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# Geomicrobiology of Blood Falls: An Iron-Rich Saline Discharge at the Terminus of the Taylor Glacier, Antarctica

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

**Key words:** Antarctica, McMurdo Dry Valleys, subglacial environment, saline lakes, pliocene sea, microbial diversity

## 1. Introduction

The subglacial environment has long been studied for its role in chemical weathering and its influences on glacier stability, sediment erosion, transport and deposition (Benn and Evans, 1998). Subglacial chemical weathering was originally thought to consist of strictly inorganic proton- and hydroxyl-promoted dissolution reactions (Raiswell, 1984; Chillrud et al., 1994). However, Sharp et al. (1999) challenged this idea with evidence for active subglacial microbial populations. Sharp and colleagues measured sulfide oxidation and cell division in basal ice samples from

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Arctic glaciers. Skidmore et al., (2000) studying the subglacial environment of Canadian glaciers, detected nitrate reduction, sulfate reduction and methanogenesis. Such microbially mediated redox reactions would provide important proton sources that could enhance subglacial silicate weathering. The metabolic by-products of some microorganisms, such as organic acids or extracellular polysaccharides, might also contribute to subglacial weathering via ligand-promoted dissolution. Welch and Ullman (1999) found that ligand-promoted weathering reactions were particularly effective at feldspar dissolution at low temperatures (between 5 and 35 °C). In a subglacial setting, the source of organic carbon for microbial life could be sedimentary material overridden by the advancing glacier and finely ground by subsequent abrasion processes. In most cases, this sedimentary material is permafrost soil (Sharp et al., 1999; Skidmore et al., 2000). There is a growing consensus that life plays an important role in earth surface processes, however, there is still a paucity of data on the role of microbes in rock weathering, and specifically subglacial processes (Lüttge and Conrad, 2004).

Blood Falls is thought to be a remnant marine feature originating below the Taylor Glacier in the McMurdo Dry Valleys, southern Victoria Land, Antarctica. It is arguably the oldest liquid water feature in the Taylor Valley dating back to a time when the valley network was fjord-like. During the Pliocene Epoch (3–5 million years ago), the Earth experienced warmth greater than today (Kennett and Hodell, 1993). This warming event resulted in the incursion of marine waters from the Ross Sea embayment (Denton et al., 1993). When these Pliocene waters retreated from the valley network, a remnant sea remained near the Taylor Glacier terminus. The present major ion chemistry of the Blood Falls outflow records the signature of its geological history. Data from chloride isotopes strongly support a marine origin of Blood Falls with its bulk chemistry resembling that of cryoconcentrated seawater (Lyons et al., 1999; 2001). A modest expansion of the Taylor Glacier during the late Pliocene or Pleistocene (Marchant et al., 1993) likely covered this concentrated sea, leaving a liquid water remnant of Pliocene marine waters sealed beneath the Taylor Glacier.

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

## 2. Study Area

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

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

The prominent coloration of Blood Falls holds true today, though release events appear to be episodic. The volume and physical extent of Blood Falls outflow from the glacier terminus has varied over time, as has the concentration of ions present in the outflow (Black et al., 1965; Keys, 1979; Lyons et al., in press). Black and Bowser (1968) documented an advance of the Taylor Glacier between 1965 and 1966 of "a few meters", an event which caused slumping of a portion of the glacier terminus into Lake Bonney. The authors further reported, "The advance produced the fractures at the northeast corner of the glacier through which the saline discharge took place." Other investigators made similar observations of an "upwelling of highly saline waters" at the Taylor Glacier snout in the early sixties (Hamilton et al., 1962; Black et al., 1965). The last major discharge occurred between February and August 1991 and flowed several hundred meters over

*Table I.* The major ionic ratios of Blood Falls outflow as compared with seawater and West Lobe Lake Bonney at 38 m depth

| | $Na^+$:$Cl^-$ | $Mg^{2+}$:$Cl^-$ | $Ca^{2+}$:$Cl^-$ | $SO_4^{2-}$:$Cl^-$ | DIC:$Cl^-$ | $K^+$:$Cl^-$ | $Cl^-$:$Br^-$ |
|---|---|---|---|---|---|---|---|
| Blood Falls | 0.88 | 0.200 | 0.085 | 0.980 | 0.040 | 0.016 | 569 |
| Seawater | 0.86 | 0.097 | 0.019 | 0.052 | 0.004 | 0.190 | 649 |
| West Bonny 38 m | 0.78 | 0.175 | 0.027 | 0.020 | 0.034 | 0.019 | 451 |

the surface of the Lake Bonney ice cover, afterwards high iron levels were detected in the deep (25 m) saline waters of the west lobe (Priscu, personal observation).

The major ion geochemistry of Blood Falls outflow waters is summarized in Table I and compared with ratios of seawater and the west lobe of Lake Bonney at 38 m depth. Blood Falls has a Na:Cl (0.88) and Cl:Br (569) ratio similar to that of seawater (0.86 and 649, respectively). A seawater signature is also found in the major ion chemistry of the bottom waters of the west lobe of Lake Bonney. Blood Falls is enriched in magnesium (Mg:Cl = 0.200) and calcium (Ca:Cl = 0.085) with respect to seawater (0.097 and 0.019, respectively) and possibly represents the effects of weathering and evaporation experienced by these waters over time. Discharge from Blood Falls in 1991 was so large that by August, a reddish-orange slush covered extensive areas of shoreline next to the glacier and water samples collected between 20 and 25 m in the west lobe during October contained such high iron levels that a noticeable iron oxide precipitate developed when exposed to air (the iron was reduced under *in situ* lake conditions) (Priscu, personal observation). A similar reddish-brown residue is also observed on filters used to collect water samples from depths below 20 m in the west lobe of Lake Bonney today.

