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Collaborative Research: What Limits Denitrification and Bacterial Growth in Lake Bonney, Taylor Valley, Antarctica?

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Collaborative Research: What Limits Denitrification and Bacterial Growth in Lake Bonney, Taylor Valley, Antarctica?

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Worked for more than 160 Hours: Yes

Contribution to Project:

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Worked for more than 160 Hours: Yes

Contribution to Project:

Post-doc

Graduate Student

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Technician, Programmer

Other Participant

Research Experience for Undergraduates

Organizational Partners

Princeton University

This is a collaborative project with Dr. Bess Ward at Princeton University.

Other Collaborators or Contacts

None.

Activities and Findings

Research and Education Activities:

Research Focus

Lake Bonney, Antarctica is a permanently ice-covered lake in the Taylor Valley of East Antarctica (77°S, 168°W). It has been thoroughly characterized in terms of its biology and physical characteristics (Priscu and Spigel, 1998) and is included in an ongoing Long Term Ecological Research Program in the McMurdo Dry Valleys. Lake Bonney has two lobes, each about 40 m deep, separated by a narrow passage with a sill depth of 12 - 13 m. Thus the surface waters of the two lobes are able to exchange, and the circulation has

been described as a set of linked gyres (Priscu and Spigel, 1998). Water enters the system at the foot of the Taylor Glacier in the West Lobe and exits at the east end of the East Lobe or is lost through sublimation of the ice surface. The chemocline depth in both lobes is deeper than the sill depth, so there is no communication of the deep waters between the two lobes. The water below chemocline depth in both lobes is suboxic to anoxic, and early reports documented high concentrations of bioactive and other trace metals in the hypolimnion (Boswell et al., 1967a, Boswell et al., 1967b, Weand et al., 1976). The most peculiar feature of the lake is the chemistry in the deep water, which is substantially different between the two lobes, both in total salt content and in concentration and distribution of several inorganic nitrogen species (Lyons et al., 2000; Priscu, 1995).

The recent geochemical history of the lake at least partially explains the high salt and metal concentrations. There is evidence that the geologic history of the East (ELB) and West (WLB) Lobes of Lake Bonney differ significantly (Lyons et al., 1998). Oxygen and deuterium isotopic measurements in lake waters of the Dry Valleys indicate a climate shift in this region from δ warm-wet to δ cold-dry conditions \sim 3000 years ago. As a consequence, the East and West Lobes of Lake Bonney became hydrologically separated at the sill as the δ cold-dry conditions increased the net evaporation. While freshwater inflows from Taylor Glacier apparently sufficed to maintain ice-cover on the West Lobe (B. Lyons, pers. comm.), sublimation loss quickly transformed the East Lobe into an exposed, hypersaline lake. These conditions persisted for more than a millennium until climatic conditions became warmer \sim 1000 years ago. The increased freshwater inflows from the Taylor Glacier then raised the West Lobe level until freshwater flow over the sill capped the hypersaline East Lobe. Thus the deep water chemical distributions in the East Lobe represent the partial dilution of the old deep brine layer into the overlying fresh layer. Some of the chemical characteristics of the two lobes are summarized in Table 1. Although sulfate is a major ion in both lobes, and oxygen is greatly depleted in the hypolimnion of both lobes, detection of hydrogen sulfide has never been reported for either lobe.

In the West Lobe of Lake Bonney, the chemocline occurs at about 15-17 m and oxygen is depleted below that depth. Ammonium accumulates to a maximum of 300 μ M in the deep water and nitrate and nitrite both show small maxima (25 and <1 μ M, respectively) in the region of the chemocline. Nitrous oxide is present at relatively low levels, again with a small maximum (about 1 μ M) near the chemocline (Priscu et al., 1996; Ward and Priscu, 1997; Voytek et al., 1999). In the East Lobe, oxygen is depleted below 18-20 m and ammonium accumulates to a maximum of about 150 μ M in the deep water. In contrast to the West Lobe, however, nitrate and nitrite both accumulate in the deep water, reaching maximum total concentrations of nearly 200 and 40 μ M, respectively. Nitrous oxide shows a record-breaking maximum near the chemocline of about 40 μ M (Priscu et al., 1996). Clearly the nitrogen cycle of the East Lobe is unusual for a stratified aquatic system; suboxic conditions in both lobes would be expected to result in denitrification, and subsequent depletion of nitrogen oxides. The different nitrogen distributions have been interpreted to infer that denitrification occurs in the West Lobe but not in the East. Priscu (1997) has demonstrated that denitrification can be detected in the West Lobe but not in the East Lobe.

