

## PROJECT DESCRIPTION

**Note to Reviewers.** We have never submitted a collaborative proposal over FASTLANE before and some of the instructions we received were confusing. We hope that all of the pieces (for example detailed budgets from Jellison and Zehr, who are our collaborators) are in the master copy of this proposal that was sent out for review.

## RESULTS FROM PRIOR NSF SUPPORT

**James T. Hollibaugh.** OCE 89-14921, "LMER site for the study of biogeochemical reactions in estuaries at Tomales Bay, California," 10/89-9/94, \$863,139; OCE 93-15639, "An investigation of the relationship between community composition and metabolic capabilities of coastal bacterioplankton communities," 10/93-9/96, \$320,000; OPP 95-00237, "Diversity and metabolic capabilities of Arctic bacterioplankton," 1/95-12/96, \$160,000; OPP 96-25131, "Diversity and metabolic capabilities of Arctic bacterioplankton - SCICEX 96," 5/96-4/98, \$161,000 (re-issued as OPP 97-96261, 5/97-4/99, no change in funding level); and OPP 98-09971, "Diversity and metabolic capabilities of Arctic bacterioplankton - SCICEX 97," 5/98-4/00, \$165,000. Publications acknowledging support from these awards for which I am an author are listed in the Bibliography. Approximately 50 more papers and theses also acknowledge support from these awards. We have also presented numerous papers on these projects at national and international meetings (see ASLO, AGU, ERF, ECSA, ISME abstract volumes) and at seminars at a number of institutions. The human resources impact of the support has been 5 postdocs, 6 graduate students, 3 undergraduate students, 5 high school students and 3 technicians.

The data collected during these studies are available in lab notebooks and Excel or DBase files kept at the Department of Marine Sciences. The data LMER data set can be accessed through the LMER web page: <http://www.mbl.edu/html/ECOSYSTEMS/lmer/tomalbay/tomalbay.html> Archive samples of DNA (see body of proposal, below) collected during OCE 93-15639, OPP 95-00237; OPP 96-25131; and OPP 98-09971 are stored frozen at -80 °C in extraction buffer. These samples continue to give reproducible PCR/DGGE patterns. Subsamples are available to other investigators on request. So far we have filled requests for Mono Lake (Dr. B.B. Ward, Princeton Univ.) and atoll and Arctic Ocean (Dr. J. Fuhrman, USC) samples.

A major goal of OCE 93-15639 was to develop a method that would allow us to characterize the phylogenetic composition of natural microbial assemblages rapidly and simply. We adopted an approach based on denaturing gradient gel electrophoretic (DGGE) separation of ca 200 bp fragments produced by polymerase chain reaction (PCR) amplification of a region of the 16S rRNA gene (positions 341-534 on the E.coli gene, these primers are relatively specific for Bacteria). The method was developed and tested in northern California coastal waters (Murray 1994; Murray et al. 1996). We then applied it to the analysis of microbial assemblages from a number of environments including Mono Lake, coral reef atolls, and sediments. It has also been used extensively with samples from the Arctic Ocean (OPP awards).

**Robert S. Jellison.** DEB 95-08733, "LTREB: Responses of a saline lake to environmental change from seasonal to decadal time Scales," 9/95-8/00, \$250,689. In 1995 as this study began, high runoff and restricted diversions led to the onset of persistent chemical stratification (meromixis) in Mono Lake. Thus, a portion of our research effort focused on the character and probable duration of meromixis. This required accurate determination of the density characteristics of Mono Lake brine (Jellison et al. In press) and hydrodynamic modeling analysis of long-term chemical stratification (Jellison et al. 1998). Our analysis indicates that meromixis is likely to persist for several decades and will affect many aspects of the plankton dynamics. Major differences in internal nutrient cycling, and the phytoplankton and zooplankton dynamics between meromictic and monomictic years were noted (Jellison and Melack 1998). Results of particulate elemental ratios indicate that phytoplankton were more nutrient stressed following the onset of meromixis. This led to declines in both primary and secondary productivity. Nearly all aspects of the life-history of the only macrozooplankton in the lake, *Artemia monica*, were

affected. Rotifers, which were last reported in Mono Lake in the mid-1960s have re-appeared and their seasonal dynamics are being monitored and analyzed. Collaborative efforts with avian researchers at Mono Lake have begun and results indicate lowered productivity in gull and grebe populations during meromixis (*In prep*).

A major collaborative effort was made to characterize vertical mixing processes in Mono Lake as this is integral to understanding ongoing changes in nutrient dynamics and associated changes in primary productivity and secondary productivity. This effort involved a series of surveys at different times of the year in which physical microstructure profiling was conducted in concert with conductivity, temperature, nutrient, and phytoplankton profiles. These efforts indicated that most of the vertical fluxes of heat and nutrients occurred near shore due to boundary mixing initiated by high wind events (MacIntyre et al. in press). An inert tracer (SF6) study was conducted in 1998 and will be expanded and repeated in 1999.