## 3. Methods

### 3.1. SAMPLE COLLECTION

Samples were collected from subglacial outflow waters associated with Blood Falls discharge during austral summers from 1999 to 2003. Samples near the outlet of Blood Falls were collected for enumeration and bacterial activity in 2000 and 2001. To capture a profile of releases from Blood Falls into Lake Bonney, a transect of samples (TransX) was collected in December (see Figure 1), 2002 starting at the source of the outflow (Trans1) and out onto the moat ice surrounding the lake with increasing distance from the source (Trans2–5). Trans2 was collected from basal ice below the source, Trans3 was collected from waters at the shoreline where Blood Falls meets the moat ice, Trans4 was collected 5 m from the shoreline just below moat ice that melted and refroze daily, and Trans5 was collected under 0.4 m of moat ice approximately 70 m from the shoreline before the moat ice meets the

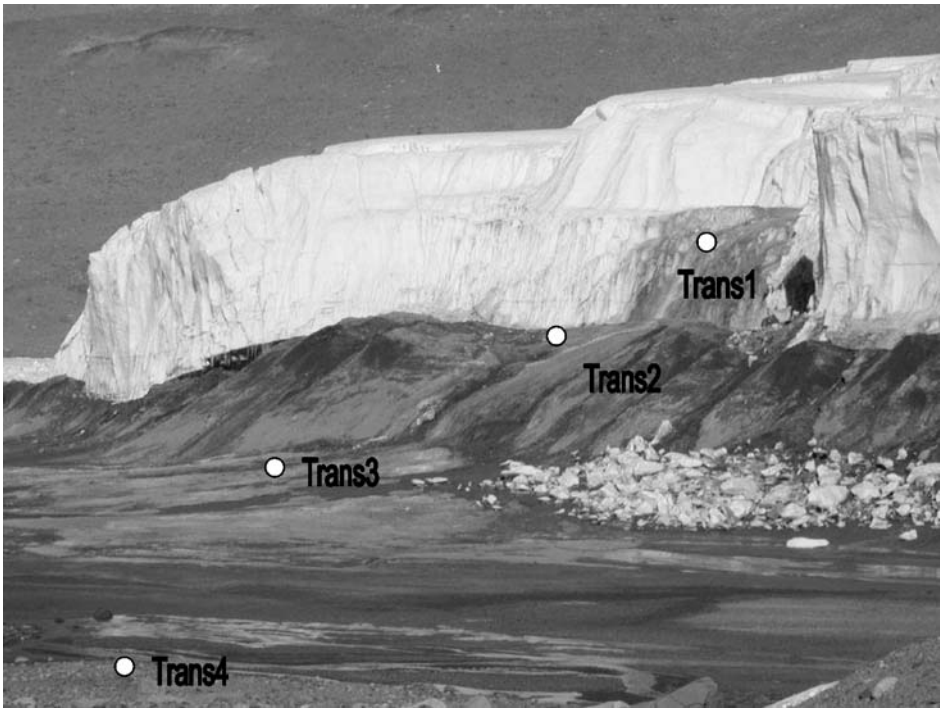


*Figure 1.* Blood Falls at the terminus of the Taylor Glacier in December 2002. Transect sample collection points are marked. Trans5 is not visible in this image.

permanent ice cover of Lake Bonney. Autoclaved metal spatulas were used to scrape ice samples into sterile plastic bags (whirl-Pak, Nasco Plastics, New Hamburg, Ontario, Canada) or sterile plastic conical tubes (Falcon). Subsequent manipulations were carried out aseptically in a positive flow hood. Subglacial flow water was collected in clean Nalgene bottles for geochemical analysis. All ice samples were melted slowly at 2–4 °C in sterile containers before analysis. Samples for analysis of dissolved organic carbon were collected in acid-washed, combusted (450 °C for $>4$ h) amber glass bottles. Dissolved inorganic carbon samples were collected in gas tight glass serum vials. Samples collected for bacterial enumeration were treated with formalin (2% final concentration) to prevent a change in bacterial numbers during storage (about 2 weeks) before counting.

### 3.2. GEOCHEMISTRY

pH was measured with a calibrated Beckman Φ12 pH meter. $E_h$ was measured using a Hach ORP combination electrode (Hach Company, Loveland, CO) connected to a Beckman Φ12 digital voltmeter, with corrections for the

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

Conductivity was measured at 1-meter depth intervals in the west lobe of lake Bonney using a Seabird SBE-25 CTD (Sea-Bird Electronics Inc., Bellevue, Washington). Salinity was calculated using the UNESCO salinity algorithm available in the Seabird data acquisition software package (Sea-Bird Electronics, 1998). Modifications for the high salinity concentrations found below the halocline of Lake Bonney are described in Spigel and Priscu (1996). Total iron was determined as described by Fulton et al. (2004) using the ferrozine assay.

