Mono Lake Microbial Observatory
Year 4 Annual Report
March 2003

Project title: "Collaborative Proposal: Microbial Observatory at an alkaline, hypersaline, meromictic lake (Mono Lake, California)." Five years, October 1, 1999 - September 30, 2004. This project was funded through 3 awards: MCB 99-77886 to the University of Georgia, J.T. **Hollibaugh** PD and S.B. **Joye** co-PI; MCB 99-77892 to the University of California, Santa Cruz, J.P. **Zehr**, PD; and MCB 99-77901 to the University of California, Santa Barbara, R.S. **Jellison**, PD.

Purpose. The Mono Lake Microbial Observatory (ML MObs) examines chemical distributions, biogeochemical processes and microbial community dynamics in Mono Lake. Mono Lake provides contrasting environments over depth and along isopycnals in which to study how environmental factors (redox, light, salinity, etc.) affect the composition and activities of microbes. Mono Lake is also an exotic, if not extreme, environment: high salinity (>80 g/L) and pH (9.8). Within this context we studied how microorganisms are distributed spatially and temporally and how they interact within the strong chemical gradients of the lake's water column.

Accomplishments. Our activities over the past year have focused on obtaining a better record of the seasonal progression of the lake's biogeochemical properties, particularly methane oxidation and arsenic biogeochemistry. To this end, we obtained complete profiles (15-20 depths) of biogeochemical and microbiological variables at Station 6 in the deepest part of Mono Lake in February, March, April, May, August, September and November.

At each sampling time, oxygen (O_2) profiles were determined using a YSI oxygen probe. A CTD was used to document the distribution of temperature, conductivity, chlorophyll (via fluorometry) and suspended particles (transmissometry). Water samples were collected from discrete depths using a Niskin bottle. Samples for the nutrient and sulfide analyses were immediately filtered and stored in gas tight glass bottles. Samples for arsenic speciation were immediately frozen in liquid nitrogen, stored frozen in liquid nitrogen or dry ice and shipped on dry ice to a commercial laboratory (Frontier Geosciences) for immediate analysis. Samples for rate assays were collected into gas tight bottles (rinsed and overfilled), which were subsequently sealed without a headspace. Ion specific microelectrodes, gas chromatography (FID), and colorimetric analyses were used to quantify hydrogen sulfide (S2-), methane (CH4), and ammonium (NH4) concentrations, respectively. Water samples for experiments and other analyses (for example, estimates of arsenic reducer populations by MPN) were collected into gas tight bottles and returned to the laboratory via air-freight.

Significant spatial and temporal changes in the distribution of dissolved oxygen and reduced metabolites (ammonium, methane, arsenite and hydrogen sulfide) were observed during the development of thermal stratification between February (winter) and August (summer) 2002. During winter (February), the oxycline, thermocline and chemocline were coincident. Concentrations of reduced metabolites were low in the mixolimnion but were significant in the monimolimnion. During early spring (March), the thermocline and chemocline began to separate. A broad sub-oxic, non-sulfidic zone was present between the developing thermocline and the permanent chemocline during

April and May. By June, oxygen was depleted and reduced species had accumulated below the thermocline and the oxycline corresponded closely to the chemocline. In August, a zone of apparent methane production and sulfate reduction was found between the chemocline and base of the oxycline. Fall overturn commenced in September and by the mid-November sampling, water column distributions of reduced substances were similar to those encountered in March.

Methane oxidation rates exhibited a dramatic seasonal cycle. Samples for rate assays were collected in parallel with geochemical samples. Methane oxidation rates were measured via C^3H_4 in the low methane surface waters and with $^{14}CH_4$ in the deep, methane rich waters. In August 2002 we also measured sulfate reduction rates using $^{35}SO_4$ to compare rates of organic matter degradation with rates of methane oxidation. The peak of aerobic methane oxidation was consistently located towards the bottom of the oxycline. Aerobic methane oxidation rates correlated with methane and oxygen concentrations and occurred in a zone where $[O_2] < \sim 2$ mg/L and $[CH_4] > \sim 2$ µM. Anaerobic methane oxidation (AOM) rates also exhibited interesting monthly variability with the highest rates (> 1.5 µM/day) in February and August and the lowest rates in April and May. The highest rates of sulfate reduction (SR) (> 2 µM/day) were present in deep anoxic (38 m) waters but activity was measurable in all anoxic water samples. Following the fall die-off of *Artemia*, there was a large deposition of labile carbon to deep waters (as evidenced by the accumulation of *Artemia* skeletons on top of the sediments at Station 6). As a consequence of this organic carbon influx, deep water sulfate reduction rates increased by 10X while AOM rates decreased by 70%. These data suggest that seasonal variation in rates of AOM and SR are tightly regulated by the lability of organic carbon in the deep waters.

