.4% relative abundance; 8.0% *Betaproteobacteria*), and the *Actinobacteria* (10.2% relative abundance) (Lanoil, unpublished). This suggests that although phyla differ in their abilities to incorporate thymidine, it is likely that the dominant members of the TV lakes communities are capable of thymidine incorporation.

I carried out experiments during November 2009 to examine the assumptions of isotope dilution (concentration series) and bottle effects (incubation time), and to

determine the effects of incubation temperature and irradiance on TdR and Leu in FRX and Leu in ELB.

Experiments were conducted using water from the depths of maximum BP (9 m in FRX and 13 m in ELB). All incubations were replicated at least twice, and the coefficient of variance ($CV = ((\text{Standard deviation}/\text{mean}) * 100)$) was calculated for all replicate samples. Average CV's ranged from 2.9 (ELB Leu light/dark experiment) to 60.9 (FRX TdR temperature gradient experiment) (Appendix A.3). Results with $CV < 20$ were used in further statistical analyses and models. Results with $CV > 20$ were used in making general statements only.

Temperature experiments used a gradient ranging from 2 to 20°C established using an aluminum thermal gradient block with re-circulating water baths attached to either end of the block (80). Samples (two live treatments and one kill at each temperature, for each isotope) were incubated with 20 nM final concentration [^3H]methyl-thymidine or [^3H]leucine in the dark for 20 hours. The energy of activation (E) of leucine incorporation was calculated in the ELB Leu experiment using the Arrhenius equation:

$$V = Ae^{-E/RT}$$

where v is equal to metabolic activity (rate of leucine incorporation) A is the frequency factor for a particular reaction (constant), E is the activation energy of the reaction (J mol^{-1}), R is the gas constant ($8.134 \text{ J mol}^{-1} \text{ K}^{-1}$), and T is the temperature (K) of the reaction. E was determined from the slope of a linear regression of $\ln v$ against $1/T$ for the linear part of the curve leading up to the temperature optimum.

Substrate concentration experiments used eight concentrations of TdR and Leu, ranging from 2 to 20 nM. Samples at each concentration were run in duplicate, with single kills at 5 nM, 10 nM and 20 nM. The experiments were incubated at $\sim 4^{\circ}\text{C}$ in the dark for 20 hours to mimic the protocols used during routine lake bacterial productivity assays.

The linearity of thymidine and leucine incorporation was tested by adding 20 nM of radio labeled thymidine or leucine to 9 duplicate 1.5 ml samples. One set of duplicate tubes was “killed” by the addition of cold TCA at time zero, and all samples were incubated at $\sim 4^{\circ}\text{C}$ in the dark. The other set of duplicate tubes was removed and killed at each time point, every 2 to 3 hours for 24 hours.

The influence of light on TdR and Leu was tested using two sets of triplicate live treatments and duplicate killed controls amended with 20 nM of radio labeled thymidine or leucine to each tube. One set was incubated in the light, and one set in the dark for 20 hours at $\sim 4^{\circ}\text{C}$.

Following completion of incubations, all samples were processed as described in the previous section.

Bacterial Enumeration

Samples were collected from the Niskin bottle into 20 ml scintillation vials, and fixed with sodium borate buffered formalin (5% final concentration) in the field. Vials were stored in the dark at 4°C until enumeration by epifluorescence microscopy (28) with SYBR® Gold (Invitrogen corp., CA, USA). Three laboratory assistants (Alyssa Doering, Hayden Wilson, and Janet Lynch) who were trained according to the Priscu Research

Group protocol were responsible for bacterial enumeration. A separate set of experiments carried out by the Priscu Research Group showed that the use of SYBR gold did not require a correction for loss of cellular abundance as was seen in earlier studies on the TV lakes counted with Acridine Orange (76).

Loss Rates

Average bacterial cell losses and gains during the periods between sampling were calculated over the epilimnion, metalimnion, and hypolimnion of FRX, ELB, and WLB, with the following equations (30):

$$\langle L \rangle = \langle P \rangle - \langle dB/dt \rangle$$

where L is equal to the loss rate (cells $\text{m}^{-2} \text{d}^{-1}$; positive values indicate loss and negative values indicate gain) P is equal to heterotrophic bacterial productivity (cells $\text{m}^{-2} \text{d}^{-1}$), B is the standing stock of cells measured by epifluorescence microscopy (cells m^{-2}) and t is time (day). Carets indicate averages. Conversions to areal rates were not weighted for lake morphometry; hence areal rates reflect integrated rates under a square meter for each layer at the sampling site only. $\langle P \rangle$ was calculated using the following equation:

$$\langle P \rangle = 0.5(P_i + P_{i+1})$$

where P_i and P_{i+1} are equal to P at successive sample points. $\langle dB/dt \rangle$ is equal to:

$$\langle dB/dt \rangle = (B_{i+1} - B_i) / \Delta t$$

where B_i and B_{i+1} are equal to B at successive sampling points. Specific loss rates (day^{-1}) were determined by dividing $\langle L \rangle$ by $\langle B \rangle$ for the corresponding interval.

To determine P , I converted bacterial thymidine and leucine uptake (nM TdR d^{-1} or nM Leu d^{-1}) to bacterial cell production (cells $\text{m}^{-2} \text{d}^{-1}$) using the aforementioned

conversion factors (see Methods- *Heterotrophic Bacterial Productivity*). *B* was determined directly, as described above in Bacterial Enumeration.

Statistical Analyses

I used the geometric mean (GM) Model II regression technique to estimate the relationships between BP TdR and BP Leu, between BP TdR and PPR, and between BP Leu and PPR. GM Model II is the preferred method when both variables are subject to natural variability and/or both variables are subject to measurement error (38). To estimate the relationship between BP and date, I used Generalized Least Squares (GLS) Regression, which included an appropriate correlation correction to account for the effect of autocorrelation between depths (see *Results* for corrections used). Statistical analyses were conducted using the R software package (<http://www.r-project.org/>).

Relationships between BP and environmental variables and specific loss rates and environmental variables were examined using correlation techniques. Non-parametric Spearman Rank correlations were used to satisfy the assumptions of normality (Shapiro-Wilk test; $\alpha=0.05$) and constant variance (visualized by plotting the residuals vs. the fitted values resulting from a linear model including all of the variables).

RESULTS

Physical and Chemical Limnology

The perennial ice covers on the lakes ranged from 2.4 and 5 m thick during the study (Appendix A), and water column depths at sampling sites ranged from ~19 m (FRX) to 42 m (WLB), on average (*See Methods - Table 1*). FRX had the thickest ice cover (4.04 ± 1.17), and the ELB (3.40 ± 0.24) and WLB (3.52 ± 0.28) ice covers were less variable.

All three lakes are permanently thermally and chemically stratified (Appendix B.4). All three lakes were $\sim 0^{\circ}\text{C}$ at the ice-water interface. Temperatures in FRX increased through the epilimnion to an average of 2.4°C (s.d. = 0.27) and then decreased through the metalimnion and hypolimnion to an average low of 2.1°C (s.d. = 0.005). In ELB, temperatures increased through the epilimnion and metalimnion to an average maximum of 4.9°C (s.d. = 0.04), and then decreased, dropping to an average minimum of -2.0°C (s.d. = 0.25) at the bottom of the lake (37-39 m). WLB had the coldest water column of the three lakes, with a maximum average temperature in the epilimnion of 2.0°C (s.d. = 0.20), which then decreased to an average of -4.2°C (s.d. = 0.08) in the deep, salty waters below 35 m.

The conductivity of all three lakes ranged from freshwater near the ice-water interface to brackish in the deeper waters; these conductivity layers were separated by a distinct chemocline (Table 1; Appendix B.4). ELB had the highest conductivity, with deep waters (below 25 m) reaching 115 mS cm^{-1} , and FRX had the lowest, with the

bottom waters (below 17 m) reaching 8.5 mS/cm. Maximum conductivity in WLB was 81 mS/cm below 35 m.

Each lake is characterized by a relatively nutrient-rich hypolimnion overlain by a nutrient-poor epilimnion (Appendix B.2 and B.3). Linear regression analyses showed that seasonal decreases in NH_4^+ concentrations were statistically significant ($\alpha = 0.05$) in all three strata of ELB and WLB during the course of the season (Table 2). The percent decrease was greatest in the epilimnia of both lakes (ELB 0.39% day⁻¹; WLB 0.46 % day⁻¹) compared to the metalimnia (ELB 0.17% day⁻¹; WLB 0.26 % day⁻¹) and the hypolimnia (ELB and WLB 0.09% day⁻¹). FRX had the steepest NH_4^+ gradient with respect to depth, but a seasonal pattern was not readily detectable for any of the depth layers defined.

Table 2. Results of linear regressions of ammonium-N on date in ELB and WLB.

ELB			
	Linear equation	R²	p-value
Epilimnion	$y = 22594 - 0.6x$	0.73	0.0008
Metalimnion	$y = 110005 - 2.9x$	0.72	0.0008
Hypolimnion	$y = 619079 - 16.0x$	0.68	0.0015
WLB			
Epilimnion	$y = 43679 - 1.1x$	0.80	0.0004
Metalimnion	$y = 450550 - 11.8x$	0.91	0.0002
Hypolimnion	$y = 648005 - 16.6x$	0.79	0.005

Concentrations of NO_2^- and NO_3^- were one to three orders of magnitude lower in the FRX epilimnion throughout the season (NO_2^- average: 4.44 mg m⁻², range 2.60 to 6.52 mg m⁻²; NO_3^- average: 5.39 mg m⁻², range: 2.23 to 8.88 mg m⁻²) than in the WLB epilimnion (NO_2^- average: 20.66 mg m⁻², range 15.86 to 28.80 mg m⁻²; NO_3^- average:

1035.98 mg m⁻², range: 926.84 to 1252.20 mg m⁻²) and the ELB epilimnion (NO₂⁻ average: 41.02 mg m⁻², range 20.26 to 64.04 mg m⁻²; NO₃⁻ average: 1252.15 mg m⁻², range: 1097.26 to 1479.17 mg m⁻²) (Appendix B.2). No seasonal patterns were detectable in any of the defined layers.

Soluble reactive phosphorus (SRP) decreased in the ELB epilimnion over the course of the season, and the trend was statistically significant (slope = -0.067; R² = 0.35; p = 0.05). A seasonal pattern was not detectable in either of the other lakes or for the other layers defined (Appendix B.3).

I calculated seasonal dissolved inorganic nitrogen (DIN = NO₂⁻ + NO₃⁻ + NH₄⁺) to SRP ratios in all three layers of FRX, ELB, and WLB and compared them to Redfield ratios (64). DIN:SRP in FRX was consistently below Redfield ratio (7:1 by weight) in all layers, while WLB DIN:SRP was two orders of magnitude above Redfield ratio in all three layers. No seasonal patterns were detected in either lake. In ELB, the ratios were two orders of magnitude above Redfield, and decreased in the metalimnion (slope = 0.8; R²=0.30; P=0.05) and hypolimnion (slope = -0.3; R²=0.50; P=0.006) during the season.

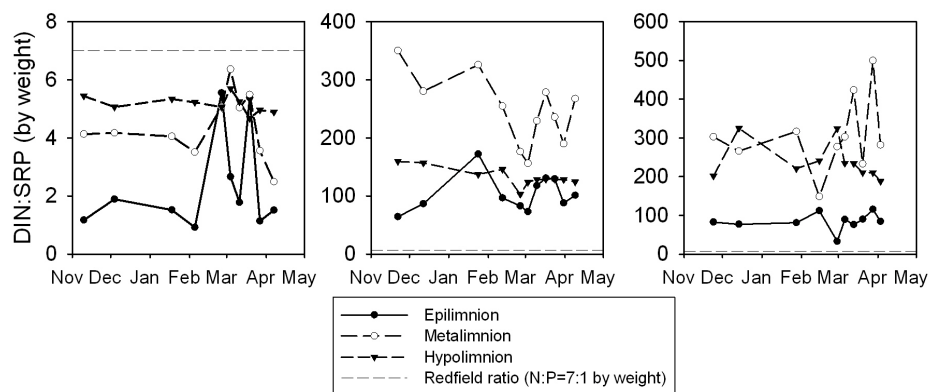


Figure 2. DIN:SRP ratios by weight. SRP = soluble reactive phosphorous. DIN is equal to NO₂⁻ + NO₃⁻ + NH₄⁺. Values are integrated over the layers given in the legend. A = FRX, B = ELB, C = WLB. The dashed line indicates Redfield ratio (7:1 by weight).

Seasonal average DOC concentrations in the hypolimnia were one (FRX: 49.1 g m^{-2} ; and WLB: 72.2 g m^{-2}) or two (ELB: 150 g m^{-2}) orders of magnitude greater than in epilimnia (FRX: 9.3 g m^{-2} ; ELB: 5.9 g m^{-2} ; WLB: 8.0 g m^{-2}). DOC decreased significantly with a linear trend over the course of the season in the WLB hypolimnion (slope = 0.14; $R^2 = 0.88$; $p < 0.001$). No significant trend was detected in any other layer, or in either of the other lakes (Figure 3).

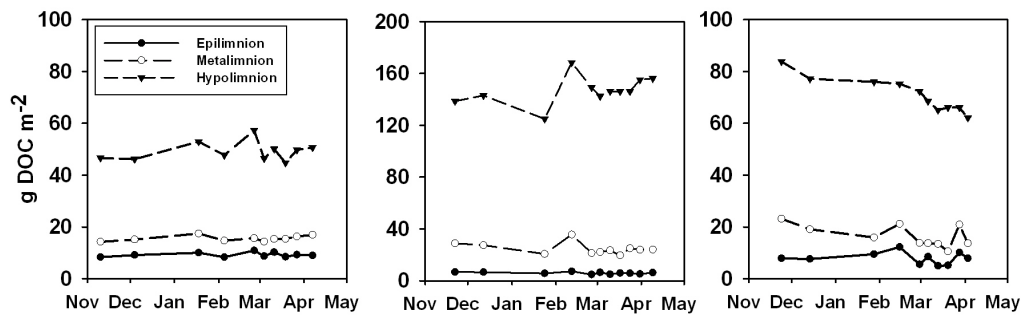


Figure 3. Seasonal dissolved organic carbon (DOC) profiles. A= FRX, B = ELB, C = WLB. Values are integrated over the layers shown in the legend. Note the different scale for ELB.