### 3.3. MICROBIOLOGY

Heterotrophic marine agar (Bacto) and oligotrophic R2A (Difco) agar plates were prepared to examine the presence of colony forming units (CFUs) in transect samples. Plates were preincubated at room temperature for 48 h to test for contamination, and then pre-chilled to 2 °C before inoculation. Aliquots (100 $\mu$L) of Blood Falls outflow from each transect collection were serially diluted, plated and incubated at 2 °C for three weeks. All transfers

were done on ice in a positive-flow hood. Plate counts on both media types were made after a three-week incubation. Environmental (*in situ*) cell abundance was determined on 0.2 $\mu$m-filtered samples as described by Lisle and Priscu (in press). Samples collected in 2001 were stained with 4',6-diamidino-2-phyenylindole (DAPI) (Sigma). Samples collected in December 2002 were enumerated with Sybr Gold DNA stain (Molecular Probes).

### 3.4. BACTERIAL ACTIVITY

Heterotrophic bacterial activity was measured using $^3$H-thymidine (20 nM) and $^3$H-leucine (20 nM) incorporation according to the methods described in Takacs and Priscu (1998). Formalin (5% final concentration) treated replicates served as controls (kills). Incubation was terminated by the addition of cold trichloroacetic acid (TCA) to a final concentration of 5%. Samples were incubated in the dark at 0–1 °C. The effect of temperature on the productivity of the natural bacterial assemblages from Blood Falls outflow was determined by incubating triplicate samples (10 mL) with 10 nM of $^3$H-leucine for 24 h at varying temperatures using the methods described above. From the $^3$H-leucine incorporation data, doubling rates were calculated (Takacs and Priscu, 1998)

The activity of two extracellular enzymes, alkaline phosphatase (AP, EC 3.1.3.1) and leucine aminopeptidase (LAP, EC 3.4.11.1), were measured across a broad substrate range (2–140 $\mu$M) in order to determine the potential for multi-phasic uptake systems. Bacteria use extracellular enzymes to breakdown complex molecules into units of assimilable size so that they can be transported across their cell walls. Because bacteria rely on enzymes to acquire these monomers, examining enzyme activity provides an overall view of dissolved carbon and nutrient dynamics for a system. Enzyme measurements were made according to the method of Foreman et al. (1998). Stock solutions of substrates were prepared in 5 mM, pH 8, bicarbonate buffer. Substrates for the assays were 4-MUF-phosphate and L-Leucine 7- amido-4-methyl-coumarin, respectively. Enzyme activities were expressed as nmoles of substrate hydrolyzed per liter of water per day, using emission coefficients calculated from standards and correcting for quench effects. Enzyme kinetics were assumed to follow the Michaelis–Menton model. Kinetic parameters were estimated from linear regressions of the Eadie-Hofstee equation, which is a linearization of the Michaelis–Menton model:

$$V = -K_{\mathrm{m}}(V/[S]) + V_{\max},$$

where $V$ is the reaction rate, $S$ the substrate concentration, $V_{\max}$ the maximum velocity of the reaction under substrate saturating conditions, and $K_{\mathrm{m}}$ is the Michaelis–Menton constant or half saturation constant, and is equal to

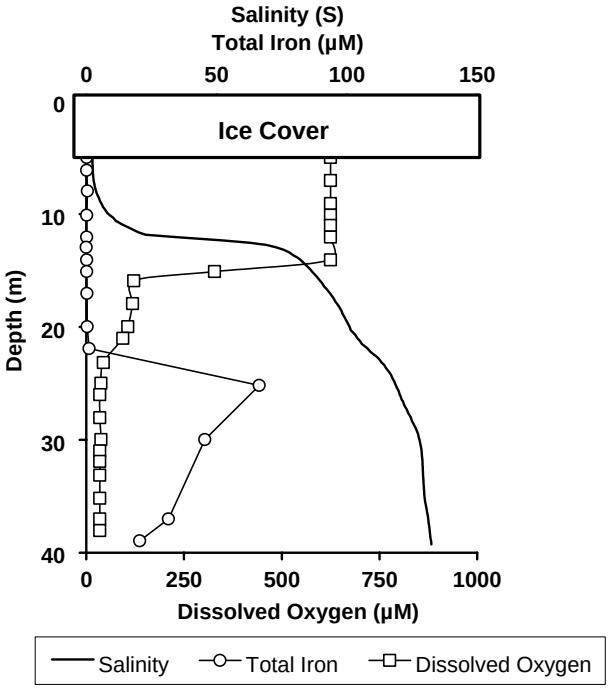


*Figure 2.* Geochemical profile for West Lobe Lake Bonney. The lake is covered by 4.5 m of permanent ice. The solid line represents salinity, open circles represent total iron and open squares show dissolved oxygen.

the concentration at which the rate of the reaction equals half of the maximum rate. The reaction rate ($V$) was measured for each enzyme over a range of substrate concentrations with three analytical replicates per assay. The Eadie–Hofstee equation was used to generate $V_{max}$ and $K_m$ values, $V$ vs. [$S$] is plotted in Figure 4.