Integration of methane oxidation activity in oxic, oxycline and anoxic waters was used to evaluate changes in the relative importance of aerobic versus anaerobic methane oxidation. During February and August, over 95% of methane oxidation was observed in the anoxic waters. However, during March through June, aerobic methane oxidation became more important. During May, the lowest aerobic oxidation accounted for almost 75% of methane oxidation. By August, the rates of anaerobic methane oxidation had increased and accounted for 99% of the total depth integrated methane oxidation. In August, rates of methane oxidation and sulfate reduction were almost equal (indistinguishable from 1:1 given the error bars on both measurements) in anoxic waters below the chemocline. We hypothesize that sulfate reduction and methane oxidation are closely coupled in this system during summer. However, during spring, high rates of primary production (spring bloom) may lead to increased labile organic matter flux to deep waters, which could de-couple methane oxidation and sulfate reduction. We plan to examine the interactions between sulfate reduction and methane oxidation in more detail in the future. Additional work in progress includes molecular characterization of methane oxidizing bacterial community structure associated with rate profile features including PCR/DGGE, FISH, and lipid biomarker analyses.

Arsenic speciation closely followed the distribution of oxygen in the lake, with As(V) being rapidly reduced to As(III) as oxygen concentration declined to 0. The dominant form of As in oxic waters is arsenate while As(III) in anoxic waters is present as arsenate or mono-, di- or tri-thioarsenite, depending on sulfide concentration. Other As species, including organic As compounds, accounted for less than 5% of the total inventory. As(V) and As(III) distributions overlapped in the suboxic zone in March and April, suggesting possible kinetic control on arsenic reduction. Based on MPN assays using lactate and acetate as supplemental electron donors, abundance of arsenate reducers were consistently low ($<10^4$/mL), peaking just above the chemocline, which was not always where the highest arsenate reduction rates (as indicated by mass balance analysis) occurred. We also consistently observed oxidized As species in the form of arsenate in the lake's bottom waters. In collaboration with Dr. Ron Oremland of the USGS in Menlo Park, we documented anaerobic arsenite oxidation in Mono Lake using Fe(III) or nitrate as an electron acceptor and we have isolated and characterized a facultative anaerobic bacterium that is capable of anaerobic arsenite oxidation using nitrate as an electron acceptor.

We also continued work on other aspects of the lake's microbiology and biogeochemistry begun in previous years. We have isolated into pure culture an organism closely related to the marine *Synechococcus* strains. We obtained the inoculum for this culture from the bottom water of station 6 (dark, sulfidic) and are exploring its physiological capabilities and phylogenetic relationships to other *Synechococcus*-like organisms. We have characterized the vertical distribution of genes (*cbb*L and *cbb*M)encoding RubisCO, an enzyme that catalyzes carbon fixation in many organisms, at Station 6. We also began a study of chitin degradation in the lake. Chitin is an important biopolymer in Mono Lake because it is copiously produced by the large biomass of *Artemia* that live in the lake during the summer. Our investigations include isolating chitin-degrading bacteria, characterizing the seasonal cycle of chitinase activity *in situ* using artificial substrates and examining the diversity of chitinase genes (*chi*A) in the lake.

Outreach. In addition to the seminars and presentations listed below, we continue to work with the Mono Lake Committee, to make information on the Mono Lake MObs project available on their web site (see web address above). We have taken them out on the lake so that they could capture some of our activities on film and provided them with examples of data along with explanatory text written for the general public. They, in return, have been very good about maintaining their web site and providing us with access to it.