The McMurdo LTER project (<http://huey.colorado.edu/LTER>) has reported bacterial abundance and production rates in depth profiles from central stations in both lobes of Lake Bonney for many years. The long running time series of bacterial production measurements show interannual variability in bacterial parameters, but a seasonal cycle generally exhibits increased abundances and productivity in November and December (Takacs and Priscu, 1998). In the West lobe, the bacterial abundance maximum (15 \hat{u} 16 m) usually coincides in depth with the productivity maximum, but in the East Lobe, a strong abundance maximum at 20 - 25 m is consistently deeper than the productivity maximum at 12 \hat{u} 15 m (Takacs and Priscu, 1998). The productivity data consistently show detectable bacterial activity in and below the chemocline of the West Lobe but more rarely

and at very low levels at analogous depths in the East Lobe.

The reason for the collapse of the nitrogen cycle in the East Lobe could be specific to some process, i.e., denitrification, in the nitrogen cycle itself, or it could be due to overall limitation, toxicity or inhibition of essentially all microbial activity in the water. Our previous investigation of the role of bioactive metals in regulating denitrification in cultured bacteria and permanently ice-covered Lake Bonney in the Taylor Valley of East Antarctica produced three important findings that form the basis of this proposal:

- Growth experiments demonstrated that cultured denitrifying bacteria could be limited by Cu or Fe, and that nitrogen oxides, either nitrite or nitrous oxide, accumulated in the medium due to limitation at the nitrite and nitrous oxide reduction steps, respectively.
- Manipulations of metal availabilities using chelators, additions of substrates and cultured bacteria all failed to elicit a response from the natural microbial communities in the lake. No denitrification or thymidine incorporation was detected in the subchemocline waters of the East Lobe of Lake Bonney, while analogous experiments detected an active denitrifying community in the West Lobe.
- Silver and iron were the only metals that showed dramatic distribution differences between the two lobes of the lake. Silver concentrations were up to 150-fold higher in the East than in the West. Concentrations of Cd, Pb, Cr, Ni and Zn in suboxic East Lobe waters were a factor of 2-5-fold higher than in the West Lobe and Fe concentration was 200 times lower in the East Lobe.

Low Fe concentrations may exacerbate the potential toxicity of the other metals, so a general metal toxicity is a possibility for the inhibition of denitrification. Silver, on the other hand, has the potential to specifically inhibit denitrification because of its ability to interfere with Cu binding in redox proteins, such as nitrite reductase and nitrous oxide reductase (Nos). High Ag concentrations might prevent the functioning of Nos in the same way that simple Cu limitation does, therefor causing the buildup of nitrous oxide and a nonfunctional N cycle.

Other factors are likely also at work in limiting bacterial activity in Lake Bonney. For example, it is still not known whether oxygen concentrations low enough to trigger denitrification prevail in the East Lobe of the lake. Oxygen concentrations will be measured using a newly developed optode mounted on the CTD. Ag toxicity, general metal toxicity and oxygen concentration will be investigated for their effect on denitrification in Lake Bonney by using a suite of 'sentinel' strains of denitrifying bacteria (isolated from the lake) incubated in Lake Bonney water and subjected to various treatments. The physiological responses of these strains to changes in metal and oxygen concentration will be quantified by flow cytometric detection of single cell probes whose sensitivity and interpretation has been optimized for the sentinel strains.

The relationships between metals and denitrification that we discover here are expected to shed light not only on Lake Bonney's unusual nitrogen cycle and, more generally, on the potential role of metals in regulation of microbial nitrogen transformations.