## 4. Results

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

### 4.1. BIOGEOCHEMISTRY

The outflow from Blood Falls is basic, all points along the transect had a pH of 8.0 or higher (Table II). The highest pH, 9.32, was recorded at the sample point furthest from the source (Trans5). Dissolved oxygen was between 140

*Table II.* Summary of biogeochemical analysis performed on each transect point during the 2002 austral summer. ND = not detected, TNTC = too numerous to count, NA = not available

| Transect | pH | DO ($\mu$M $O_2$) | Eh mV | DIC ($\mu$M C) | DIN ($\mu$M) | SRP ($\mu$M) | DIN: SRP | DOC ($\mu$M C) | CHL ($\mu$g $L^{-1}$) | Bacteria ($10^4$ cells $mL^{-1}$) | Colony Growth | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | R2A | | Marine | |
| | | | | | | | | | | | $10^{-1}$ | $10^{-3}$ | $10^{-1}$ | $10^{-3}$ |
| 1 | 8.16 | | 230 | | 0.22 | 0.09 | 2.4 | 771 | 1.4 | 1.08 | 32 | 1 | TNTC | 0 |
| 2 | 8.00 | | 247 | | 0.26 | 0.38 | 0.7 | 211 | 0.2 | 0.8 | 75 | 0 | TNTC | 0 |
| 3 | 8.80 | 215 | 250 | 476 | 9.98 | 0.05 | 200 | 47.5 | 15.0 | 0.31 | TNTC | 0 | 241 | NA |
| 4 | 9.28 | 220 | 250 | 165 | 9.40 | 0.08 | 118 | 52.0 | 18.5 | 1.10 | TNTC | 0 | TNTC | 0 |
| 5 | 9.32 | 140 | 252 | 70.8 | 8.37 | 0.08 | 105 | ND | 11.8 | 1.07 | TNTC | 0 | 30 | 0 |

and 220 $\mu$M $O_2$ for the three measured transect points (Trans3–5), however, mixing with surface air might have occurred while the samples were collected. Two Eh values for each transect sampling point were collected and averaged. The average Eh value for Trans1 is 230 mV, Trans2 was higher (247 mV) and both Trans3 and Trans4 were about 250 mV. Trans5 was slightly higher at 252 mV. DIC values were obtained for Trans3–5 and were lowest at the sample site furthest from the source (Trans5). Trans3 was 476 $\mu$M C (while Trans5 was 70.8 $\mu$M C). DOC at the source (Trans1) of Blood Falls was high (9.25 mg C $L^{-1}$ or 771 $\mu$M) when compared to values typical of marine waters (40–80 $\mu$M) (Williams, 2000). Blood Falls DOC concentration is more comparable with that of highly productive systems receiving allochthonous inputs such as black water stream flow systems ranging from 250–3480 $\mu$M C (Meyers and Edwards, 1990). DOC quickly decreases to below the detection limits of our methods (40 $\mu$M C) by Trans5 (Table II). SRP was highest (0.38 $\mu$M) in Trans2, which is basal ice directly below the source. All other transect samples were below 0.1 $\mu$M and may be a result of abiotic loss of phosphorus via adsorption to iron hydroxides. DIN values in Trans1 and 2 were 0.22 and 0.26 $\mu$M, which are lower than values reported for the perennial ice cover of east lobe Lake Bonney (between 2 and 10 $\mu$M) (Priscu et al., 1998). DIN in Trans3–5 was between 8.4 and 10.0 $\mu$M, which are similar to values found in the lake ice covers. 100 percent of the DIN in Trans1–2 was in the reduced form ($NH_4^+$), whereas DIN in transects further from the source were comprised mainly ($>60\%$) of nitrate ($NO_3^-$) the most oxidized form. Increases in DIN may be a result of the biological fixation of atmospheric nitrogen by diazotrophic cyanobacteria. The increase in the fraction of DIN in the oxidized form correlates strongly with an increase in Chlorophyll-a ($r = 0.97$). Farther downstream from the source of Blood Falls outflow, CHL increases from 1.4 $\mu$g $L^{-1}$ (Trans1) to 18.5 and 11.8 $\mu$g $L^{-1}$ (for Trans4 and 5 respectively). This increase in CHL correlates weakly (correlation $r = 0.25$) with a decrease in DIC, suggesting that photoautotrophs may be colonizing the outflow once nutrients supplied by Blood Falls are available. DIN:SRP increased from 0.7 in the upper transect points (Trans1–2) to 200 in the lower transect points (Trans3–5). These ratios imply phosphorus deficiency in the flow where it enters Lake Bonney. Phosphorus deficiency is a feature of the upper water column of Lake Bonney (Dore and Priscu, 2001).

### 4.2. MICROBIOLOGY

Prokaryotic cell morphologies included rods, cocci, dipplococci and diplorod cell types. Cells ranged in length, width and volume between 0.59–0.91 $\mu$m, 0.44–0.45 $\mu$m and 0.11–0.16 $\mu m^3$ respectively. Based on these results a total bacterial biovolume between $1.7 \times 10^3$–$9.5 \times 10^3$ $\mu m^3$ $ml^{-1}$ was calculated.

The average density of cells from environmental Blood Falls outflow was $10^4$ cells $ml^{-1}$, ranging from $0.3 \times 10^4$ to $1.1 \times 10^4$ cells $ml^{-1}$. Bacterial colonies grew on both oligotrophic R2A plates and high nutrient marine agar plates (Table II). Colonies appeared within two weeks of incubation at 2 °C. Percent recovery from the two heterotrophic media types (R2A and marine agar) was generally low. Inoculum from Trans1 and Trans5 demonstrated a 2.9 and 2.8% recovery, respectively, based on total cell counts when grown on R2A media. Trans3 had 78% recovery when grown on marine agar. 78% recovery seems unusually high and is suspect. Subsequent isolation and transfers indicated that most colonies were capable of growth at 15 °C. Pigmented pink, yellow and orange colonies as well as opaque and translucent colonies grew on both media preparations incubated at 2 °C. Growth occurred on plates incubated at room temperature ($\geq$22 °C), however colonies were less dense and for the most part non-pigmented.