Underwater PAR (10 m) decreased in ELB at a rate of $0.3\% \text{ day}^{-1}$ until 13 February, and then decreased at a rate of $2\% \text{ day}^{-1}$ until the end of the season. In WLB underwater PAR (10 m) decreased slowly until 16 February ($0.6\% \text{ day}^{-1}$), and more rapidly ($1.7\% \text{ day}^{-1}$) from 16 February until the end of the season. Underwater PAR (7m) increased at a rate of $2.6\% \text{ day}^{-1}$ in FRX until 6 February, and then decreased ($1.7\% \text{ day}^{-1}$) through the end of the season (Figure 4). On average, underwater PAR decreased 94% between February and April in all lakes.

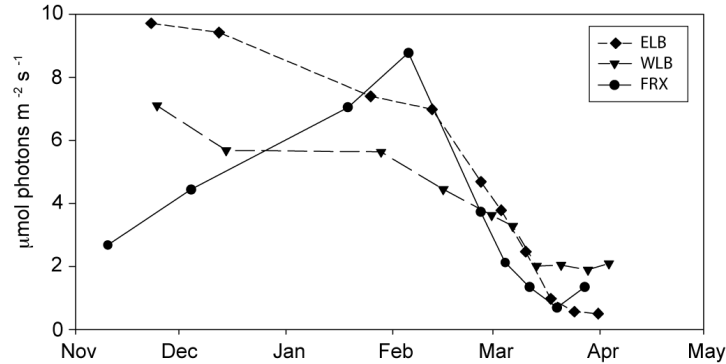


Figure 4. Seasonal underwater photosynthetically available radiation (PAR). PAR was measured at 10 m in ELB and WLB and 7m in FRX.

Biological Parameters

Primary Productivity and Chl-*a*

Primary productivity (PPR) increased in the FRX and ELB epilimnion (FRX: 0.6% % day⁻¹, max PPR 12.8 mg C m⁻² d⁻¹; ELB: 0.7% % day⁻¹, max PPR 17.1 mg C m⁻² d⁻¹) until 27 and 13 February, respectively. PPR then decreased steadily in ELB (1.7% d⁻¹) until the final time point on 1 April. In FRX, PPR decreased sharply (14% day⁻¹) between 27 February and 5 March and remained an order of magnitude below the maximum until the last time point on 28 March. In the FRX metalimnion, PPR increased (4.6% day⁻¹) until 19 January (4.8 mg C m⁻² d⁻¹) and then decreased at a rate of 1.4% day⁻¹ until the end of the season. The ELB metalimnion PPR maximum occurred on 13 December (6.7 mg C m⁻² d⁻¹) and then decreased by 0.9% day⁻¹ until the end of the season. In WLB, the maxima for both the epilimnion (31.5 mg C m⁻² d⁻¹) and the metalimnion (1.38 mg C m⁻² d⁻¹) occurred on 29 January. PPR then decreased by 1.9 and 1.4% d⁻¹, respectively, until the end of the season (Figure 5).

Integrated chlorophyll-*a* (chl-*a*) concentrations in the FRX epilimnion ranged from 14.3 to 19.6 mg m⁻² between 11 November and 19 January (Figure 6). Starting in mid-January (19 January), epilimnion chl-*a* concentrations increased at a rate of 0.8% d⁻¹ (p=0.04) until the end of the season. Integrated chl-*a* concentrations in the FRX metalimnion decreased over the course of the season (-0.2% d⁻¹; p= 0.02), while concentrations in the hypolimnion increased (0.75% d⁻¹; p= 0.002). In ELB and WLB, integrated chl-*a* concentrations increased during the season in the epilimnion (ELB: 1.1% d⁻¹, p<0.001; WLB: 0.6% d⁻¹, p<0.001) and the hypolimnion (ELB: 0.96% d⁻¹, p= 0.001; WLB: 0.08% d⁻¹, p= 0.02), but did not change significantly in the metalimnion. Seasonal average chl-*a* concentrations in the epilimnion exceeded hypolimnion concentrations in all lakes by one (FRX) to two (ELB and WLB) orders of magnitude. The seasonal average in the WLB epilimnion (42 mg m⁻²) twice the seasonal average in FRX, and 2.3 times the seasonal average in ELB.

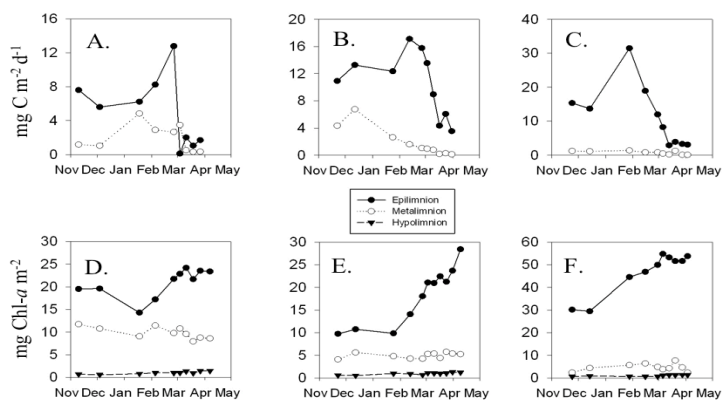


Figure 5. Seasonal primary productivity (PPR) and chlorophyll-*a* (chl-*a*) profiles. Values are integrated over the layers shown in the legend. No PPR measurements were undertaken in the hypolimnia. A & D = FRX; B & E = ELB; C & F = WLB. Note the differences among scales.

These results show that while there was a clear loss of PPR starting in late January (WLB) and mid- to late February (FRX and ELB), chl-*a* concentrations increased in the epilimnia of all three lakes until the end of the season.

Heterotrophic Bacterial Productivity

Thymidine and Leucine Method Validation Experiments: No statistically significant relationship ($\alpha=0.05$) between thymidine incorporation rate and temperature, substrate concentration, or incubation time was apparent in the FRX TdR dataset, likely due to high standard errors and variability amongst data points (Figure 6; Appendix A.3). TdR was 38% higher in the dark than in the light, but the difference was not significant (student's t-test; $\alpha=0.05$). The temperature optimum for TdR was 18°C. High variation amongst replicates (average CV = 60.9) prevented fitting of the Arrhenius equation to determine energy of activation.

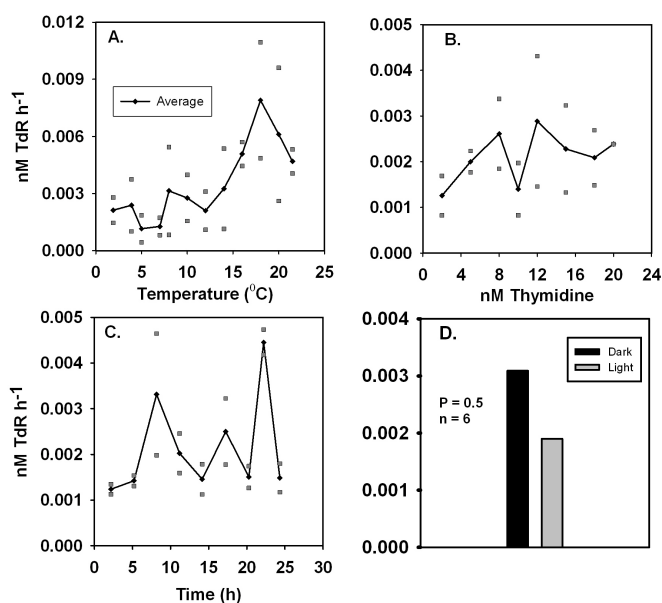


Figure 6. Lake Fryxell (FRX) thymidine (TdR) method validations. A = temperature gradient; B = substrate concentration gradient; C = time series; D = light vs. dark incubations. P-value is the result of a student's t-test.

Results of the FRX Leu temperature gradient, substrate concentration, time-series, and light/dark experiments are shown in Figure 7. High variance amongst samples prevented fitting a linear model or Arrhenius equation to the FRX Leu temperature series, but it appears that incorporation rates increased until reaching an optimum temperature of 14°C. These results indicate that microorganisms incorporating leucine in FRX are operating below their temperature optima.

The substrate concentration gradient showed that rates of leucine incorporation increased between 2 and 5 nM, but that concentrations of greater than 5 nM had no effect on rates of substrate incorporation. These results indicate that concentrations of up to 5 nM leucine are sufficient to measure leucine incorporation rates by microbial communities in the sample from FRX.

Results of the time-series experiment indicate that rates of leucine incorporation decreased moderately (slope = -0.002; $p < 0.001$) during the first 11 hours of incubation. Between 11 and 14 hours, leucine incorporation decreased at a rate of 2.89 nM Leu h⁻¹, and then remained linear until the end of the incubation. These results indicate that incubation periods of longer than 11 hours may underestimate leucine incorporation.

Leucine incorporation was 30% higher in the light than in the dark, but the difference was not significant (student's t-test; $\alpha = 0.05$).

Results of the ELB Leu temperature series experiments (Figure 8) showed that maximum incorporation was measured for samples incubated around 18°C, and then decreased as temperature increased. This indicates that organisms are operating below their temperature optima in ELB. Incubating experiments at 4°C may underestimate

potential Leu in ELB. The energy of activation, determined using the slope of the line $\ln(v)$ vs. $1/T$ (see *Methods*), was $74.62 \text{ kJ mol}^{-1}$.

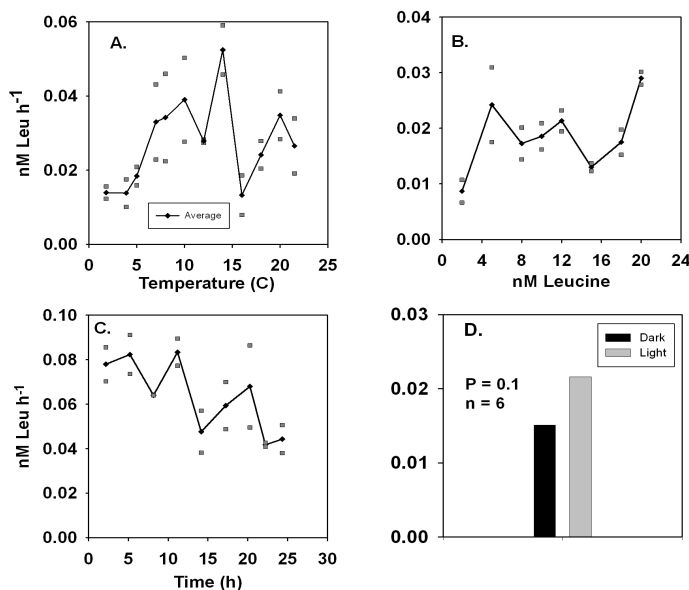


Figure 7. Lake Fryxell (FRX) leucine (Leu) method validations. A = temperature gradient; B = substrate concentration gradient; C = time series; D = light vs. dark incubations. P-value is the result of a student's t-test.

Results of substrate concentration experiments show that incorporation is approximately linear over the course of the concentration gradient, although a significant ($\alpha = 0.05$) least-squares fit was not achieved. These results suggest that as little as 2 nM leucine is sufficient to measure leucine incorporation in ELB, but that higher concentrations do not negatively influence incorporation.

Results of the time-series experiment show a linear relationship between Leu incorporation and time (linear regression; $R^2 = 0.8$; $p < 0.0001$). Incorporation decreased by $\sim 7.6 \times 10^{-5} \text{ nM Leu h}^{-1}$ during the time series. This indicates that 20 h incubations underestimate Leu in ELB by $\sim 32\%$. Results of the light/dark experiment show that leucine incorporation is significantly higher in the dark than the light ($p < 0.001$; $n = 6$).

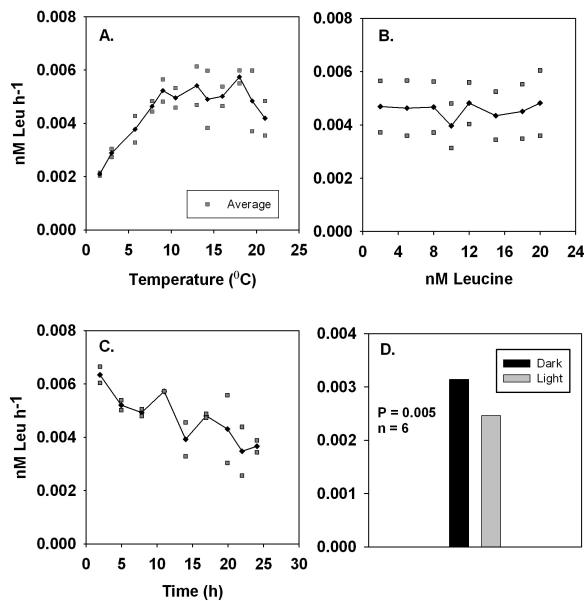


Figure 8. East Lobe Bonney (ELB) leucine (Leu) method validations. A = temperature gradient; B = substrate concentration gradient; C = time series; D = light vs. dark incubations. P-value is the result of a student's t-test.

Summer Fall Comparison: To determine whether there was an overall change in BP between the summer and fall seasons, I compared average summer and fall BP in the epilimnion, metalimnion, and hypolimnion in each of the lakes (Table 3), using the Mann-Whitney test. No significant differences ($\alpha = 0.05$) were found in any of the lakes. General trends were noted as follows.

- In FRX, TdR was greater in the summer than in the fall by between 12 and 54%. Fall Leu was twice summer Leu in the metalimnion, and 60% greater than summer Leu in the hypolimnion. In the epilimnion, summer Leu was 46% greater than fall Leu.
- In ELB, TdR was below detection in the hypolimnion during both the summer and fall. TdR and Leu were greater (8% to 120%) in the fall than

in the summer in all layers of ELB.

