Mono Lake Microbial Observatory
Year 3 Annual Report

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 extreme environment: high salinity (>80 g/L) and pH (9.8). Within this context we studied how microorganisms are distributed vertically and seasonally and how they interact within the strong chemical gradients.

Accomplishments. Six stations distributed across the lake were sampled in May and July 2000; February, April 2001 and August 2001. The stations encompass both shallow and deep water and range from the center to the edge of the lake.

Physical, chemical and biological variables and rates of biogeochemical processes were measured along vertical profiles taken at 2 deep-water sites (stations 3 and 6) that span both the oxy- and chemo-clines but differ in their interaction with the internal seiche of the lake (station 3 exhibits more spatial and temporal variability). Rates and inventories were compared to archive data to provide information about seasonal variation and the effect of changing stratification regimes on microbial activity. For example, methane oxidation rates measured in 2001 are 5 times the rates measured in 1994 and 1997.

DNA samples collected at all of these stations and depths (a total of 220 samples) have been subjected to PCR/DGGE analysis (See Mono Lake Committee web page at www.monobasinresearch.org/research/#BIOLOGY) to determine the spatial and temporal variability of lake microbial populations. Eighteen samples were chosen for more detailed phylogenetic analysis based on cloning and sequencing 16S rRNA genes. Approximately 400 clones have been sequenced, resulting in the identification of 71 different Bacterial sequences.

The sequences we obtained contained representatives of the major classes of Bacteria with the exception of the â-Proteobacteria. Community composition changed little throughout

the year. Surface waters appear to be dominated by Actinobacteria (50% of the sequences); however, these sequences fell into 3 distinct clades of closely related sequences. In contrast, hypolimnion assemblages appeared to be dominated by Bacillus/Clostridia-like sequences (53% of 54 randomly selected clones contained sequences with affinity to this group); however, this group exhibited great diversity: no two clones were similar at 99% or greater. We also identified a sequence with equal affinity to *Prochlorococcus* and *Synechococcus* in Mono Lake bottom waters. These samples contain an orange-fluorescing, rod-shaped cell (ca 2 x 0.75 um) in high abundance ($>10^9$/L). Despite its unusual distribution (maximum population in dark, anoxic bottom waters), the organism appears to be a photoautotroph that either tolerates or requires high sulfide concentrations. We are in the process of purifying it from enrichment cultures.

In addition to surveys, we have also conducted studies of specific processes. Collaborators J. Colin Murrell and J.C.M. (Hans) Scholten, Department of Biological Sciences, University of Warwick, UK, have worked with Dr. Joye to characterize organisms involved in anaerobic methane oxidation in anoxic waters and, as a result have isolated, identified and characterized approximately 40 sulfate reducing bacteria (SRB) from Mono Lake. Absolute rates of aerobic methane oxidation are greater than anaerobic rates but depth-integrated rates show that anaerobic oxidation accounts for up to 80% of total methane oxidation. This work is being prepared for publication. In a related study, collaborator Dr. Antje Boetius, Max Plank Institute for Marine Microbiology, Bremen, Germany, has examined samples from Mono Lake for the presence of SRB and Archaeal consortia that might be involved in anaerobic methane oxidation.

We have also performed enrichment experiments designed to identify bacteria responsible for significant biogeochemical reactions. These experiments have included amendments with various electron acceptors, with size fractions of DOC and with trace metals. We have identified organisms responding to these perturbations by PCR/DGGE and cloning/sequencing. One result of these experiments is that they suggest the growth of aerobic heterotrophic bacteria is limited by the availability of trace metals, specifically Co, Mo and Ni. Another is that there is an active, microbially mediated As-cycle in Mono Lake.

We have obtained partial sequences of nitrogenase genes from three zones in the lake: the epilimnion, the oxycline and the anoxic hypolimnion. Our results provide the first evidence for the presence of a diverse array of novel planktonic heterotrophic bacteria capable of nitrogen fixation in Mono Lake. Despite the widespread occurrence of *nif* genes in Mono

Lake, ^{15}N experiments conducted in mid-summer (greatest N-limitation) indicated that nitrogen fixation was not occurring in either the mixolimnion or the monimolimnion.