### 4.3. BACTERIAL ACTIVITY

Bacteria in Blood Falls outflow incorporated thymidine into trichloroacetic acid insoluble products at an average rate of $1.5 \times 10^{-3}$ nM $d^{-1}$. There was considerable variation between the samples, with rates ranging from 0.4 to $3.0 \times 10^{-3}$ nM $d^{-1}$ (standard deviation = $1.1 \times 10^{-3}$). Doubling times ranging from 37 to 54 days at 0 °C were calculated based on thymidine incorporation experiments. The effect of temperature on leucine incorporation was determined at 0, 5, 10, 15, 20 and 30 °C (Figure 3). The activation energy ($E_a$) over the range of temperatures tested, and the $Q_{10}$ for metabolic activity between 0 and 30 °C was 8.8 kcal $mol^{-1}$ and 1.74, respectively.

Alkaline phosphatase (AP) and leucine amino-peptidase (LAP) activities were assayed in two samples from Blood Falls collected in 2001. The first sample was obtained from slushy ice, which corresponds to the approximate location of Trans1, and the second from a sediment slurry (obtained from an area between Trans1 and Trans2 in Figure 1). These two samples show similar patterns of activity to one another, however both AP and LAP activity were greater in the sediment slurry than in the ice (Figure 4).

## 5. Discussion

### 5.1. BIOGEOCHEMICAL DATA

Blood Falls water is a source of organic carbon and nutrients for subglacial microbial growth, in outflow waters and in Lake Bonney (Table II). The pH of Blood Falls outflow was basic (8.00–9.32), which results in part from the high DIC ($HCO_3^- + CO_3^=$) values of these waters. DOC at the source of Blood Falls (Trans1) is 771 $\mu$M C, about nine times greater than that of the

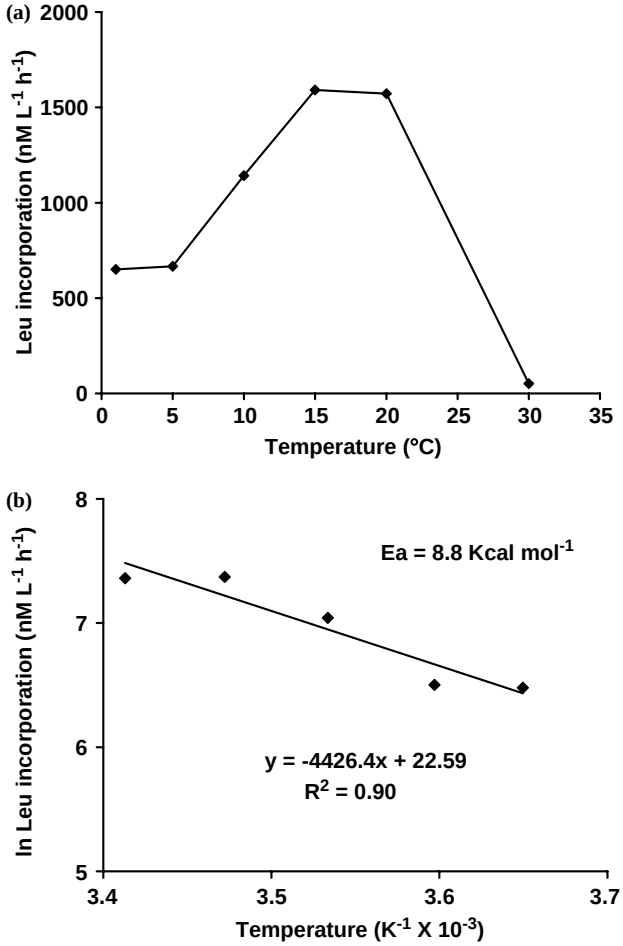


*Figure 3.* Effect of temperature on Blood Falls outflow plotted arithmetically (a) and with the Arrhenius equation (b).

open ocean indicating that the Blood Falls subglacial environment is a source of DOC. Whether the DOC detected in Blood Falls accumulated from ancient valley runoff or biological activity prior to the isolation of this marine feature by the advance of the Taylor Glacier or is a result of extant subglacial microbial productivity is unknown. However, the high DOC values (771 $\mu$M C) strongly imply ancient or contemporary *in situ* production of organic carbon, as there are no other available allochthonous sources. Regardless of its origin, this DOC provides a vital substrate for heterotrophic bacteria both within Blood Falls outflow and Lake Bonney.

Dissolved oxygen was higher than would be expected for subglacial waters that appear to be anaerobic during high flows (Keys, 1979; Priscu, personal observation). However, samples were collected in the dynamic zone of glacier

melt (Trans3) where the moat ice is actively thawing and refreezing (Trans4 and 5). These areas are in direct contact with the atmosphere and atmospheric exchange has surely occurred. The increase in DIN in Trans3–5 suggests an input of nitrogen which might come from microbial nitrogen fixation. Nitrogen fixation activity has been detected in microbial aggregates associated with the Lake Bonney ice cover (Paerl and Priscu, 1998). Additionally, specific nitrogen-fixing cyanobacterial (i.e., *Nostoc sp.*) and heterotrophic microorganisms were identified in ice-cover samples through nitrogenase gene (*nifH*) amplification (Olsen et al., 1998).