- In WLB, Leu was below detection in the hypolimnion during both the summer and fall. Fall TdR exceeded summer TdR except in the hypolimnion, where TdR was greater in the summer by 22%. Fall Leu also exceeded summer Leu, except in the metalimnion, where Leu was 12% greater in the summer.

Table 3. Comparison of summer and fall BP rates in FRX, ELB, and WLB. Summer-Fall = summer average BP minus fall average BP. % Difference is between summer and fall averages.

LAKE FRYXELL				
	Thymidine		Leucine	
	<u>Summer-Fall</u>	<u>% Difference</u>	<u>Summer-Fall</u>	<u>% Difference</u>
Epilimnion	0.6	54	0.7	47
Metalimnion	0.3	12	-0.7	-101
Hypolimnion	0.005	20	-0.003	-60
EAST LOBE BONNEY				
	Thymidine		Leucine	
	<u>Summer-Fall</u>	<u>% Difference</u>	<u>Summer-Fall</u>	<u>% Difference</u>
Epilimnion	-0.1	-28	-0.1	-8
Metalimnion	-0.08	-81	-0.06	-12
Hypolimnion	-	-	-0.01	-120
WEST LOBE BONNEY				
	Thymidine		Leucine	
	<u>Summer-Fall</u>	<u>% Difference</u>	<u>Summer-Fall</u>	<u>% Difference</u>
Epilimnion	-0.2	-25	-0.4	42
Metalimnion	-0.03	-95	0.03	12
Hypolimnion	0.01	22	-	-

These results show that BP in each of the lakes responds differently to the change in season. TdR activity decreased during the fall relative to the summer in FRX. This was the only lake that showed consistently higher activity during one season when

compared to the other, and this may suggest that FRX BP is the most seasonally dynamic with regard to TdR. It should be noted, however, that the trends were not statistically significant at $\alpha=0.05$.

Primary Productivity vs. Bacterial Productivity: Results of Model II regressions (Geometric Mean) showing the relationship between BP and PPR are presented in Figures 9 and 10. None of the BP-PPR relationships were significant, except for in the ELB metalimnion. TdR and PPR were weakly, negatively correlated in this layer ($r = -0.63$, $p=0.04$). These results indicate a weak relationship between BP and PPR.

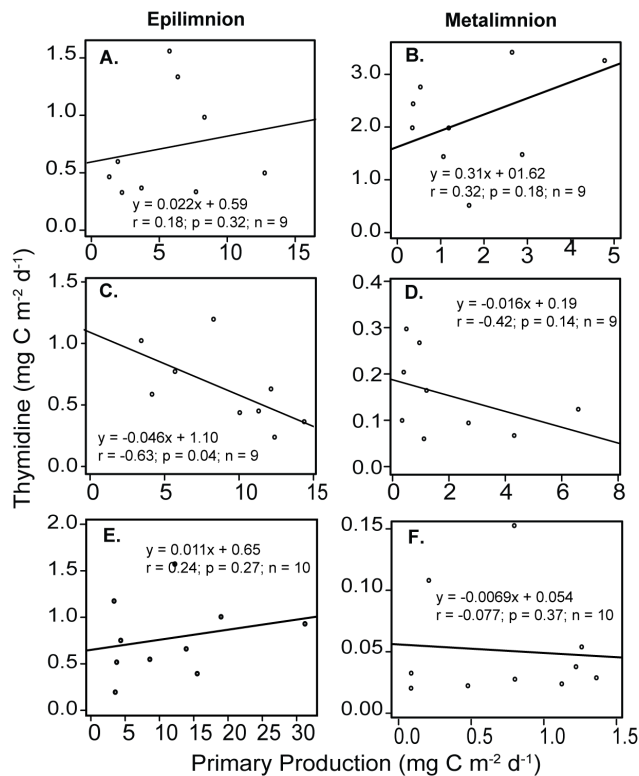


Figure 9. Bacterial productivity (BP; TdR) plotted against primary productivity (PPR) for the epilimnion and metalimnion of each lake. A&B = FRX, C&D = ELB, E&F = WLB. The solid line represents the Model II regression. The equation to the line, in addition to the r-value, p-value, and n are given on each plot.

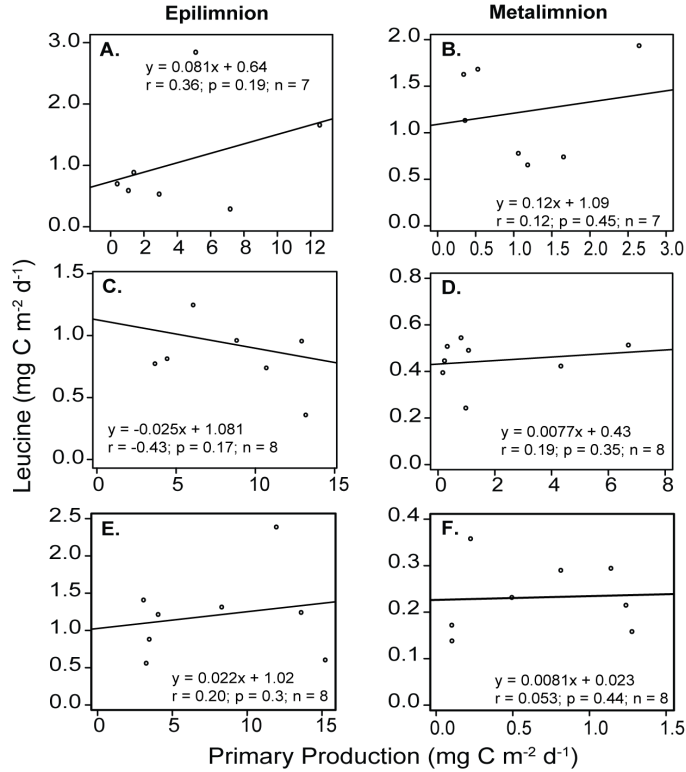


Figure 10. Bacterial Productivity (BP; Leu) plotted against Primary Productivity (PPR) for the epilimnion and metalimnion of each lake. A&B = FRX, C&D = ELB, E&F = WLB. The solid line represents the Model II regression. The equation to the line, in addition to the r-value, p-value, and n are given on each plot.

Season-long Trends in Bacterial Productivity: To determine whether there were statistically significant patterns in bacterial productivity over the course of the season, linear models based on natural log transformed bacterial productivity data from all sampling dates and depths were fit using the Generalized Least Squares (GLS) method. Correlation corrections (spherical, linear, or exponential) were added based on each model as appropriate in order to account for autocorrelation between depths. The type of correction was selected based on the Akaike information criterion, or AIC (2). The model with the lowest AIC was deemed to have the best fit. In all cases, the GLS regression

with correlation correction had significantly better fit than GLS regression with no correlation correction (ANOVA; $\alpha=0.05$). These analyses showed no significant linear relationship between BP and date throughout the season, for any lake.

I examined BP data, integrated over the epilimnion, metalimnion, and hypolimnion of each lake for general seasonal trends in BP. The results are summarized in Figure 11. In FRX, integrated TdR reached a maximum of $3.4 \text{ mg C m}^{-2} \text{ d}^{-1}$ on 27 February, in the metalimnion. That was also the maximum rate, for both TdR and Leu, measured in any of the lakes. Integrated Leu was greatest on 5 December, also in the metalimnion. TdR and Leu minima were found in the hypolimnion, where rates were below detection on 11 November and 5 March, respectively.

In ELB, integrated TdR and Leu maxima occurred in the epilimnion on 11 March and 10 April, respectively. Overall minima were measured in the hypolimnion, where TdR was below detection all season, and Leu was below detection in late February. For each layer, the Leu maximum was measured on 10 April, the last sample point of the season. TdR:Leu ratios were the lowest of the season on 10 April as well.

In WLB, TdR and Leu maxima both occurred on 1 March in the epilimnion, and minima (below detection) were measured in the hypolimnion. Epilimnion TdR gradually increased (slope of least squares fit = 0.01 ; $r^2 = 0.86$) until reaching a maximum on 1 March, decreased dramatically between 1 and 7 March ($9.4\% \text{ d}^{-1}$), and then decreased (slope of least squares fit = -0.05 ; $r^2 = 0.98$) during the remainder of the

season. Leu was not measured during January and February, making determination of early-season trends difficult, but it appears to follow an overall pattern similar to TdR.

Several large (27% to 91%) decreases in BP were detected between the sixth (FRX and ELB - 27 February; WLB - 1 Mar) and seventh (FRX – 5 Mar; ELB – 4 Mar; WLB – 7 Mar) sample points. I refer to this as the “March crash”. In FRX and ELB, BP generally rebounded by the eighth sample point (12 Mar – FRX; 11 Mar – ELB; 14 Mar – WLB). Results are described below.

In FRX, TdR decreased by 27% in the epilimnion during the March crash, and never recovered to the maxima reported in December and January. The highest post-crash rate was less than half of the December maximum. In the FRX metalimnion, TdR decreased by 85% during the crash, and rebounded to within 19% of the pre-crash maximum by the next sample point. Rates remained high until the final sample point, when they decreased again. There was a 91% decrease in TdR in the hypolimnion during the crash, followed by a rebound to within 20% of pre-crash rates. Overall, TdR integrated over the hypolimnion is more difficult to interpret due to greater variability.

FRX Leu showed a similar pattern to TdR, with a 70% decrease in the epilimnion being followed by an apparent leveling-off until the end of the season. Leu integrated over the metalimnion decreased by 63% during the crash, and then rebounded to within 13% of pre-crash rates. Leu in the metalimnion stayed within 42% of the season maximum through the end of the season.

In ELB, TdR integrated over the epilimnion decreased by 34% during the March crash, and then rebounded to nearly twice the pre-crash maximum. Rates remained

above or at the pre-crash maximum until 10 April, when TdR dropped to its lowest rate of the season. TdR was more variable following the March crash than prior to the crash (S.D. = 0.4 and S.D. = 0.2, respectively). In the ELB metalimnion, TdR decreased by 62% during the crash, and then rebounded to 40% greater than the pre-crash maximum. Rates remained high until 1 April 2008.

ELB Leu integrated over the epilimnion decreased by 47% during the March crash, and then rebounded to 100% of the pre-crash maximum. Rates were at least 80% of pre-crash rates for the rest of the season, and spiked at the final sample date (10 April), when they reached the season maximum. Leu integrated over the metalimnion showed a similar pattern, where it decreased by 52% during the March crash, rebounded to greater than the pre-season maximum, and spiked to the season maximum on 10 April.

In WLB, TdR integrated over the epilimnion decreased by 66% during the crash, and then rebounded to within 25% of the pre-crash maximum. Rates then decreased during the remainder of the season, until falling to the season minima at the final sample point (4 April). This pattern seems to suggest a continual increase in productivity until early March, followed by a gradual decrease, and may indicate a seasonal trend, rather than a sharp crash and rebound. The pattern is similar in the metalimnion, with an 86% decrease during the crash followed by a rebound and gradual decrease through the end of the season. Leu follows the same general trend.

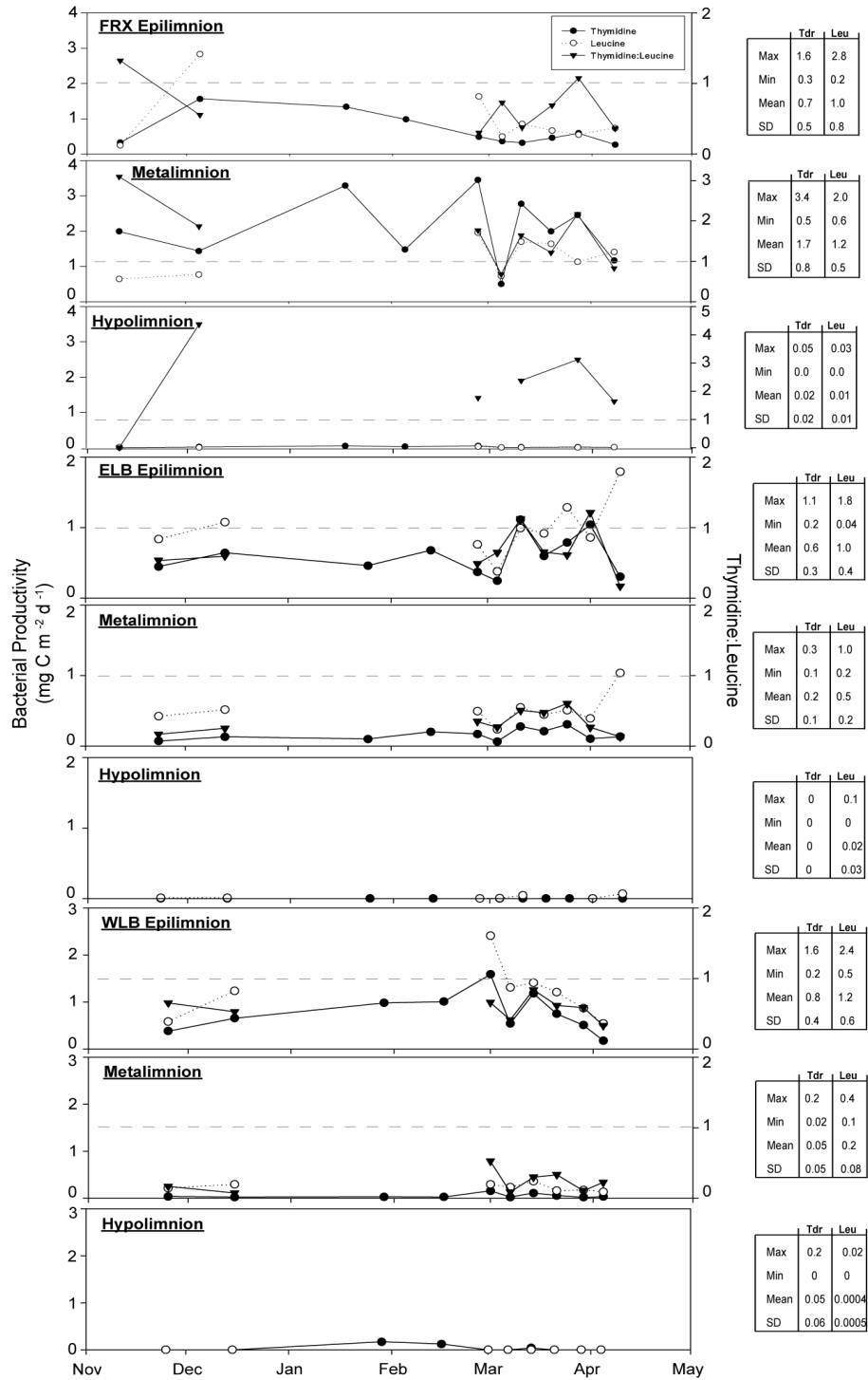


Figure 11. Seasonal trends in bacterial productivity. The dashed line represents a 1:1 thymidine (Tdr) to leucine (Leu) ratio. Summary statistics are given to right of each plot. Ratios are not given for the hypolimnia of ELB and WLB because Tdr and/or Leu were below detection for a majority of the season.