Human resources. The human resources impacted by this funding (cumulative since the beginning of the project) include 4 research associates (Shaheen Humayoun, Nasreen Bano, Sandy Roll, Vladmir Smarkin); 3 postdocs (Francoise Lucas, Grieg Steward, Bethany Jenkins); 2 technicians (Lydia Vasquez, Matt Erickson); 6 graduate students (Gary Lecleir, Steve Carini, Rosalynn Lee, Samantha Lugo, Andi Stephens, Charles Budinoff, Beth Orcutt); 6 undergraduates (Chris Weaver, Briana Ransom, Shomari Ruffin, Beth Orcutt, Kelley Cornett, Eric Vaillant) and 3 high school students (Ryan Hollibaugh, Jennifer Nation, Joshawna Nunnery).

Collections. We are maintaining samples of the crude DNA collected during the project in our (JTH, SBJ and JZ) laboratories along with the clone libraries generated during our analyses. Sequences have been deposited in GenBank. R. Jellison has designed and implemented a fully relational database of limnological data collected from Mono Lake using Microsoft Access. In addition to the actual data, this database includes a full suite of metadata and user-friendly search routines. This ACCESS database provides an efficient research tool for rapid querying of a wide array of diverse data and is shared among collaborating researchers via distribution on CDs.

Publications. Eight papers that are published or in review acknowledge support from Mono Lake Microbial Observatory awards; 7 additional papers are in the final stages of preparation. Twenty papers based on results of this work have been presented at several national and international meetings. Publications and presentations (cumulative since the beginning of the project) are listed below.

Peer-Reviewed Publications

Hollibaugh, J.T., P.S. Wong, N. Bano, S.K. Pak, E.M. Prager and C. Orrego. 2001. Stratification of microbial assemblages in Mono Lake, California, and response to a mixing event. Hydrobiologia 466: 45-60. MCB 99-77886

Lucas, F. and J.T. Hollibaugh. 2001. Shifts in the composition of estuarine sediment bacterial assemblages in response to amendments with selenate and acetate. Environmental Science and Technology 35: 528-534. MCB 99-77886

Hoeft, S. E., F. Lucas, J.T. Hollibaugh, and R.S. Oremland. 2002. Characterization of bacterial arsenate reduction in the anoxic bottom waters of Mono Lake, California. Geomicrobiology Journal 19: 23-40. MCB 99-77886

Oremland, R.S., S.E. Hoeft, J.M. Santini, N. Bano, R.A. Hollibaugh and J.T. Hollibaugh. 2002. Anaerobic oxidation of arsenite in Mono Lake water and by a facultative chemoautotroph, strain MLHE-1. Applied and Environmental Microbiology 68: 4795-4802. MCB 99-77886

Humayoun, S., N. Bano and J.T. Hollibaugh. 2003. Phylogenetic composition of the bacterioplankton from an alkaline, hypersaline lake, Mono Lake California. Applied and Environmental Microbiology 69:1030-1042. MCB 99-77886 and MCB 99-77901.

Carini, S.A., B. N. Orcutt, and Joye, S.B. In press. Interactions between aerobic methane oxidation and nitrification in coastal sediments. Geomicrobiology Journal. MCB 99-77886

S. Jiang, G.S. Steward, R.E. Jellison, W. Chu, S. Choi. Submitted. Abundance, distribution and diversity of viruses in an alkaline hypersaline lake, Mono Lake, California. Applied and Environmental Microbiology. MCB 99-77886 and MCB 99-77901.

Steward, G., J.P. Zehr, R. Jellison, J.P. Montoya, and J.T. Hollibaugh. In press. Vertical distribution of nitrogen-fixing phylotypes in a meromictic hypersaline lake. Microbial Ecology. MCB 99-77892, MCB 99-77886 and MCB 99-77901.