The N:P ratio in the source waters of Blood Falls indicate that phosphorus should not be limiting, however alkaline phosphatase activity strongly suggests that bioavailable phosphorus is low and that bacteria present at the source of Blood Falls are actively producing enzymes to scavenge less-available sources of phosphorus. Measurement of AP activity has often been used as an indicator of phosphorus deficiency in aquatic ecosystems given that it has been found produced in ample amounts when inorganic phosphorus becomes limiting (Chróst and Overbeck, 1987; Stewart and Wetzel, 1982). De-repression of AP activity due to low inorganic phosphorus concentrations is not always immediate because bacteria may have intracellular pools of ortho- and polyphosphate in the their cells (Chróst, 1991). Bacterial communities in the lakes of the Taylor Valley are stimulated by inorganic P additions (Dore and Priscu, 2001) and bacterial P limitation has been demonstrated in other lacustrine systems (Elser et al., 1995).

Biological analysis of Blood Falls discharge reveals the presence of a viable, active microbial assemblage. Growth on low nutrient, low salt agar (R2A) plates suggest that a fraction of the consortium is living *in situ* in a halotolerant fashion, or that organisms present in glacial melt ice have been selectively enriched. Primarily, translucent and opaque colonies grew at room temperature incubations, while a variety of pigmented colonies were observed at lower (below 15 °C) incubation temperatures. It is not clear what relationship exists between the production of pigments and growth temperature, however similar results were observed by Skidmore et al. (2000) when growing Artic subglacial sediment samples on R2A. A fraction of the isolates obtained from Blood Falls grew at both low (2 °C) and higher temperatures ($\geq$22 °C), suggesting a psychrotolerant response to temperature, therefore the Blood Falls assemblage has members capable of growth under increased temperatures. However, a distinct fraction of the population was unable to grow at higher temperatures. The ability of isolates from Blood Falls to grow on marine agar suggests that some portion of the microbial consortium can grow under simulated marine conditions. This supposition is further supported by the phylogenetic relatedness (93% similarity based on partial 16S rRNA data) of an isolate from the Trans3 marine agar plate to that of *Schwenella frigidamarina* (Mikucki, unpublished). *S. frigidamarina* is a

facultative iron-reducer that has been isolated from sea ice and Antarctic lakes of marine salinity (Bowman, et al., 1997).

### 5.2. BACTERIAL ACTIVITY

Heterotrophic incorporation of thymidine in Blood Falls outflow (average = $1.5 \times 10^{-3}$ nM per day) is low when compared to bacterial production in other lakes in the Taylor Valley such as Lake Fryxell (0.453 nM per day) and west Lake Bonney (0.05 nM per day) (Takacs and Priscu, 1998). Blood Falls bacterial production does however, fall within the range for samples from the Sargasso Sea or the Gulf Stream ($3.6–11 \times 10^{-3}$ nM per day and $2.4–13.2 \times 10^{-3}$ nM per day, respectively) (Rivkin and Anderson, 1997). Long doubling times such as those we computed for bacteria from Blood Falls (37–54 days) are not uncommon for microbes from cold environments. The doubling time for Blood Falls is comparable to rates from other environments below freezing, Rivkina et al. (2000) calculated a doubling time of 40 days for permafrost bacteria incubated at −15 °C. Bacterial activity in icy environments may ultimately be limited by the presence of liquid water. For example, in the permanent ice cover of Lake Bonney, liquid water only exists within the ice for 80–150 days during the austral summer (Fritsen and Priscu, 1999), yet heterotrophic, photosynthetic and diazotrophic microorganisms are metabolically active during this brief period (Priscu et al., 1998).

The fraction of the microbial assemblage capable of incorporating leucine demonstrated a psychrotolerant response to temperature, growing fastest at 30 °C (Figure 3), but was still able to metabolize at near freezing temperatures. Consequently, these bacteria would never realize their true growth potential *in situ*. The energy of activation and $Q_{10}$ are similar to many other bacterial assemblages ranging from psychrophilic to thermophilic (Priscu and Goldman, 1984; Priscu et al., 1989 and Thamdrup et al., 1998) indicating common thermodynamic responses from the various enzyme systems.

Metabolic activity of microbial assemblages can also be demonstrated by examining the activity of extracellular enzymes. The majority of natural dissolved organic matter is in polymeric form, while only a small portion is composed of readily utilizable monomers such as amino acids or urea (Thurman, 1985). Utilization of polymeric substrates requires extracellular hydrolysis; hence many organisms maintain adequate substrate availability through the deployment of enzymes. LAP is a broad range proteolytic enzyme that signals the hydrolysis of proteins to amino acids, which releases both nitrogen and carbon compounds. Demonstration of LAP activity indicates that organisms from Blood Falls are trying to acquire nitrogen. Interestingly, LAP activity in this study was found to be greater than activity within either the East or West Lobes of Lake Bonney's water columns (Foreman, unpublished). Alkaline phosphatase (AP) activity

shown in the samples is indicative of the use of phosphomonesters by the microbial assemblage and may be related to phosphorus deficiencies. Dore and Priscu (2001) demonstrated AP activity in the bacterial size fraction of both lobes of Lake Bonney, providing further evidence of phosphorus limitation.

Blood Falls showed no evidence of multi-phasic kinetic responses based on AP or LAP activity, as shown in Figure 4. In fact, the activity ($V$) vs. concentration of substrate plots show almost linear progressions, indicating that the communities would be responsive to elevated substrate levels in their environments. This lack of multiple kinetic systems also supports the notion that substrate availability in the subglacial environment below Taylor Glacier is temporally stable, and not subject to episodic fluxes of nutrients.