These data show that BP varied throughout the season and amongst lakes. No statistically significant correlation could be determined between BP and date, but general trends indicate some bacterial community responses to the changing seasons.

BP in all three lakes decreased during early March, and then rebounded in the FRX metalimnion and the ELB epi- and metalimnia. In WLB, the March crash preceded a late season decline in BP.

Bacterial Abundance

Bacterial cell densities, as abundance integrated over the epilimnion, metalimnion, and hypolimnion of each lake are shown in Figure 12. Cell densities in FRX were $2-7 \times 10^{12}$ cells m^{-2} in all three strata (one to two orders of magnitude greater than in the ELB and WLB meta- and hypolimnia), throughout the season. Maximum densities were recorded on 5 March (the date of the March crash in BP) in all three strata, indicating the BP crash in FRX was not due to a decrease in biomass.

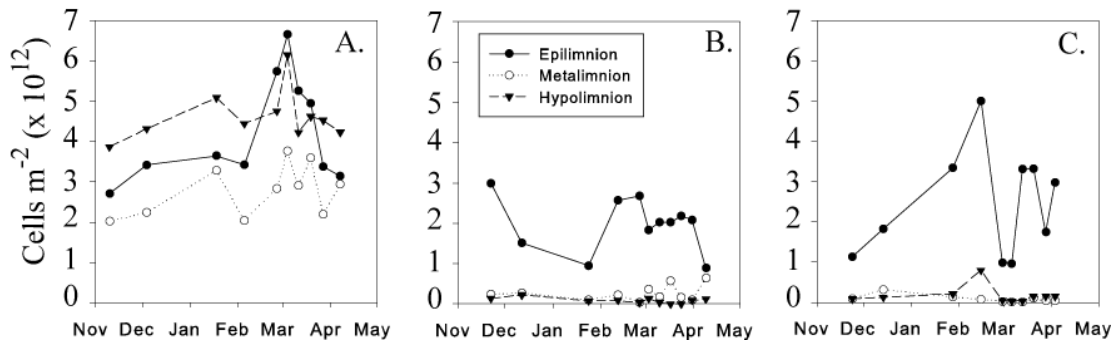


Figure 12. Seasonal trends in bacterial abundance for each lake. A = FRX, B = ELB, C = WLB. Cell counts are integrated over the layers indicated in the legend.

Cell densities in ELB were between 0.9 and 3×10^{12} cells m^{-2} in the epilimnion, between 0.4 and 7×10^{11} cells m^{-2} in the metalimnion, and between 0.3 and 3×10^{11} cells m^{-2} in the hypolimnion. Minimum densities in the ELB metalimnion and hypolimnion were recorded on 27 February. In WLB, cell densities were between 0.9 and 5×10^{12} cells m^{-2} in the epilimnion, between 5×10^9 and 10×10^{10} cells m^{-2} in the metalimnion, and between 3×10^{10} and 8×10^{11} cells m^{-2} in the hypolimnion. Maximum cell densities were reached on 16 February in both the epilimnion and hypolimnion of WLB. One of the metalimnion sample vials from 16 February was damaged in transit from the field; thus the integrated cell density could not be calculated for this date. Minimum densities were recorded on 1 March for all three strata in WLB.

Minimum densities in ELB and WLB were measured on the date immediately preceding the March BP crash. Abundance in ELB, but not WLB, recovered by the time of the March crash.

Average bacterial abundances (cells ml^{-1}) for individual sample depths are reported in Appendix C.1.

Specific Activity (SA)

Cell specific activity (SA; $\text{mg C cell}^{-1} \text{ day}^{-1}$) calculated individually for TdR and Leu, are shown throughout the water column of each lake in Figures 13-15. The highest SA was measured with Leu in the FRX metalimnion, where the average (4.7×10^{-10} $\text{mg C cell}^{-1} \text{ day}^{-1}$) exceeded the ELB and WLB averages (4.4×10^{-12} and 2.4×10^{-11} $\text{mg C cell}^{-1} \text{ day}^{-1}$, respectively) by one to two orders of magnitude. The lowest average SA (TdR)

was measured in the FRX hypolimnion (4.0×10^{-15} mg C cell⁻¹ day⁻¹), and was two to three orders of magnitude less than in ELB or WLB, respectively.

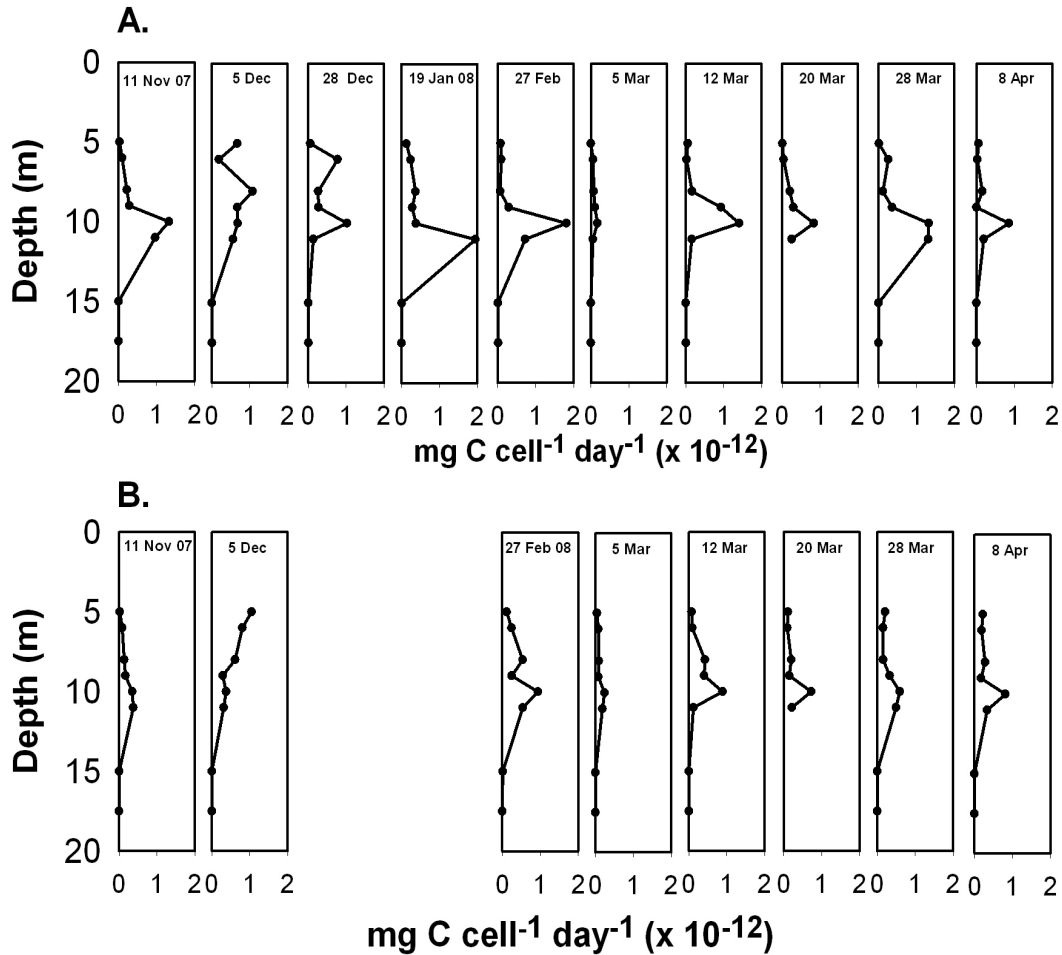


Figure 13. Depth profiles for specific activity (SA) in Lake Fryxell (FRX). A= Thymidine (TdR), B = Leucine (Leu). Leu was not assayed on 29 December or 19 January.

Peak TdR SA occurred on 27 February in the metalimnia of FRX and ELB (1.3×10^{-12} mg C cell⁻¹ day⁻¹; 4.5×10^{-12} mg C cell⁻¹ day⁻¹) and on 14 March in WLB (2.7×10^{-11} mg C cell⁻¹ day⁻¹). Leu SA was highest in FRX and ELB on 27

February (7.4×10^{-10} mg C cell⁻¹ day⁻¹; 1.2×10^{-11} mg C cell⁻¹ day⁻¹) and on 14 March in WLB (1.1×10^{-10} mg C cell⁻¹ day⁻¹).

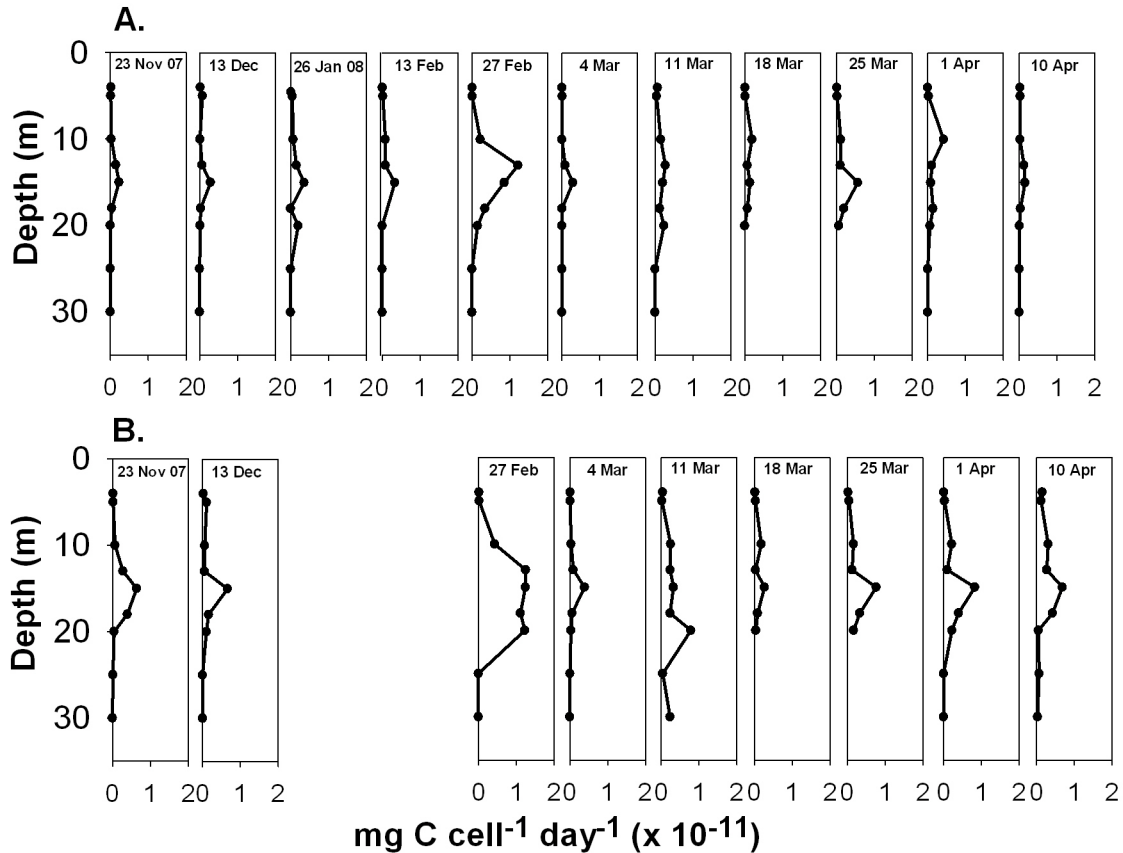


Figure 14. Depth profiles for specific activity (SA) in ELB. A= Thymidine (TdR), B = Leucine (Leu). Leu was not assayed on 26 January or 13 February.