Scholten, J.C.M, S.B. Joye, J.T. Hollibaugh and J. Colin Murrell. Submitted. Molecular analysis of the sulfate reducing and methanogenic community in a meromictic lake (Mono Lake, California) by targeting 16s rRNA, methyl coM-, aps- and dissimilatory sulfite reductase genes. Applied and Environmental Microbiology. MCB 99-77886 and MCB 99-77901

Published Abstracts and Seminars

Hoeft, S. E., F. Lucas, J.T. Hollibaugh, and R.S. Oremland. Characterization of bacterial arsenate reduction in the anoxic bottom waters of a meromictic, hypersaline, alkaline soda lake: Mono Lake, California. International Symposium on Environmental Biogeochemistry, Warsaw, Poland, August 2001. MCB 99-77886.

Hollibaugh, J.T., G. Rocap, N. Ahlgren, S.W. Chisholm, J. Nelson and R.S. Jellison. An unusual Synechococcus from Mono Lake, California. American Society of Limnology and Oceanography Annual Meeting, Victoria, B.C., Canada, June 2002. MCB 99-77886.

Humayoun, S., G. Lecleir, N. Bano, J.T. Hollibaugh. Composition of bacterial assemblages from alkaline, hypersaline Mono Lake, California. International Symposium on Microbial Ecology, Amsterdam, the Netherlands, September 2001. MCB 99-77886.

Jiang, S., G. Steward, R. Jellison, S.B. Joye and J.T. Hollibaugh. (2001). Abundance and diversity of viruses in an alkaline hypersaline lake - Mono Lake, California. American Society of Limnology and Oceanography Annual Meeting, Albequerque NM, February 2001. MCB 99-77886, MCB 99-77892 and MCB 99-77901.

Joye, S.B. Biogeochemistry of methane in alkaline, hypersaline Mono Lake. Seminar presented to the University of Georgia, Department of Microbiology, Athens GA, June 2001. MCB 99-77886.

Joye, S.B., S. A. Carini, and J. T. Hollibaugh. Molecular ecology and biogeochemistry of methane cycling in an alkaline, hypersaline lake. International Symposium on Microbial Ecology, Amsterdam, the Netherlands, August 2001. MCB 99-77886.

Joye, S.B. Molecular biogeochemistry: Linking the distribution of microbes to their biogeochemical function in the environment. International Symposium on Environmental Biogeochemistry, Warsaw, Poland, august 2001. MCB 99-77886.

Joye, S.B., S. A. Carini, and B. Orcutt. Methane oxidation and the distribution of methanotrophs in the environment. American Society for Microbiology Annual Meeting, Atlanta GA, May 2001. MCB 99-77886.

Joye, S.B. Achaean analogs: Methane biogeochemistry in an alkaline soda lake: Mono lake, California. Carnegie Institution of Washington Geophysical Laboratory, Washington DC, Sept 2000. MCB 99-77886.

Lucas, F.S., J. Switzer-Blum, R.S. Oremland, and J.T. Hollibaugh. Identification of mRNA differentially transcribed by *Sulfurospirillum barnesii* SES-3 during selenate versus nitrate respiration. International Symposium on Microbial Ecology, Amsterdam, the Netherlands, September 2001. MCB 99-77886.

Stephens, A.Q., G.F. Steward and J.P. Zehr. Computer simulation of TRFLP using functional gene sequences and implications for microbial community analysis. American Society for Limnology and Oceanography Summer Meeting. Victoria, B. C. Canada, June 2002. MCB 99-77892

Steward, G.F., Jellison, R.S., Hollibaugh, J.T., Joye, S.B., and Zehr, J.P. Detection of novel and diverse nitrogenase genes suggests potential for pelagic diazotrophy in alkaline, hypersaline Mono Lake. American Society for Limnology and Oceanography Annual Meeting, Albuquerque NM, February 2001. MCB 99-77886 MCB, 99-77892 and MCB 99-77901.

Steward, G. F., Zehr, J. P., Jenkins, B. D., Montoya, J. P., Jellison, R. S. and Hollibaugh, J. T. 2003. Diverse nitrogen-fixers, but undetectable nitrogen fixation in alkaline, hypersaline, meromictic Mono Lake, California. ASLO Aquatic Sciences Meeting. Salt Lake City, Utah, February 2003. MCB 99-77892, MCB 99-77901 and MCB 99-77886

Carini, S.A. and S.B.Joye. Patterns of aerobic methane oxidation and methanotroph community composition during development of thermal stratification in Mono Lake, California. ASLO Aquatic Sciences Meeting. Salt Lake City, Utah, February 2003. MCB 99-77886.