Microbes that take advantage of inorganic energy might also live in the Blood Falls environment. Skidmore et al. (2000) reported the presence of nitrate and sulfate reduction, as well as methanogenic activity in subglacial basal ice and waters. In addition to the visibly high levels of iron in Blood Falls, sulfur compounds are also present. For example, dimethylsulfide (DMS), which originates from microbial oxidation of dimethylsulfoniopropionate (DMSP), a major metabolite of marine algae, has been detected in Blood Falls outflow (Priscu, unpublished data). Preliminary analysis of DNA extracted from Blood Falls subglacial flow waters detected the presence of 16S rDNA sequences whose nearest relative (98% sequence similarity) is *Thiomicrospira*, a sulfur oxidizing bacteria (Mikucki, unpublished data). Moreover, clones representing *Thiomicrospira* were the most dominant members found in our clone library. Two broad ecological classes of sulfur-oxidizing bacteria exist, those that grow at acidic pH and those that grow at near neutral (pH = 6–8) (Madigan et al. 2000). *Thiomicrospira* is a representative of the genera that operates at neutral pH and its presence is consistent with Blood Falls outflow *in situ* pH and sulfate data. Incubation experiments conducted at 4 °C by Sharp et al. (1999) showed production of sulfate in arctic subglacial slurries after one week. Microbially mediated sulfur oxidation may be an important subglacial biological process occurring under microaerophilic conditions underneath the glacier and at the glacier surface interface.

### 5.3. IMPORTANCE OF BLOOD FALLS TO LAKE BONNEY GEOCHEMISTRY AND MICROBIOLOGY

The west lobe of Lake Bonney is highly stratified with respect to salts and nutrients (Figure 4). Keys (1979) estimated that Blood Falls discharges an average of 2,000 $m^3$ per year of saline water (episodic discharges reach 6,000 $m^3$ per year). The density of Blood Falls discharge (1,100 kg $m^{-3}$) is also identical to that of the deep waters of the west lobe of Lake Bonney,

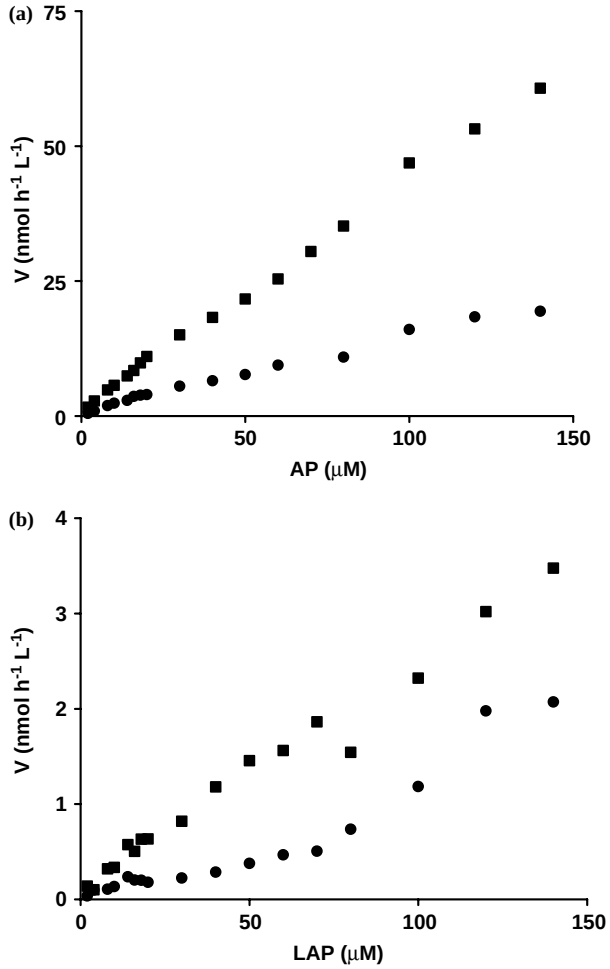


*Figure 4*. Enzymatic activity of alkaline phosphatase (a) and leucine-aminopepsidase (b) in Blood Falls ice melt from the source (solid circles) and slush ice slurry (solid squares).

1100 kg $m^{-3}$ (Spigel and Priscu, 1996) indicating that Blood Falls discharge would sink beneath the lake's chemocline at 14 m. Lyons et al. (1998) concluded that Blood Falls contributed up to 95% of the total chloride flux to the West Lobe of Lake Bonney in the 1994–1995 flow season. Total iron and salts provided by Blood Falls accumulate below the chemocline. This contribution of Blood Falls to the deep waters of Lake Bonney is further corroborated by similarities in bacterial size (biovolume) between Blood Falls and the deep waters of the west lobe. Image analysis of bacteria in Blood Falls reveal small (range of length, width and volume = 0.59–0.91, 0.44–0.45 and 0.11–0.16 $\mu m^3$) cells resembling those found below 22 m in the west lobe; cells in the deep water of other lakes in the Taylor Valley have bacteria that