There were also changes in SA during the time of the March crash. SA (Leu) decreased by an order of magnitude in the FRX epilimnion, and SA (TdR) decreased by an order of magnitude in the epilimnion and metalimnion of FRX. March 4 (March crash) SA (TdR and Leu) in the epilimnion and metalimnion of ELB decreased by an order of magnitude compared to 27 February. SA (Leu) increased between 1 March and

14 March (34.6% d⁻¹) in the WLB metalimnion, and then decreased from 14 March to 29 March (6.5% d⁻¹).

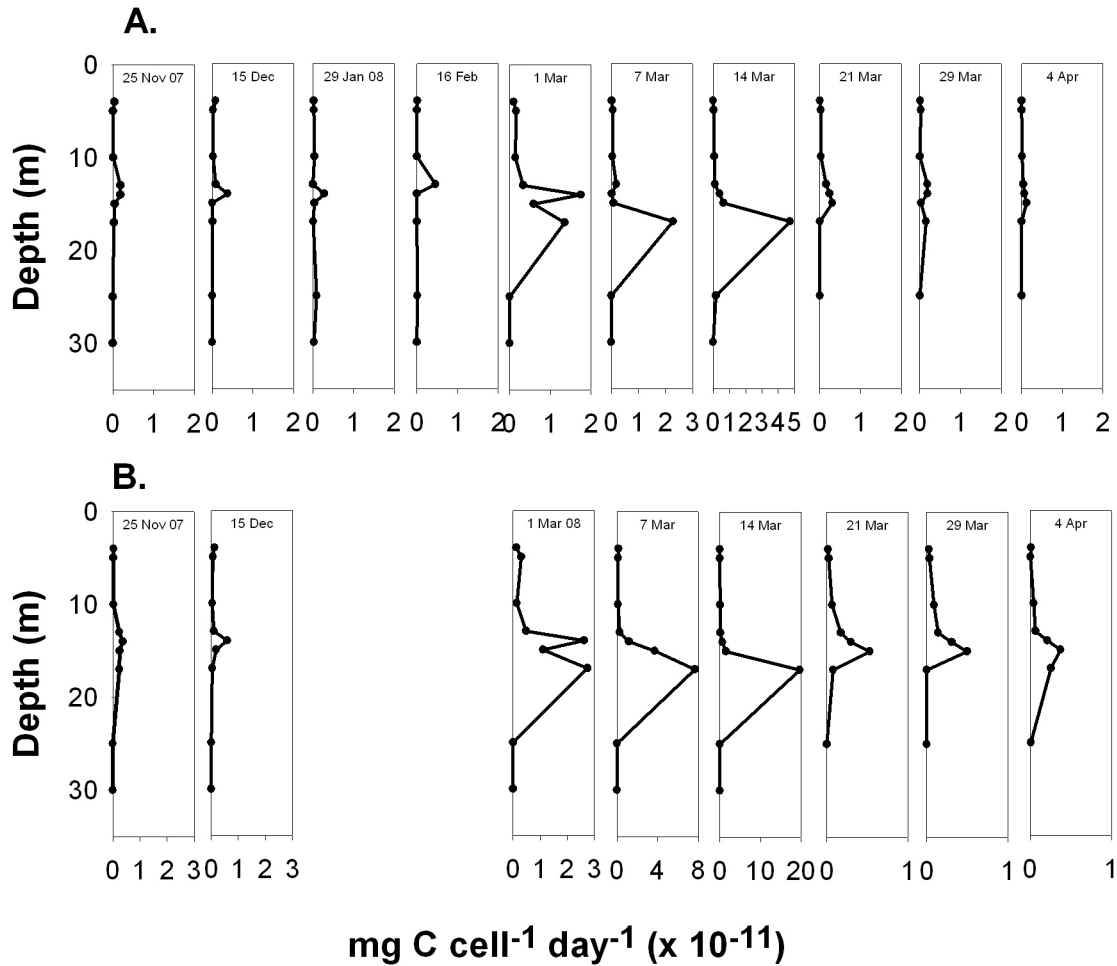


Figure 15. Depth profiles for specific activity (SA) in WLB. A= Thymidine (TdR), B = Leucine (Leu). Leu was not assayed on 29 January or 16 February.

Loss Rates

Seasonal average bacterial cell loss rates (cell m⁻² d⁻¹) and specific loss rates (day⁻¹) calculated for both TdR and Leu and averaged for the epilimnion, metalimnion, and hypolimnion of ELB, WLB, and FRX are shown in Appendix C.2. The highest cell loss rates were measured in the metalimnion of FRX, which were an order of

magnitude greater than in ELB or WLB. The highest specific loss rates were measured in the metalimnion of WLB. On average, a specific gain per day and a gain in cells $\text{m}^{-2} \text{d}^{-1}$ were measured in the hypolimnion of ELB.

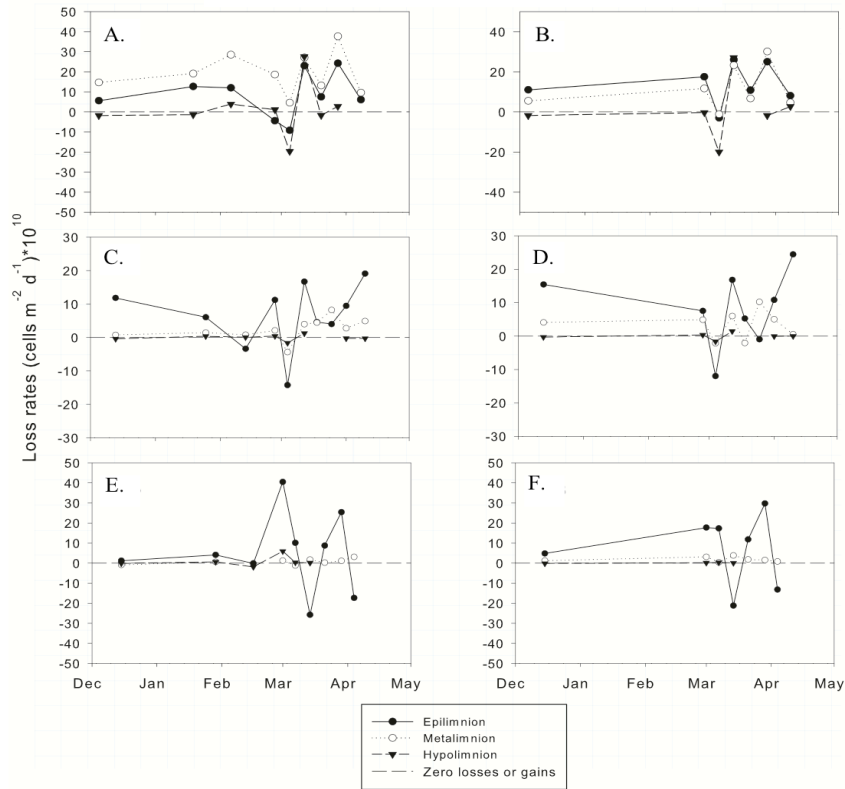


Figure 16. Seasonal loss rates. FRX (A&B), ELB (C&D), and WLB (E&F). Calculated based on TdR (A,C,E) and Leu (B,D,F). Values above the dotted line indicate losses, while values below the dotted line indicate gains. Note the differences in scales.

Seasonal trends in loss rates are shown in Figure 16. Loss rates were positive or approximately zero in all lakes during the summer, except for in the FRX hypolimnion. Gains of between approximately 1 and 20×10^{10} cells $\text{m}^{-2} \text{d}^{-1}$ were calculated for the FRX and ELB water columns during the interval leading up to the March BP crash.

Losses of between 10 and 20×10^{10} cells $\text{m}^{-2} \text{d}^{-1}$ were calculated for the WLB epilimnion leading up to the March crash.

Comparison of Rates of DNA and Protein Synthesis

The results of GM model II regression analyses of the relationship between TdR and Leu are shown in Figure 17. In WLB, TdR and Leu were significantly positively correlated in the epilimnion ($r=0.81$, $p=0.03$), with Leu tending to be greater than TdR. In the FRX metalimnion, TdR and Leu were significantly negatively correlated ($r=-0.60$, $p=0.05$). TdR and Leu were not negatively correlated in any other lake.

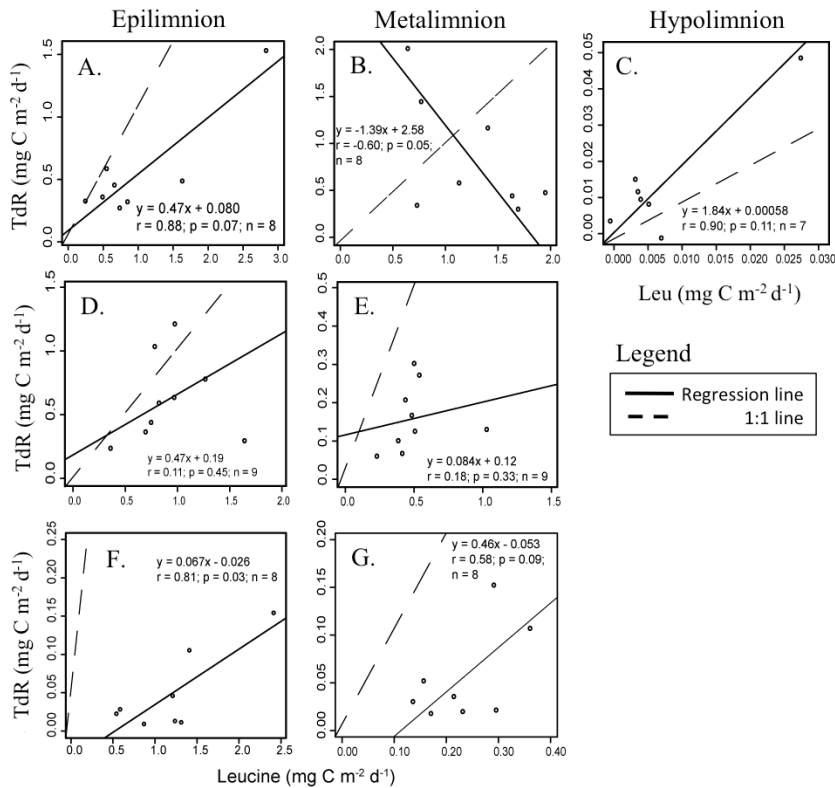


Figure 17. BP (TdR) plotted against BP (Leu). FRX = A, B, C; ELB = D, E; WLB = F, G. The solid line is the result of Model II regression; the dashed line indicates a 1:1 TdR:Leu ratio. TdR and/or Leu were below detection for a majority of the season in the ELB and WLB hypolimnia. Note the differences in scales.

Correlation between Biological and Environmental Parameters

Statistically significant ($\alpha=0.05$) results of Spearman Rank correlation analyses are shown in Table 4. I analyzed correlations between biological parameters specific to bacterioplankton (cell counts, TdR, Leu, TdR Specific Loss, and Leu Specific Loss), biological parameters specific to phytoplankton (PPR, chl-*a*), and environmental parameters (DO, DOC, NH_4^+ , NO_2^- , NO_3^- , SRP, and DIN:SRP) in the epilimnion, metalimnion, and hypolimnion of each lake.

Table 4. Results of Spearman Rank Correlations between 13 biological, physical, and environmental parameters. Only significant results ($\alpha=0.05$) are shown.

LAKE FRYXELL					
EPILIMNION		METALIMNION		HYPOLIMNION	
Variables	r-value p-value	Variables	r-value p-value	Variables	r-value p-value
Cells:DO	0.66, 0.03	Cells: NO_3^-	0.64, 0.04	Cells: NO_2^-	0.61, 0.05
Cells: SRP	-0.62, 0.05	TdR: NH_4^+	0.76, 0.009	-	-
EAST LOBE BONNEY					
EPILIMNION		METALIMNION		HYPOLIMNION	
-	-	-	-	-	-
WEST LOBE BONNEY					
EPILIMNION		METALIMNION		HYPOLIMNION	
Variables	r-value p-value	Variables	r-value p-value	Variables	r-value p-value
Cells: NO_2^-	-0.64, 0.04	Cells:PPR	0.73, 0.01	-	-
-	-	Cells:SRP	0.65, 0.04	-	-
-	-	Cells: NO_3^-	-0.78, 0.005	-	-

No significant correlations were found in ELB, and WLB was the only lake where a significant correlation between a bacterial parameter (cell counts, metalimnion) and a phytoplankton parameter (PPR) was found ($r = 0.73$, $p = 0.01$). Cell counts in all three layers of FRX were correlated to either nutrients or DO. Similarly, cell counts in the epilimnion and metalimnion of WLB were also correlated with nutrients. The only significant relationship between BP and an environmental parameter was between TdR and NH_4^+ in the FRX metalimnion ($r = 0.76$, $p = 0.009$).

DISCUSSION

The winter ecology of Antarctic terrestrial ecosystems is poorly understood, and the current study is part of the first investigation of Taylor Valley lake ecology during the summer-winter transition. A key hypothesis addressed by this study is that heterotrophic bacterial communities are indirectly affected by the onset of winter darkness via the loss of new organic carbon from primary production. Although I contend that DOC released by phytoplankton during photosynthesis and death/lysis imposes the greatest control on BP, factors including nutrient limitation, temperature, and grazing may also regulate BP.

Seasonal trends in BP

If BP were directly dependent on PPR alone, I would expect summer BP to be greater than fall BP. To test this hypothesis, I divided the sampling season into “summer” and “fall” based on the date of the first sunset (20 February 2008). Sample points prior to the sunset were considered to be summer, and all sample points after the sunset were considered to be fall. In ELB, and WLB TdR maxima occurred in the fall, while maxima for both TdR and Leu in FRX were measured in the summer. BP was highly variable during the season, with standard deviations often approaching the mean (see *Figure 11*). Takacs and Priscu (76) noted that BP tended to increase in TV lakes as the seasons progressed from spring through summer, and our results replicate this general trend during the summer, showing BP increasing between the early and mid-summer sample points.

A sharp decrease in BP was observed during early March (the “March crash”). In FRX and ELB, the crash was not related to a loss in bacterial cell numbers, as cell densities were highest during the crash. This was reflected in the loss rates, which showed gains of between 1×10^{10} and 20×10^{10} cells $\text{m}^{-2} \text{d}^{-1}$ leading up to the crash. Specific activity, however, was at a minimum in both FRX and ELB during the crash. It appears that, in FRX and ELB, the March crash can be attributed to a decrease in activity per cell. In WLB, specific activity increased during the crash, but cell densities were an order of magnitude lower than at previous time points. This was also reflected in the loss rates, which were high (between 0.3×10^{10} and 1×10^{11} cells $\text{m}^{-2} \text{d}^{-1}$) in WLB leading up to the crash.

There are several possible explanations for the observed decrease in physiological state that occurred during the drop in BP in FRX and ELB. Coveney and Wetzel (15) noted a similar sharp decrease and then rebound of BP in their seasonal study of Lawrence Lake, Michigan. In that study, the decrease was related to a sudden, seasonal precipitation of solutes from the water column. The BP crashes in FRX and ELB could not be attributed to any sudden change in the physical or chemical environment; rather a suite of chemical, physical, and biological factors exerts control over heterotrophic bacterial processes. Cell specific activity (and thus, BP) rebounded following the March crash, which indicates that the crash was due to a temporary condition. Temporal shifts in environmental parameters, including oxygen concentrations, temperature, inorganic nutrient availability, and amount and quality of DOC, have been reported to influence microbial community structures and activities in other systems (17, 29, 57, 65, 81, 88). It

is possible that bacterial productivity was initially suppressed by the decrease in PPR, but then adapted, perhaps through a shift in bacterial community structure or through a shift to a different DOC substrate type.