Joye, S.B., S.A. Carini, V. Samarkin, J. T. Hollibaugh, and R.S. Jellison. Spatial and seasonal patterns of the anaerobic oxidation of methane in Mono Lake, California.. ASLO Aquatic Sciences Meeting. Salt Lake City, Utah, February 2003. MCB 99-77901 and MCB 99-77886.

Carini, S. and S.B. Joye. Seasonal variations in aerobic methane oxidation rates in Mono Lake (USA) as a function of geochemical variables and methanotroph community composition. AGU/EUG/EGU meeting Nice, France, April 2003. MCB 99-77886

Joye, S.B., S. Carini, V. Samarkin, R. Jellison and J.T Hollibaugh. Seasonal decoupling of sulfate reduction and anaerobic oxidation of methane in Mono Lake, California. AGU/EUG/EGU meeting Nice, France, April 2003. MCB 99-77886 and MCB 99-77901.

Hollibaugh, J.T., S. Humayoun, N. Bano and R.E. Jellison. Diversity of bacteria in alkaline, hypersaline Mono Lake, California, during a period of meromixis. ASLO Aquatic Sciences Meeting. Salt Lake City, Utah, February 2003. MCB 99-77901 and MCB 99-77886.

Budinoff, C. and J.T. Hollibaugh. Ecology and physiology of a phylogenetically unique *Synechococcus* from Mono Lake. ASLO Aquatic Sciences Meeting. Salt Lake City, Utah, February 2003. MCB 99-77886.

Scholten, J.C.M, S.B. Joye, J.T. Hollibaugh, A.J.M. Stams and J.C. Murrell. Anaerobic degradation of glycine-betaine in Mono Lake, a moderately, hypersaline, alkaline environment. ASLO Aquatic Sciences Meeting. Salt Lake City, Utah, February 2003. MCB 99-77886.

Works in Progress

Hepperle, D., N. Bano and J.T. Hollibaugh. In preparation. Picocystophyceae, a new class of the green algae (Chlorophyta). Protista. MCB 99-77886.

Steward, G.F., Zehr, J.P., and Stephens, A.Q. Seasonal and depth distribution of nitrogen fixation genes in Mono Lake, California. MCB 99-77892.

Oremland, R.S., J.F. Stolz and J.T. Hollibaugh. In preparation. The microbial arsenic cycle in Mono lake, California. invited review, FEMS Microbiology Ecology. MCB 99-77886

Zehr, J.P., Jenkins, B.D., Short, S.M., Omoregie, E.O., Bebout, B. M. and Steward, G.F. (2003) Nitrogenase gene diversity and microbial community structure: a

cross-system comparison. *Environmental Microbiology*. MCB 99-77892 and MCB 99-77901.

Hollibaugh, J.T., S.B. Humayoun, N. Bano, G. Lecleir, B. Ransom, R.S. Jellison and S.B. Joye. In preparation. Spatial and temporal variation of the bacteria community in alkaline, hypersaline Mono Lake, California. Aquatic Microbial Ecology. MCB 99-77886 and MCB 99-77901.

S. Carini and S.B. Joye. In preparation. Seasonal variations in aerobic methane oxidation rates in Mono Lake (USA) as a function of geochemical variables and methanotroph community composition. Applied and Environmental Microbiology. MCB 99-77886

S.B. Joye, S. Carini, V. Samarkin, J.T. Hollibaugh and R.S. Jellison. In preparation. Seasonal decoupling of sulfate reduction and anaerobic oxidation of methane in an alkaline, hypersaline lake, Mono Lake, CA (USA). To be determined. MCB 99-77886 and MCB 99-77901.

Hollibaugh, J.T., S.B. Joye, R.E. Jellison, L. Vasquez, N. Bano, H. Gurleyuk and D. Wallenschlager. In preparation. Distribution of arsenic species in alkaline, hypersaline Mono Lake, California, in response to seasonal stratification and oxygen depletion. Geochimica Cosmochimica Acta. MCB 99-77886 and MCB 99-77901.