The sequences we obtained share <87% similarity with other nitrogenase sequences indicating that the diazotrophic communities in the Mono Lake are very different from other environments studied so far. Terminal restriction fragment length polymorphism (TRFLP) and cluster analyses of our first 25 sequences suggest that the diazotroph assemblages in upper mixed layer and oxycline samples are more similar to each other than to assemblages from anoxic bottom waters (Steward et al. 2001). In the upper waters many sequences clustered with the â- and ã-Proteobacteria while others were related to *nif*H genes from anaerobic bacteria such as ä-Proteobacteria and green sulfur bacteria.

The *nif*H genes from the deep anoxic waters clustered mostly with sequences from known anaerobes involved in sulfur cycling but two clustered with a deeply branching group having no cultivated representatives. Analysis of *nif*H genes from macroscopic microbial aggregates along the shore of the Lake supported prior evidence (Oremland R.S. 1990 Appl. Environ. Microbiol. 56:614-622) that a filamentous non-heterocystous cyanobacterium is most likely responsible for much or all of the N_2-fixation in these aggregates. The *nif*H sequence we obtained from the aggregates is most closely related to that from a cyanobacterial isolate in the genus *Phormidium* (91% identity).

Once the diversity has been well covered at the sequence level, the bulk of the samples will be screened by the more rapid TRFLP analysis to examine seasonal and spatial variability in the community of diazotrophs. Our initial sequencing efforts will provide the necessary database to properly interpret variations in the TRFLP patterns. Samples have also been archived from each trip for RNA extractions. These will be analyzed by RT-PCR to determine which, if any, and when the potential diazotrophs are actively expressing nitrogenase. DNA microarrays that will facilitate analysis of Mono Lake samples are being developed with funds from other projects .

Outreach. In addition to the seminars and presentations listed below, we have worked with an NGO, 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 include 3 research associates (Shaheen Humayoun, Nasreen Bano, Sandy Roll); 2 postdocs (Francoise Lucas, Grieg Steward); 4 graduate students (Gary Lecleir, Steve Carini, Rosalynn Lee, Samantha Lugo, Andi Stephens); 4 undergraduates (Chris Weaver, Beth Orcutt, Briana Ransom, Shomari Ruffin, Kelley Cornett) 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 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. Seven papers that are published or in review acknowledge support from this grant; 4 additional papers are in the final stages or preparation. Eleven papers based on results of this work have been presented at several national and international meetings. Publications and presentations are listed below.

Peer-Reviewed Publications

Carini, S.A. and Joye, S.B. Submitted. Molecular ecology and biogeochemistry of aerobic methane oxidation in Mono Lake, California. Geomicrobiology Journal. MCB 99-77886.

Hollibaugh, J.T., P.S. Wong, N. Bano, S.K. Pak and C. Orrego. In press. Stratification of microbial assemblages in Mono Lake, California, and response to a mixing event. Hydrobiologia. MCB 99-77886.

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

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.

Lucas, F.S. J. Switzer-Blum, R.S. Oremland and J.T. Hollibaugh. Submitted. Identification of mRNA differentially transcribed by Sulfurospirillum barnesii SES-3 during selenate versus nitrate respiration. Geomicrobiology Journal. 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.

Humayoun, S., N. Bano and J.T. Hollibaugh. Submitted. Phylogenetic composition of the bacterioplankton from alkaline, hypersaline Mono Lake California. Applied and Environmental Microbiology. MCB 99-77886.

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. Archaean 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.

Works in Preparation

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

Hollibaugh, J.T., G. Lecleir. Co, Mo and Ni availability limit the growth of aerobic heterotrophic bacteria in alkaline, hypersaline Mono Lake, California. Limnology and Oceanography. MCB 99-77886.

Oremland, R.S., S.E. Hoeft, N. Bano, R.A. Hollibaugh and J.T. Hollibaugh. Anaerobic oxidation of arsenite by a chemoautotrophic bacterium isolated from Mono Lake, California. Applied and Environmental Microbiology. 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.

Zehr, J. P., Steward, G. F., Omoregie, E.O., Bebout, B. M. Nitrogenase phylogenetic diversity in aquatic environments: implications for genetic diversity of aquatic microbial assemblages. MCB 99-77892.