DOC and PPR: Controls on BP

The conventional “microbial loop” view of the aquatic food web is that planktonic microbial communities are supported by dissolved organic carbon (DOC) produced by phytoplankton (5). Such coupling between phytoplankton production and bacterial production has been described in various ecosystems ranging from Antarctic marine environments to tropical Lake Tanganyika (53, 54, 75). It has been suggested that strong bacterial dependence on phytoplankton carbon production occurs mainly in areas that are isolated from allochthonous inputs of organic carbon, such as the open ocean (53), although the bacteria-to-phytoplankton relationship is apparently confounded by grazing in some Antarctic marine systems (8).

The permanent ice cover of the TV lakes in combination with the nutrient poor surrounding soils, mean that outside sources of carbon are limited. Takacs et al. (79) estimated, based on summer measurements, that allochthonous and authochthonous (PPR) carbon sources are two to three times less than BP in lakes Fryxell and Hoare, and about 1.6 times greater than BP in Lake Bonney, annually. In no case was PPR alone enough to sustain measured levels of BP. My results, which showed a significant correlation between BP and PPR only in the epilimnion of ELB, support the idea of coupling between BP and PPR in that layer alone (see *Figures 9 and 10*). Tight coupling

of BP and PPR was not apparent in any of the other lakes during the study; however, there is evidence that bacterioplankton were affected by changes in DOC availability in WLB. DOC decreased significantly in WLB during the season (*see Figure 3*), and followed a similar pattern to PPR, although there was no significant correlation between the two (*Figure 18*). The March crash was contemporaneous with decreasing PPR in late February and early March. In WLB, cell densities reached a seasonal low (by an order of magnitude) at the time of the crash, and were positively correlated with PPR (*see Table 4*). While causation cannot be inferred from correlation alone, it seems reasonable to suggest that the decrease in cell density noted in WLB at the time of the crash was related to the decrease in PPR.

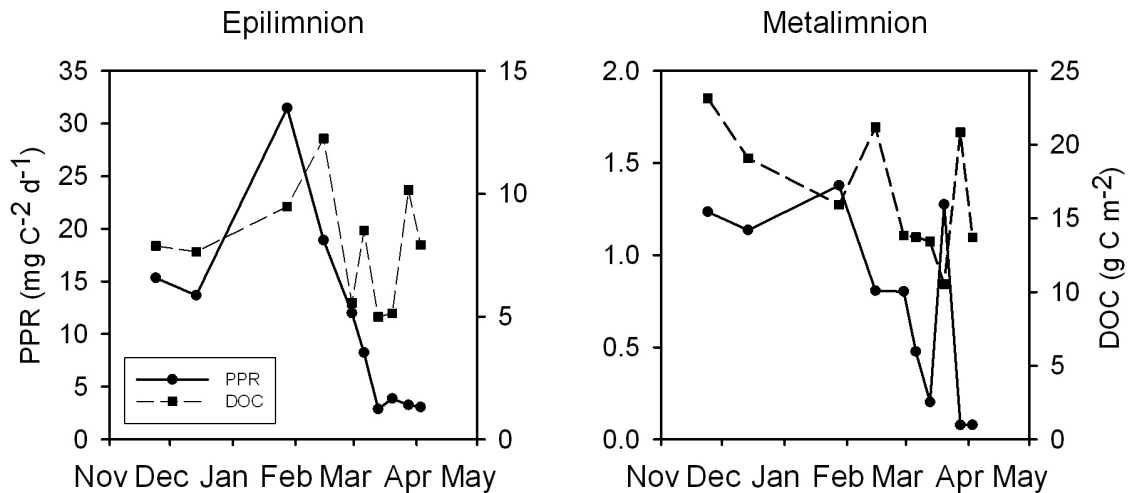


Figure 18. Seasonal trends in West Lobe Bonney PPR and DOC. WLB was the only lake with a significant decrease in DOC during the season, and DOC and PPR trends were similar, although the correlation was not statistically significant ($\alpha=0.05$).

Decreasing PPR during February and March would have diminished the pool of labile, fresh carbon available for heterotrophic growth only if the phytoplankton percent extracellular release of organic carbon (PER) did not increase throughout the season. Past

studies found that between 6 and 30% of PPR by TV lake phytoplankton goes to extracellular organic carbon (EOC) during the summer (52, 72). In these studies, PER varied among depths and lakes, but was not measured as a seasonal parameter, and the composition of the EOC was not determined.

Baines and Pace (6) synthesized 16 studies of PPR and EOC, which included 225 observations on both marine and freshwater systems and found that EOC was primarily constrained by PPR; however, the two were inversely related. Vegter and De Visscher found a positive correlation between EOC and PPR in a freshwater lake (71, 82), and a positive correlation between the two was also found in estuarine systems (71). Bjørnsen (9) noted that a portion of EOC results from the passive diffusion of low-molecular weight compounds such as mono- and oligosaccharides through the cell membrane, and that diffusion was proportional to phytoplankton carbon biomass (5% of total phytoplankton carbon biomass day⁻¹). Compounds of higher molecular weight may also be important phytoplankton exudates (56), and their contribution cannot be explained by the passive diffusion hypothesis; however, low molecular weight compounds have been found make up as much as 85% of total PER (21). Maurin et al. (47) noted that low molecular weight compounds (<700 Da) accounted for 66% of the phytoplankton EOC consumed by heterotrophic bacteria. These studies indicate that the diffusion of low molecular weight organic compounds is related to PPR, and that these compounds are important for heterotrophic growth.

Total EOC is not always directly proportional to PPR. The amount and quality of EOC has also been linked to nutrient availability (6, 11, 46, 57). Bratbak and Thingstad

(11), using a model system containing bacterial and phytoplankton cultures in a chemostat, showed that PER increased under phosphorus-limited conditions. Similarly, Mykkestad (55) found increased PER with N to P ratios in excess of the Redfield ratio (7, by weight). Nutrient limitation has also been found to affect the composition of the exudates, with P-depleted compounds favored under P-limited conditions (55). Obernosterer and Herndl (57) studied the effect of P-limitation on the quality and subsequent bacterial uptake of phytoplankton excreted DOM and found that while PER was greater under P-limited conditions than under balanced N:P ratios, the resultant DOM was not as readily utilized by bacteria.

Lake Bonney phytoplankton are known to be P-limited (20), with the greatest limitation occurring near the ice-water interface. If PER by Lake Bonney phytoplankton were controlled by nutrient deficiency, I would expect PER to be greater in the shallow than the deep waters of the lake; however, a previous study found that PER at the deep chlorophyll maxima of each lake (9 m in FRX and 15 m in ELB and WLB) was greater than at the ice-water interface (52). This may suggest that phytoplankton PER is not directly related to nutrient deficiency in these TV lakes; however, P-deficiency may affect the quality of the excreted DOM (57).

Assuming that PER is positively correlated to PPR (as shown by Vegter and De Visscher; (82)), the labile organic carbon pool would have decreased during the transition to polar night. Alternatively, if PPR and PER were inversely correlated (as shown by Baines and Pace; (6)), the percentage of PPR going to PER would have increased during the polar night transition. A conservative estimate, based on the maximum PER (30%)

for TV lakes measured by Moore (52), of the average EOC during March 2008 is between 2.18 and 0.14 mg C m⁻² d⁻¹ for the epilimnia and metalimnia of FRX, ELB and WLB. The range exceeds that of average BP during the same period in the ELB and WLB epilimnia by an order of magnitude, suggesting that EOC may have been enough to support BP in that layer. BP exceeded PPR in the metalimnia of ELB and WLB by between 30 and 50%. In FRX, the estimated EOC was an order of magnitude below BP in the metalimnion, and accounted for between 57% and 92% of BP in the epilimnion, indicating that even if PER increased to 30% of PPR, other carbon sources would still be necessary to support BP. Using 6% PER, the minimum measured by Moore (52), EOC from phytoplankton accounts for only between 7% and 56% of BP in ELB and WLB. Further, BP measurements underestimate the total bacterial carbon demand, which also includes bacterial respiration. If bacterial respiration (BR) is estimated according to the method of del Giorgio and Cole (25),

$$BR = 3.7 \times BP^{0.41}$$

as done by Takacs et al. (79) to estimate annual carbon budgets in the TV lakes, maximum EOC accounts for <50% of the total bacterial carbon demand. It is unlikely, even if PER increased, that EOC by phytoplankton provided enough organic carbon to support bacterial demand during the polar night transition.

The overall evidence does not support a model in which BP is unequivocally brought to a halt by the loss of new organic carbon from primary production. Possible explanations for this are that either the heterotrophic nanoplankton of these TV lakes obtain organic carbon from sources other than contemporaneous phytoplankton

production, or that their activities are limited by something other organic carbon. Takacs et al. (79) concluded that wintertime BP must be dependent on either the decomposition of particulate organic matter, or on the bulk DOC pool. Low-molecular weight DOC compounds such as fulvic acids, which are products of microbial activity, account for about 20% of the total DOC pool in the TV lakes (49). Most of the remainder of the pool is presumed to be recalcitrant (79).

Decomposition of biomass during the winter may also be an important carbon source for heterotrophic bacterioplankton. chl-*a* concentrations increased throughout the season, and did not decrease as PAR and PPR decreased during the transition to polar night. The increase was statistically significant in the epilimnia of ELB and WLB (linear regression; $r^2 = 0.83$ and 0.92 , respectively; $p < 0.0001$ for both). Lizotte et al. (43) noted temporal separation in the development of PPR and chl-*a* maxima in ELB during the winter-spring transition. Lizotte et al. (43) also detected photosynthetic activity early in the spring, indicating that some standing stock of phytoplankton cells is maintained during the winter.

Using ELB epilimnion integrated spring chl-*a* concentrations estimated from Lizotte et al. (43) of 4 mg m^{-2} in combination with late season chl-*a* concentrations from this study of $\sim 30 \text{ mg m}^{-2}$ I calculate that approximately 86% of phytoplankton biomass was lost during the winter. Based on the average C:Chl ratios in ELB calculated by Sharp (72); $70 \text{ g C (g chl)}^{-1}$) approximately 1000 g C m^{-2} would be made available to heterotrophs in the ELB epilimnion through decomposition of phytoplankton stocks during the winter. Using the average (February to April) mean Leu BP rate measured in

the ELB epilimnion ($1 \text{ mg C m}^{-2} \text{ d}^{-1}$) as a high estimate of BP during the winter (May to November total 184 mg C m^{-2}), BP is less than 1% of the total potential supply from phytoplankton decomposition. These calculations assume that phytoplankton biomass and C:Chl ratio entering the winter was the same in the our study and the Lizotte study.

Chemolithoautotrophy is also a potentially relevant autochthonous source of organic carbon. It has been posited that chemolithoautotrophically fixed carbon is important in TV lake systems, and rates of dark carbon fixation in FRX have been measured at as much as 82% of phytoplankton carbon fixation (Vick, unpublished data). If these rates continue throughout the winter, they may help to sustain the heterotrophic bacterial population. It should be noted, however, that the measured rates of dark carbon fixation include possible anapleurotic reactions by bacteria and phytoplankton, which would not yield new carbon to the system.

Allochthonous carbon sources may also be important in maintaining the overall carbon pool, although addition from outside the water column is most likely to occur during the summer, when lake ice is more permeable to small particles (31) and glacial melt-water streams are running. Carbon and other nutrients may be added to the water column as particles transported through the lake ice. Jepsen et al. (31) showed that sediments moved through the FRX lake ice during the summer of 2007-2008 at rates of 1.3 cm day^{-1} on average, and that fine particles can be transported through the lake ice at $0.1 \text{ g m}^{-2} \text{ h}^{-1}$ during the summer melt season. This particle flux rate is likely significant with regard to overall sedimentation rates in the lakes (31), but the amount of carbon contributed by these processes is unknown. The lakes also receive allochthonous carbon

inputs from ephemeral glacial melt water streams, which are thought to be small in comparison to the total carbon pool (49). This suggests that autochthonous carbon sources are likely more important than allochthonous sources, particularly during the winter.

Inorganic Nutrients: Controls on BP

The availability of inorganic nutrients (N and P) can also be important in controlling BP. A study in mesotrophic temperate lake systems found that where the molar N:P ratio was <40 (or <17 by weight), BP and PPR were less likely to be coupled than in systems where the molar N/P ratio was >40 due to similar responses of bacterioplankton and phytoplankton to nutrient limitation (41). This relationship alone cannot explain the uncoupling of BP and PPR in the TV lakes (*see Figures 9 and 10*), as N:P weight ratios in the current study ranged from <17 in the shallow waters of FRX to >400 in the deep waters of WLB.

Dore and Priscu (20) found that phytoplankton in FRX, ELB, and WLB responded positively to the addition of P in nutrient bioassays and showed that bacterial alkaline phosphatase at 5 m in ELB was suppressed in response to P addition. These experimental results provide strong evidence that phytoplankton in all three lakes, and near surface bacterioplankton in ELB are P-deficient. P-deficiency would likely endure year-round, given that DIN:SRP remained one to two orders of magnitude above Redfield ratio (7 by weight) in ELB and WLB throughout the season (*see Figure 2*). DIN:SRP ratios in FRX were below Redfield ratio throughout the season, and no large-

scale seasonal trend in was detected. Thus, it is possible that bacterioplankton in these lakes are limited by the availability of inorganic nutrients to the extent that seasonal fluctuations in phytoplankton excreted DOC exert little control over BP on a seasonal basis.