Findings:

Major Findings

The central objective of this renewal proposal was to seek to solve the mystery of the collapsed nitrogen cycle and moribund condition of the East Lobe waters by probing the physiological state of the bacterial cells present in the lake and applying a new suite of methods to induce activity and identify the reason for the lack of denitrification in situ.

The metal data and experience resulting from our previous work in Lake Bonney lay the foundation for this new project. Our research strategy employ a number of different approaches but were guided by the following hypotheses.

Hypothesis 1: High dissolved concentrations of silver, and perhaps low concentrations of dissolved iron, are responsible for the low growth of denitrifying bacteria in East Lobe Bonney.

Comparison of dissolved metal concentrations in suboxic waters of the East and West Lobes of Lake Bonney obtained during the previous project indicated that Ag concentrations were substantially higher in suboxic waters of the east Lobe vs. west Lobe of Lake Bonney. Silver toxicity could be a unique factor causing the difference in denitrification between the lobes because it can specifically interact to limit nitrogenase activity. However, these data were obtained using medium resolution ICPMS by direct aspiration of diluted suboxic waters, and small shifts in Ag isotopic ratios in the east Lobe samples indicated that unknown polyatomic interferences may have been biasing these data. Using a newly published approach, we developed, verified and used an on-line preconcentration technique to specifically extract Ag from the diluted samples, thereby removing the possibility of matrix interferences. With this new approach we showed that differences in Ag concentrations in suboxic waters of the two lobes were orders of magnitude less than previously determined (West Lobe \hat{u} ~2-7 nM Ag; East Lobe - ~1-50 nM, but 7 nM at the depth expected for maximum denitrification). The first order interpretation is that Ag toxicity is not responsible for limiting curtailing denitrification in East Lobe Lake Bonney. We therefore did not proceed on plans to better characterize Ag toxicity effects on denitrifiers in Lake Bonney waters.

Hypothesis 2: Oxygen tension is too high in the mid-depth waters of the east lobe Lake Bonney for denitrification to be a significant process.

While oxygen content decreases to extremely low levels in the mid-depth waters in the East Lobe before increasing again in deep waters (a relict of previous sub-areal exposure of the hypersaline lake), it was not certain that O₂ concentrations fall to the 2 - 20 \hat{A} M O₂ threshold below which denitrification is initiated. Previous measures of the oxidation-reduction potential obtained using a combination electrode suggested that the East Lobe may be poised at an Eh too high to trigger the switch to denitrification by the resident assemblage. We measured dissolved O₂ using a newly developed, highly sensitive oxygen probe (Optode). Profiles showed that dissolved O₂ decreased from > 300 \hat{A} M in surface waters to 13.2 \hat{A} M at 17 m depth. Oxygen increased slightly at 18-21 m depth (~20-30 \hat{A} M O₂) before falling below 4 \hat{A} M in deeper waters. We therefore disproved the hypothesis that O₂ tension is too high in mid-depth and deep waters to explain the limitation of denitrification in the East Lobe of Lake Bonney.

Hypothesis 3: Inhibition or limitation by organic components limits denitrification and bacterial growth in East Lake Bonney.

Large exposed formations containing shales and other organic-rich rocks exist in the Dry Valleys (e.g. Barrett, P.J., 1991. The Devonian to Triassic Beacon Supergroup of the Transantarctic Mountains and correlatives in other parts of Antarctica. In: R.J. Tingey (Editor), *The Geology of Antarctica*. Clarendon Press, Oxford, pp. 481-488). Aerosols generated from these exposed deposits would find their way into the suboxic waters of both lobes of Lake Bonney, but the weathering and release of organics from these particles likely could have been very different in the suboxic (West) and oxic (East) hypersaline waters during the cold-dry period of 1000-3000 years ago. There remains some possibility then that organic toxicants released by these weathering processes are partly responsible for limiting denitrification. We tested this possibility by diluting Lake Bonney waters with synthetic, purified salt solutions have composition and ionic balances matched with in-situ. Results showed no stimulation of bacterial activity in these treatments.