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

| Year | Temperature (°C) | $^3$H-labeled Thymidine incorporation (nM $h^{-1}$) $10^{-3}$ | $^3$H-labeled Leucine incorporation (nM $h^{-1}$) | Doubling time ($d^{-1}$) |
|---|---|---|---|---|
| 2000 | 0 | 4.27 | 1.04 | 37 |
| 2000 | 0 | 1.63 | 0.51 | 54 |
| 2001 | 2 | 12.5 | | |

are about 40% larger by volume (Takacs, 1999). DOC concentrations of 771 $\mu$M C are more comparable to that of the chemocline of the west lobe Lake Bonney and the waters below, where DOC is 750 $\mu$M C at 14 m and increases to 2083 $\mu$M C at 38 m (Takacs et al., 2001). Eh values in the surface waters of west Lake Bonney are near 750 mV and drop to 240 mV below the chemocline (Lee et al., in press). This is the same Eh value recorded from Blood Falls outflow (average = 246 mV). Clearly the bottom waters of the west lobe of Lake Bonney (below 14 m) are strongly influenced by the Blood Falls outflow (Figure 4). Ward et al. (2003) found bacterial incorporation of thymidine was stimulated when growth conditions were diluted compared to natural Lake Bonney bottom water, suggesting that *in situ* organisms may be stressed by the high salt, high iron conditions below the chemocline. However, after six days of incubation, thymidine incorporation activity did not increase further in the diluted samples while growth continued to increase in the undiluted samples. The authors concluded that substrate limitation prevented sustained growth in the diluted samples. Perhaps the organisms at depth in Lake Bonney are adapted to sustained, slow growth in their natural environment. It is interesting to note that despite these strong similarities, the pH in the bottom waters of west Lake Bonney is acidic (pH=6) whereas that of Blood Falls is basic. This pH difference could have a significant effect on the microbial populations in Blood Falls as they enter the waters below the chemocline of west Lake Bonney and warrants further study.

### 5.5. CONTRIBUTION TO SUBGLACIAL WEATHERING

Subglacial flow influences glacier stability, sediment erosion, transport and deposition (Benn and Evans, 1998). Glacier bed movement also acts to grind and pulverize subglacial rocks making available more surface sites for chemically and microbially mediated weathering reactions. In a polar desert such as the dry valleys, subglacial processes would be slow as most glaciers have frozen beds (cold-based glaciers) (Benn and Evans, 1998). However, Blood Falls might influence the geochemical processes in the basal ice of the

Taylor Glacier snout. A mass balance study of Taylor Glacier by Robinson (1984) revealed that as much as 50% of the lower ablation area of the Taylor Glacier basal ice may be melting and can be expected to behave as temperate ice. Robinson further reported that this area of pressure melting probably occurs as the glacier passes over extensive salt deposits which have the effect of depressing the freezing-point of the basal ice, thereby extending basal melt even further. Recent ice penetrating radar data and subsequent modeling suggest the presence of water ponding or saturated sediment beneath the tongue of the Taylor Glacier between 3–6 km up glacier of the snout (Hubbard et al., in press). Modeling results indicate that this water must be hypersaline. Whether this water is chemically related to Blood Falls is unknown. This may explain the episodically high values of dissolved ions in samples collected from Blood Falls outflow. Lithium and silicon are two elements typically used to gauge water-rock interactions (Lyons and Welch, 1997; Pugh et al., 2003). Pugh et al. (2003) report an average of 242 $\mu$M Si in Blood Falls outflow during the 1999–2000 season. Lithium concentrations ranging between 47.1–91.1 $\mu$M reported by Lyons and Welch (1997) were the highest of all samples analyzed from the dry valleys lakes and streams during the 1995–1996 season. Sr isotopic measurements of Blood Falls discharge also suggest a significant weathering component as the $^{87}Sr/^{86}Sr$ values are more radiogenic than seawater, i.e., 0.71146 (Lyons, et al., 2002). In addition the suspended load measurements from streams exiting from Blood Falls yield values from 25–925 mg $L^{-1}$ (mean = 481) in 1998. Although we only have measurements from this one year, they indicate very high sediment yields related to the Blood Falls discharge. Subglacial waters are expected to have a higher solute content owing to prolonged contact with fresh bedrock and fine rock particles (Benn and Evans, 1998). It is clear from the discussion above that the glacier-brine-bedrock interactions that have led to the unusual geochemical characteristics of Blood Falls also have an important impact on the geochemistry of Lake Bonney. The initial brine trapped in the Bonney Basin has been modified by the addition of weathered products. Lake Bonney has undergone numerous size changes over the past 300 kyr (Hendy, 2000). As the lake waxes and wanes and the Taylor Glacier either advances or retreats; the brine now underneath the glacier is chemically modified and the chemistry of Blood Falls is undoubtedly the consequence of a number of important processes that have taken place since the initial emplacement of seawater in the late Tertiary.

## 6. Conclusions

Blood Falls provides a source of viable microbes, new nutrients, carbon, and energetic mineral weathering products to the Lake Bonney system that is largely limited in these biological necessities. The microbes from Blood Falls

are not only viable, but also metabolically active as demonstrated by three distinct assays for heterotrophic activity. The source of carbon utilized by these organisms could be a legacy of photosynthesis by marine phytoplankton from before Blood Falls was covered by the Taylor Glacier and cut off from sunlight. The presence of chemolithoautrophs capable of growth on sulfur compounds suggests other energy sources may be driving this unique system today. Reduced iron and ammonia are other energy sources that might be thermodynamically available to autotrophs operating subglacially.

In the absence of drilling through the Taylor Glacier, the geomicrobiological environment of Blood Falls can be inferred by studying the chemistry and microbiology of the discharge and the deep waters of west Lake Bonney. The discharge channels themselves are transition zones from subglacial to lake system and provide a glimpse of the subglacial environment, but not true *in situ* conditions. Actual samples taken from the base of the Taylor Glacier at the source of Blood Falls will allow us to determine the true geomicrobiology of this subglacial system. What is evident from our analysis of the discharge is that Blood Falls has a major geochemical and biological influence on Lake Bonney. However, it remains unclear whether microbes from Blood Falls are able to survive once transported to Lake Bonney.

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