Light and Temperature: Controls on BP

My results showed that PPR decreased as irradiance (underwater PAR) decreased during the transition to polar night. BP has been linked to irradiance in oligotrophic waters of the North Pacific Ocean, where one study found that increasing light was correlated with increasing incorporation of ^3H -leucine (14). Phytoplankton were present during incubations, so the observed increase in ^3H -leucine incorporation may have been an artifact of coincident increases in photosynthetic primary production. The authors noted that BP in the North Pacific is dependent on DOC released by “sloppy feeding” of grazers, rather than PPR, and suggest that stimulation of PPR alone would not have increased BP. Alternatively, the increase in ^3H -leucine incorporation may have been due to photoheterotrophic uptake by cyanobacteria. Light stimulation of ^3H -leucine incorporation in the North Atlantic has been correlated with photoheterotrophic activity of the common oceanic cyanobacterial genera *Prochlorococcus* and *Synechococcus* (50). Incubations in my 2007-2008 study were conducted in the dark (see *Methods*), minimizing the possible influence of photoheterotrophic activity by cyanobacteria present in the TV lakes.

In my 2010 season BP methods validations, I found that ELB Leu is significantly higher in the light than the dark ($p < 0.001$) at 13 m when phytoplankton are present in the incubation, but that there was no significant difference between light and dark incubations for TdR or Leu in FRX at 10 m. Moore (52) found that TdR was significantly higher in the light than the dark in ELB at 5 m, but higher in the dark than the light at 9 m. Because incubations were done with whole lake water, elevated BP in the light may have been due to phytoplankton production of organic carbon or photoheterotrophy by cyanobacteria. Differences between lakes and among depths indicate that BP and irradiance are not directly related in the TV lakes; rather bacterial communities are ultimately impacted by irradiance via the activities of primary producers.

Temperature is commonly thought to limit BP in marine and aquatic systems (15, 26, 83). Temperature profiles in the lakes are relatively stable, as the permanent ice cover protects them from oscillations in air temperature, so any temperature effect would be perennial rather seasonal. Temperature experiments in ELB (*see Figure 8*) showed that the temperature optimum for leucine incorporation at 13 m occurred at 18°C, about 13°C warmer than *in situ* temperature, implying that bacterial activity is suppressed by temperature at that depth. In FRX, the maximum rates of thymidine and leucine incorporation were measured at 18 and 14°C, respectively, which are between 11 and 15°C above *in situ* temperature. These results support the conclusion by Ward and Priscu (85) that bacteria in TV lakes are growing below their temperature optima. Temperature is likely to be important in regulating BP in the TV lakes year round.

Bacterial Losses and Gains: Causes and Consequences

Bird and Karl (8) found uncoupling of bacterial and phytoplankton biomass in the Gerlache Strait, Antarctic Peninsula, which was attributed to grazing on bacteria. They showed that bacterial biomass was negatively correlated with phytoplankton biomass (as chl-*a*) during phytoplankton bloom conditions due to increases in protistan grazing rates on bacteria. This is a prime example of top-down control in an Antarctic marine ecosystem. Antarctic lake ecosystems are generally thought to be subject to bottom-up controls, rather than top-down (7, 66) owing to a general lack of grazing pressure on both phytoplankton and bacterioplankton. The bulk of TV lakes grazing studies have been carried out in Lake Hoare, a non-chemically stratified lake located in the mid-TV between FRX and ELB (see *Figure 1*). Roberts et al. (66) noted that mixotrophic cryptophytes may have some impact on bacterioplankton communities in Lake Hoare but that ciliate grazing rates were not high enough to impact bacterioplankton abundance. Their overall conclusion was that top-down controls are unlikely to have a significant impact in Lake Hoare.

Marked decreases in bacterial abundance were noted in the current study in the epilimnia of ELB and WLB during late February and early March, as indicated by loss rates of 0.2 and 17×10^{10} cells $\text{m}^{-2} \text{d}^{-1}$. It is possible that phototrophic nanoflagellates (PNAN) capable of mixotrophy began to ingest bacterioplankton as PAR decreased. Preliminary results of grazing experiments using fluorescently labeled bacterioplankton as prey for cryptophytes indicate that rates of bacterivory may have increased (from 0.06 to 0.24 bacterial cell h^{-1}) during March 2008 in the shallow waters of Lake Hoare

(Thurman, unpublished data). The statistical significance of these results is not known, but if these rates were converted from cell h⁻¹ to mg carbon d⁻¹ (based on 11 fg C cell⁻¹; see *Methods*), then PNAN would have been ingesting approximately 6.3 x 10⁻¹¹ mg C d⁻¹ in late March. This estimate cannot account for the observed loss rates in any of the lakes. These results support previous conclusions that the bacterioplankton populations in the TV lakes are regulated by bottom-up controls such as organic and inorganic nutrient supply and low temperature. However, it is possible that mixotrophy is an important factor in phytoplankton winter survival, without heavily impacting bacterioplankton dynamics.

Other mechanisms of bacterial cell loss are sinking, cell death, and viral lysis. Sinking rates are low in these lakes (61, 76), and decomposition of dead cells is slow, owing to low temperatures. Virus-like particles (VLP) on the order of 10⁶ VLP ml⁻¹ are present in TV lakes (35), and lysogenic bacteria account for up to 63% of the total bacterial population in the lakes. If the onset of polar night induced lysogeny, bacterial loss rates would increase.

Physiological Responses: DNA and Protein Synthesis

³H-thymidine incorporation and ³H-leucine incorporation are commonly used as methods for measuring bacterial DNA and protein synthesis, respectively (13). Rates of TdR and Leu have been found to covary over time and space (1, 37), and TdR and Leu followed similar overall trends in the TV lakes throughout the season (see *Figure 11*). The ratio of TdR to Leu can be an indicator of the physiological state of the organisms.

TdR:Leu ratios = 1 suggest equal investments in biomass synthesis (Leu) and cell division (TdR); ratios $\neq 1$ may reveal impending changes in growth rates (12).

TdR:Leu ratios varied between lakes, and within lakes over time. The ratio was ≤ 1 in ELB and WLB throughout the season. In WLB, the ratio was consistently < 1 in all three layers. In the epilimnion of ELB, the ratio was near 1:1 early in the season, and decreased later in the season owing to a relative increase in Leu incorporation. The ratio in the metalimnion and hypolimnion in ELB was consistently < 1 . BP and cell specific BP were always lower in the deeper waters of ELB, compared to the shallow depths, and this has been suggested to be the result of low temperature, high salt concentrations (85), and the presence of toxic levels of bioactive metals at depth (84). The same factors that limit BP may also cause the organisms to favor protein production over cell division, thus shifting the TdR:Leu ratio to < 1 .

TdR:Leu was > 1 at the first sample point in the FRX epilimnion, and all season in the FRX metalimnion and hypolimnion. The FRX metalimnion was the only strata in which Model II regression detected a significant, negative correlation between TdR and Leu (*see Figure 17*). FRX also had the highest overall rates of BP of any of the lakes (TdR maximum of 6 to 20 times ELB and WLB maxima). Higher BP in FRX may be attributed, at least in part, to the increased availability of energy sources in the sulfidic oxic-anoxic interface. The interface supports active communities of sulfur-oxidizing chemolithoautotrophic and purple non-sulfur bacteria (32, 70), which may provide the heterotrophic microorganisms with a consistent supply of fresh labile carbon. This relatively carbon-rich environment may allow the microorganisms to sustain higher

growth rates, which would manifest as an increased TdR:Leu ratio. Nutrient profiles together with results from phytoplankton nutrient bioassays (62) suggest that organisms in FRX may be less P-deficient than those in ELB and WLB, which may also explain the TdR:Leu ratios >1 in this lake. P is required for DNA synthesis, but is less important when synthesizing proteins.

TdR:Leu ratios can also be indicative of shifts in cellular function. A ratio of <1 indicates a shift from population growth to survival (73). RNA synthesis is the first to increase when bacteria shift to a higher growth rate, followed by protein synthesis, and then DNA synthesis. When growth rates decrease, as in a shift towards survival mode, DNA synthesis decreases first, followed by protein and RNA synthesis (12). Such a shift would manifest as an increase of Leu relative to TdR. BP in the FRX metalimnion favored TdR over Leu (TdR:Leu >1) throughout the season, but there was a relative increase in Leu late in the season. A similar trend was noted in ELB. This apparent shift from DNA synthesis to protein synthesis may be explained a variety of ways. Heterotrophic bacterial communities may be shifting to a lower growth rate in preparation for the darkness of the approaching polar night, and the change may be manifested by a decrease in TdR:Leu.

In addition to the darkness of the polar night, microorganisms living in the TV lakes must also contend with extreme environmental conditions year-round, including low temperatures and high salinity, and nutrient deficiency (20, 62, 78). Bacterioplankton may favor protein production over replication in order to increase survival under stressful conditions (73), as suggested by TdR:Leu ratios <1 in ELB and

WLB. Bacteria living in cold environments are also known to have slow doubling times, but still need to produce protein to survive. This may shift their productivity profiles in favor of protein production, thus depressing the TdR:Leu ratio to <1 . TdR and Leu covaried in WLB during the season with TdR:Leu maintaining a relatively constant ratio of 0.58 ± 0.16 in the epilimnion and 0.22 ± 0.15 in the metalimnion (*see Figure 11*). Perhaps higher protein synthesis relative to DNA synthesis in WLB may not be due to a transient, seasonally dictated shift in growth rate, but a permanent condition resulting from environmental conditions (e.g. nutrient limitation, temperature) in the lake year-round.

Conclusions

My study is the first to sample the bacterioplankton of McMurdo Dry Valley lakes Fryxell, East Lobe Bonney, and West Lobe Bonney during the transition from summer to the polar night. Results clearly indicate that a complex suite of interactions exists between the biotic and abiotic factors in the lakes studied. The three lakes responded differently to the onset of winter, with linkages between bacterioplankton and phytoplankton EOC being strongest in WLB. There is evidence for bacterioplankton adaptation to the change in seasons in each of the lakes, although determining the importance of community succession versus physiological adaptation will require further study.

BP decreased during March in the lakes, which I attribute to decreases in cell specific activity in FRX and ELB, and a decrease in cell density in WLB. Cell specific

activity increased in WLB during the same period. The decrease in specific activity in FRX and ELB was contemporaneous with decreases in PPR, which may have impacted the labile organic carbon pool, although the overall DOC concentration in these lakes seemed unaffected by the cessation of PPR.

Bacterioplankton in FRX and ELB may have adapted to the loss of new labile carbon, either by shifting to different carbon substrates, or by reallocating carbon resource use from DNA synthesis and cell division to protein synthesis in preparation for winter. In WLB, cell stocks recovered after the crash, but specific activity and BP did not, particularly in the epilimnion. WLB was the only lake in which a decline in DOC was noted, and it seems likely that WLB bacterioplankton were carbon-starved during the onset of winter.

Data from my study indicate that bacterioplankton in these lakes respond to various factors that prevail over the season. Importantly, my data, in concert with winter-spring data indicate that bacterioplankton remain active throughout the winter when the lakes enter a period of “heterotrophy” which may be supplemented by unknown inputs from chemolithoautotrophs. The prevalence of heterotrophic processes throughout the year is the primary reason that the Production:Respiration ratios in the lakes are below unity on an annual scale (60). Bacterioplankton activity will eventually cease under this scenario unless the lakes are “reset” by major climate events.

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APPENDICES

APPENDIX A

SITE DESCRIPTION AND METHODS

Table A.1. Sampling dates and depths. Notes indicate any special circumstances.

DATE	ICE THICKNESS (m)	NOTES
Lake Fryxell		
11-Nov-07	4.7	
5-Dec-07	4.6	
28-Dec-07	-	No Leu
19-Jan-08	4.7	No Leu
6-Feb-08	-	No Leu
27-Feb-08	-	
5-Mar-08	3.6	
12-Mar-08	3.6	
20-Mar-08	3.6	No samples below 12 m
28-Mar-08	3.6	
8-Apr-08	-	
East Lobe Bonney		
23-Nov-07	3.6	
13-Dec-07	3.5	
1-Jan-08	-	No Leu
26-Jan-08	3.4	No Leu
13-Feb-08	-	No Leu
27-Feb-08	-	
4-Mar-08	3.3	
11-Mar-08	3.3	
18-Mar-08	3.3	No samples below 22 m
25-Mar-08	-	No samples below 22 m
1-Apr-08	-	
10-Apr-08	-	
West Lobe Bonney		
25-Nov-07	3.7	
15-Dec-07	3.6	
2-Jan-08	-	No Leu
29-Jan-08	3.5	No Leu
16-Feb-08	-	No Leu
1-Mar-08	3.5	
7-Mar-08	3.5	
14-Mar-08	3.4	
21-Mar-08	-	No samples below 25 m
29-Mar-08	3.4	No samples below 25 m
4-Apr-08	-	

Table A.2. Routine sampling depths for this study. X's indicate depths where samples were taken routinely. Cases where sampling diverged from this scheme are highlighted in Appendix A. BP = Bacterial Productivity; PPR = Primary Productivity; BIOMASS = bacterial abundance; CHL = chl-*a*; NUT = Inorganic N and P; DO = Dissolved Oxygen; DOC = Dissolved Organic Carbon

LAKE	SAMPLE DEPTH (m)	BP	PPR	BIOMASS	CHL	NUT DO DOC
FRX	5	X	X	X	X	X
	6	X	X	X	X	X
	7		X	X	X	X
	8	X	X	X	X	X
	9	X	X	X	X	X
	10	X	X	X	X	X
	11	X	X	X	X	X
	12		X	X	X	X
	15	X		X	X	X
	17.5	X		X	X	X
ELB	4.5	X	X	X	X	X
	5	X	X	X	X	X
	6		X	X	X	X
	8		X	X	X	X
	10	X	X	X	X	X
	12		X	X	X	X
	13	X	X	X	X	X
	15	X	X	X	X	X
	18	X	X	X	X	X
	20	X	X	X	X	X
	22		X	X	X	X
	25	X		X	X	X
	30	X		X	X	X
	35			X	X	X
	37			X	X	X
WLB	4.5	X	X	X	X	X
	5	X	X	X	X	X
	6		X	X	X	X
	8		X	X	X	X
	10	X	X	X	X	X
	12		X	X	X	X
	13	X	X	X	X	X
	14	X	X	X	X	X

Table A.2. Continued

LAKE	SAMPLE DEPTH (m)	BP	PPR	BIOMASS	CHL	NUT DO DOC
WLB	15	X	X	X	X	X
	17	X	X	X	X	X
	20		X	X	X	X
	22			X	X	X
	25	X		X	X	X
	30	X		X	X	X
	35			X	X	X
	37			X	X	X
	15	X	X	X	X	X

Table A.3. Average coefficients of variation (CV) from thymidine (TdR) and leucine (Leu) methods validation experiments.