These findings provisionally disprove the hypothesis that organic toxicants are responsible for the lack of measurable denitrification in the East Lobe of Lake Bonney.

In additions concentrations of Cd, Pb, Cr, Ni, and Zn also are 2-5 -fold higher in deep waters of the East Lobe than in West Lobe deep waters, and the uncertainties of ionic strength corrections for the model calculations leaves open the possibility that our chelator additions may not have fully complexed these metals. Although these differences are much less dramatic their combined, or synergistic effects conceivably could impede microbial activity in these waters.

Hypotheses 4: A major fraction of denitrifying bacteria in suboxic waters of the East Lobe of Lake Bonney are viable but inactive.

Denitrifying bacterial strains from Lake Bonney (fresh isolates and several previously collected strains stored at -80 C) were grown in well-defined laboratory conditions under both oxic and suboxic (denitrifying) conditions. A suite of probes (see below) were characterized in terms of their sensitivity for these isolates.

Metabolically active cells with intact cytoplasmic membranes maintain an electrical potential across the membrane. Oxonol dyes that carry a single delocalized negative charge are excluded from bacterial cells with a normal (positive) membrane potential gradient and the fluorescence of organisms exposed to them increases with increasing membrane depolarization (Novo et al., 2000). We used SYTO 13 DNA stain to quantify the abundance of bacterial cells, and propidium iodide, PI, to detect cells that have lost membrane integrity. We intended to apply the ratiometric method of Novo et al. (1999) to assay membrane potential by flow cytometry. Cells stained with SYTO 13 fluoresce green due to binding of the dye to DNA, independent of membrane potential. Cells with compromised membrane potential accumulate PI in high concentrations in the cytoplasm accompanied by a spectral shift in the fluorescence to red. The ratio of red to green fluorescence allows a measure of membrane potential that is independent of cell size. CFDA SE (also called CFSE) is a non-fluorescent dye that passively diffuses into the cytoplasm of cells. Once inside the cell, it is cleaved by intracellular esterases, becoming fluorescent. This highly amine-reactive product then forms dye-protein adducts that are retained by the cells throughout their development. Moreover CFSE is inherited equally by daughter cells after division, resulting in the sequential halving of mean fluorescence with each generation. When analyzed by flow cytometry, this sequential halving of fluorescence is visualized as distinct peaks and can be used to track division progression. We employed this dye to gain insight to the growth rates of bacteria in Lake Bonney water, and in our experimental treatments

The capacity to reduce CTC (5-cyano-2,3-ditolylyl tetrazolium chloride) to its fluorescent product formazan has been used to indicate actively respiring cells in cultures and natural aquatic samples (Rodriguez et al., 1992; Sieracki et al., 1999; del Giorgio and Bouvier, 2002). We were aware of unpublished research in which CTC was used to characterize the bacterial assemblage in Lake Bonney, but the results were inconsistent and have remained unpublished (Smith and Priscu, personal communication). Instead, we attempted to use CTC in much the same manner as proposed above for CFDA/SE. Cultured bacteria will be stained with CTC following the protocol of del Giorgio and Bouvier (2002) over a time course of exposure to environmental challenges (proposed treatments, above). Because we were staining a vigorous pure culture initially, we expected to be able to detect a decrease in activity and number of cells stained by comparison to cells in a control treatment.

There are no cell-specific indicators for denitrification. We investigated the efficacy of utilizing in-vivo determinations of NO concentrations and production as a proxy for denitrification in bacterial isolates from Lake Bonney. Nitric oxide is very unstable and no equilibrium indicators for NO are known. We tested a variety of existing in-vivo NO

indicators in laboratory cultures of fresh and cryogenically preserved Lake Bonney isolates, starting with 4,5-diaminofluorescein diacetate (DAF-2 diacetate) (Kawahara et al., 1998), (4-amino-5-methylamino-2',7'-difluorofluorescein (DAF-FM) (Kojima et al., 1999), and 1,2-diaminoanthraquinone (DAA) (Heiduschka and Thanos, 1998). Rather than quantify denitrification, the goal was to screen the experimental treatments to sense changes in, or onset of, denitrification processes.