	FRX (TdR)	FRX (Leu)	ELB (Leu)
Experiment	Avg. CV	Avg. CV	Avg. CV
Time-series	26.2	17.1	16.3
Concentration-series	47.8	19.8	4.6
Temperature-series	60.9	31.0	14.9
Light/dark	13.6	18.1	2.9

APPENDIX B

PHYSICAL AND CHEMICAL PARAMETERS

Table B.1. Inorganic Nutrients Dilution Schedule. Dilutions were applied to NH_4^+ , NO_2^- , NO_3^- , and SRP analyses.

Lake Fryxell									
11-Nov-07		5-Dec-07		28-Dec-07		19-Jan-08		6-Feb-08 to 8-Apr-08	
Depth (m)	Dilution factor	Depth (m)	Dilution factor	Depth (m)	Dilution factor	Depth (m)	Dilution factor	Depth (m)	Dilution factor
5-9	1:1	5-9	1:1	5-9	1:1	5-9	1:1	5-9	1:1
10	1:10	10	1:10	10	1:10	10	1:10	10	1:10
11	1:100	11	1:10	11	1:100	11	1:50	11	1:100
12	1:100	12	1:100	12	1:50	12	1:50	12	1:100
15	1:100	15	1:100	15	1:100	15	1:100	15	1:100
17.5	1:100	17.5	1:100	17.5	1:100	17.5	1:50	17.5	1:100
East Lobe Bonney									
23-Nov-07		13-Dec-07		1-Jan-08		26-Jan-08 to 11-Mar-08		18-Mar-08 to 10-Apr-08	
Depth (m)	Dilution factor	Depth (m)	Dilution factor	Depth (m)	Dilution factor	Depth (m)	Dilution factor	Depth (m)	Dilution factor
4-13	1:1	4-13	1:1	4-13	1:1	4-13	1	4-13	1:1
15-22	1:10	15-22	1:10	15-22	1:10	15-22	10	15-22	1:10
25	1:50	25	1:10	25	1:50	25	50	25	1:50
30-37	1:50	30-37	1:50	30-37	1:50	30-37	50	30-37	1:50
West Lobe Bonney									
25-Nov-07 to 14-Mar-08					21-Mar-08 to 29-Mar-08		4-Apr-08		
Depth (m)	Dilution factor				Depth (m)	Dilution factor	Depth (m)		Dilution factor
4-10	1:1				4-10	1:1	4-10		1:1
12-38	1:10				12-25	1:10	12-38		1:10

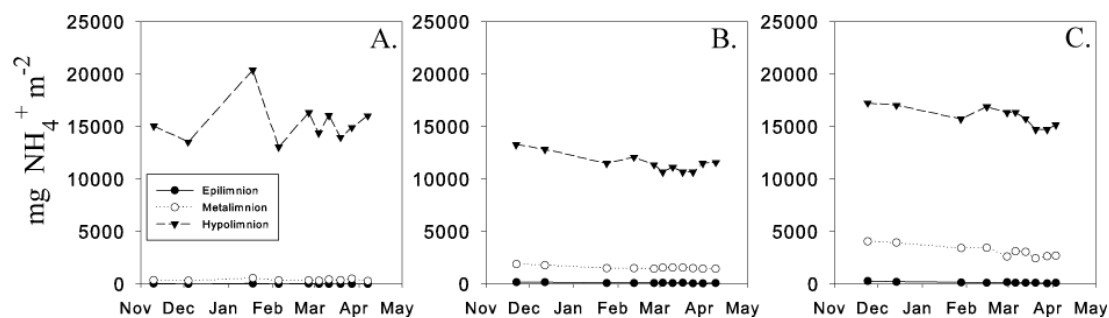


Figure B1. Seasonal ammonium profiles. Data are integrated over the layers shown in the legend. A=FRX, B=ELB, C=WLB.

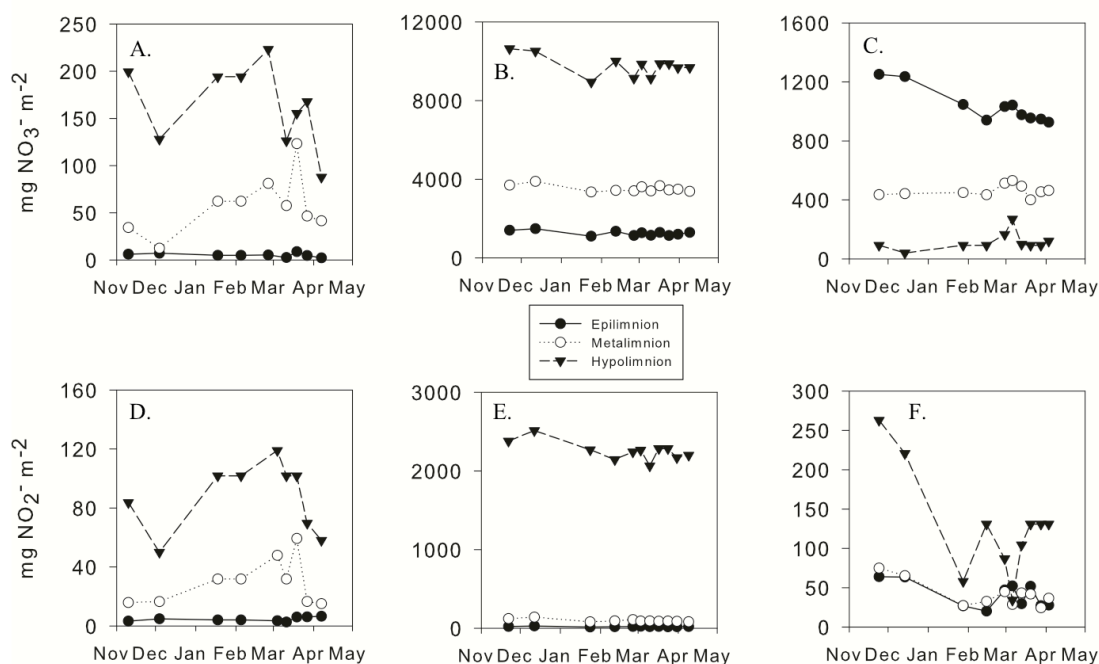


Figure B.2. Season-long nitrate and nitrite profiles. Values are integrated over the layers given in the legend. A&D = FRX; B&E = ELB; C&F = WLB.

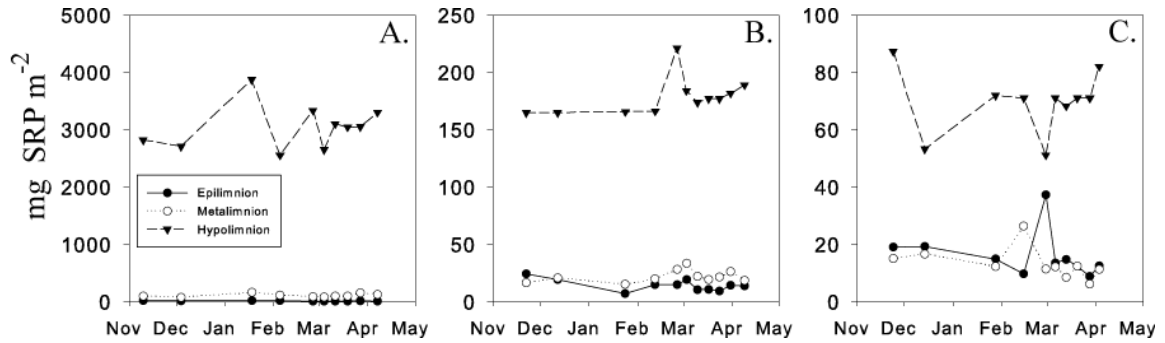


Figure B.3. Season-long SRP profiles. Values are integrated over the layers given in the legend. A=FRX, B= ELB, C=WLB

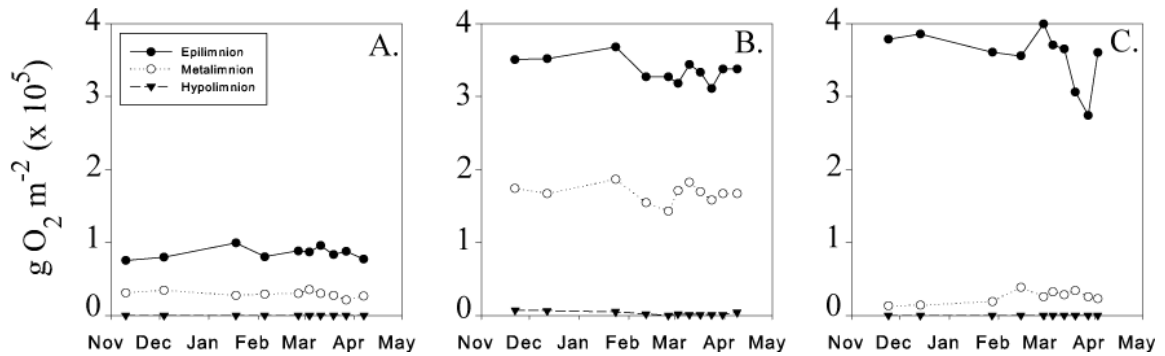


Figure B.4. Season-long dissolved oxygen profiles. Values are integrated over the layers given in the legend. Note the differences in scales among lakes. (A = FRX, B = ELB, C = WLb).

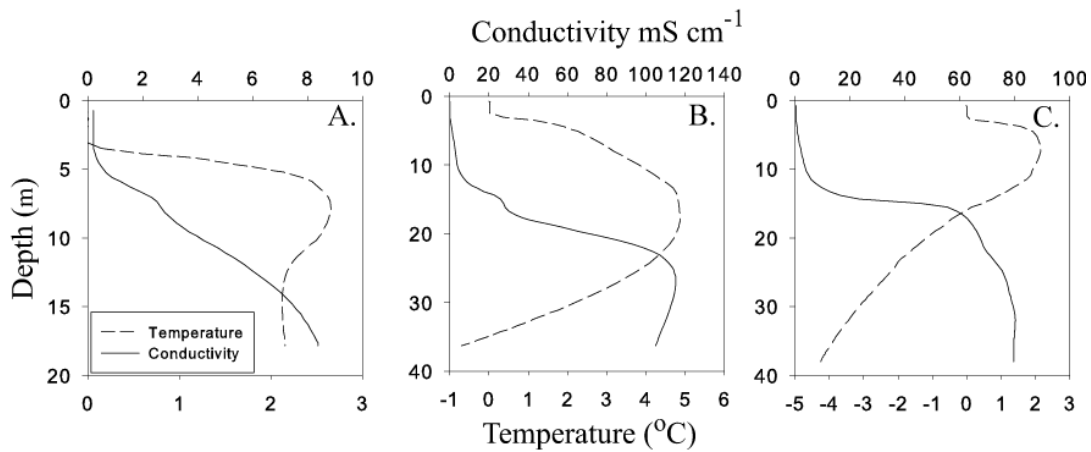


Figure B.4. Temperature and conductivity profiles from each lake A = FRX, B = ELB, C = WLb). Note the differences in scales.

APPENDIX C

BIOLOGICAL PARAMETERS

Table C.1. Cell counts (seasonal averages) for each depth in each lake.

FRX		ELB		WLB	
Depth (m)	Avg. Cells/ml	Depth (m)	Avg. Cells/ml	Depth (m)	Avg. Cells/ml
5	1.4×10^6	4	6.0×10^5	4	5.1×10^5
6	1.6×10^6	5	4.4×10^5	5	4.8×10^5
8	1.2×10^6	10	6.0×10^4	10	1.5×10^5
9	1.2×10^6	13	6.2×10^4	13	7.0×10^4
10	1.4×10^6	15	1.7×10^4	14	2.9×10^4
11	1.5×10^6	18	4.0×10^4	15	3.7×10^4
15	1.8×10^6	20	1.3×10^5	17	4.3×10^4
18	1.9×10^6	25	3.0×10^4	25	5.6×10^4
-	-	30	1.3×10^4	30	8.1×10^3

Table C.2. Seasonal average loss rates (positive) and gains (negative) and average specific losses (positive) and gains (negative) for each layer of each lake.

Lake	Avg. Bacterial cell loss rate ($\times 10^{10}$ cells $\text{m}^{-2} \text{d}^{-1}$) for TdR	Avg. Bacterial cell loss rate ($\times 10^{10}$ cells $\text{m}^{-2} \text{d}^{-1}$) for Leu	Avg. Spec. Loss ($\times 10^{-2} \text{d}^{-1}$) TdR	Avg. Spec. Loss ($\times 10^{-2} \text{d}^{-1}$) Leu
East Lobe Bonney				
Epilimnion	6.52	8.40	5.28	4.64
Metalimnion	2.48	3.29	10.01	14.75
Hypolimnion	-0.09	-0.05	-0.91	-0.58
West Lobe Bonney				
Epilimnion	5.18	6.70	2.15	4.77
Metalimnion	0.72	1.78	13.73	19.95
Hypolimnion	0.80	0.04	3.82	1.38
Fryxell				
Epilimnion	8.62	13.62	2.15	3.11
Metalimnion	19.23	11.53	6.93	4.01
Hypolimnion	1.32	0.95	0.22	0.22