Flow cytometric analyses of vertical profiles in the East lobe of Lake Bonney showed two peaks in heterotrophic abundance; one at 10m in the oxic freshwater zone, and one at 22 m associated with the sharp increase in conductivity. Eukaryotic phytoplankton were restricted to the fresh surface waters. In contrast, heterotrophic bacterial abundance was elevated only at the pycnocline in West lobe Lake Bonney. Eukaryotic phytoplankton also were restricted to fresh surface waters, but were less abundant than in East Lobe Lake Bonney.

Dilution experiments to measure the effects decreasing salinity, decreasing metals, decreasing organic matter on bacterial growth were inconclusive. Despite replication, there were no significant changes in CFSE labeled cells in the West lobe experiments, and a net disappearance of stain in the East Lobe experiments, indicating the experiment traced cell loss over time (rather than growth). There also was significant variability among the bag treatments, suggesting that mixing was not effect within the bags. Overall, despite considerable effort, the findings were not able to show any consistent positive or negative effects of the treatments examined.

Incubation experiments aimed at testing the cell-specific probes for NO production proved unsuccessful. In no case was it feasible to measure probe activity despite indications that denitrification was occurring.

In general, probe behavior in the solution phase was substantially different than in seawater, an indication of the different salt matrix and concentration. While extremely frustrating, the findings are useful in that they demonstrate that new methods will have to be developed before cell-specific techniques can be applied in high saline, anoxic water systems of subglacial lakes.

The findings include complete vertical profiles of trace metals in the West and East Lobes of Lake Bonney, and a transect between the primary stations across the Lake. These data are to be contrasted with LTER data, and earlier trace metal findings from Lake Bonney.

There currently are three manuscripts in preparation for this project. Two on trace metal distributions and one short manuscript on the Flow cytometric findings.

Training and Development:

The project supported one full time Ph. D graduate student (Eric Roy) who participated in all aspects of the work at different stages, including the second field season. Mr. Roy took responsibility for developing and testing the on-line Ag extraction/analysis and has been working on the analysis of other metals in the samples returned from Lake Bonney. Sea ice cores collected to assess the metal composition of dust transported in the Dry Valleys are being analyzed by an undergraduate student (Mr. Morgan Brunbauer) in the Maine ICPMS facility. Components of the project and broader perspectives of biogeochemistry of the Dry Valleys and adjoining regions have been incorporated into graduate courses taught but M. Wells.

Outreach Activities:

Outreach activities to communicate our research efforts, and the field of oceanography in general, have been both local and field-based. Local activities include the incorporation of Polar Research principles in undergraduate and graduate curricula as well as general public outreach efforts. Dr. Wells has participated each year in the local chapter of the international Lego-league program as a Oceanography and nanotechnology expert. These programs focus on providing elementary and middle school students opportunities to investigate, plan, and implement programmed robotic missions that emphasize environmental, marine and broader engineering and environmental knowledge bases. Dr. Wells also has devoted time to reviewing web-based educational sites on behalf of the Maine COSEE office.

Journal Publications

Books or Other One-time Publications

Web/Internet Site

Other Specific Products

Contributions

Contributions within Discipline:

The primary contribution arising from this project is the demonstration that a number of probes actively used in other marine systems are not effective as metabolic tracers in Lake Bonney waters. Other techniques will be required if we are to eventually determine what factor, or factors are responsible for the absence of denitrification in the East Lobe of Lake Bonney.

Contributions to Other Disciplines:

Contributions to Human Resource Development:

The project supported the PhD. research of Mr. Eric Roy, who will use this research experience as a significant component of his dissertation research.

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

Categories for which nothing is reported:

Any Journal

Any Book

Any Web/Internet Site

Any Product

Contributions: To Any Other Disciplines

Contributions: To Any Resources for Research and Education

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