A secondary set of objectives included analysis of sediment cores and correlation with contemporary plankton dynamics. Our analysis of organic matter accumulation during the past 170 years indicated a positive correlation between accumulation rates and salinity (Jellison et al. 1996). A set of 7 cores was collected to assess spatial variability in deposition rates and analysis indicated approximately two-fold variation in sedimentation rates across the lake (*In prep*). The diatom composition of the 170-yr core was also determined (*In prep*). Also, further analysis of more recently deposited sediments has begun and will continue through the end of the grant period.

As part of the LTREB program, meteorological (MET) and limnological (LIM) databases have been established. The MET database includes data and metadata collected from Paoha Island and from a shore station. The LIM database includes all routine monitoring data collected from 1982 to 1998 and associated metadata. Both databases are accessible at the Sierra Nevada Aquatic Research Laboratory. Web access is expected by 8/2000. This project has supported 3 graduate students and 1 post doc. Publications are listed in the Bibliography of this proposal.

**Samantha B. Joye.** OCE 96-96054 "Nitrification in aquatic sediments: Interactions with methane and hydrogen sulfide," 10/95-9/99, \$299,000. This project examines the biogeochemical cycling of nitrogen and the physiological and environmental controls on nitrification within Galveston Bay, Texas. Our objectives are to examine the influence of methane and sulfide on nitrification rates. Secondary objectives are to assess coupling between nitrification and denitrification and to determine the relative efficiency of nitrogen regeneration in Bay sediments in order to develop an improved system-level N budget. This project has supported six graduate students, two technicians, and five undergraduate students, generating one undergraduate thesis, two M. Sc. theses, and one Ph. D. dissertation. Field work was completed in October of 1998 and laboratory experiments and sample analyses should be completed by July of 1999. To date, we have published three papers (see Bibliography) and seven abstracts. We have submitted one other paper [starred in Biosketch] and four additional manuscripts are in preparation.

**Jonathan P. Zehr.** OCE 91-01399 "A Novel Molecular Approach to Nitrogen-Fixation in Marine Ecosystems," 8/91-7/94, \$298,898, D. Capone, co-PI; OCE 95-03593 "The Genetic Potential for Nitrogen Fixation in the Marine Environment," 7/95-6/99, \$405,000. Nitrogen (N) availability can limit the productivity of oceanic systems. Recent geochemical budgets suggest that N<sub>2</sub> fixation is quantitatively more important than previously believed. The objective of these projects was to detect and characterize nitrogenase genes from the marine environment. Marine N<sub>2</sub> fixation has only been detected in the cyanobacteria *Trichodesmium* and *Richelia*; however, the genetic machinery (nitrogenase gene, *nifH*) is more widespread. We used PCR to amplify *nifH* DNA from marine diazotrophs and from cyanobacterial cultures. A database of *nifH* gene sequences from diverse marine and freshwater N<sub>2</sub> fixing species was obtained then the gene sequences and deduce amino acid sequences were subjected to extensive phylogenetic analysis. This analysis provided a relatively robust phylogenetic tree from which to identify uncultivated N<sub>2</sub>-fixing organisms. Several interesting discoveries were made, including the presence of an alternative (non-molybdenum, non-vanadium-containing nitrogenase) *nifH*. Based on our work, N<sub>2</sub> fixing organisms can be detected and quantified in diverse marine environments, and the N<sub>2</sub> fixing organisms identified by phylogenetic analysis. Quantitative analysis of *nifH* genes indicated that other N<sub>2</sub>-fixing organisms could be as abundant (cell L<sup>-1</sup>) as *Trichodesmium*, which could double the potential for N<sub>2</sub>

fixation in subtropical waters. This has significant implications for the oceanic N budget. Novel nitrogen fixation genes, that are significantly different from previously sequenced nitrogenase genes, were discovered in the equatorial Atlantic and Pacific Oceans. A particularly important discovery was the presence of unicellular cyanobacterial diazotrophs in the Pacific Ocean. The finding of previously undescribed N<sub>2</sub>-fixing microorganisms in the open ocean, some of which are associated with zooplankton, is important as it provides clues to potential sources of "new" N in the sea. These grants have supported the publication of >15 presentations and have financially supported 12 undergraduate students. Publications are listed in the bibliography of the proposal.

## INTRODUCTION

### **Rationale – Why Mono Lake?**

There are a number of reasons why Mono Lake is an ideal site for a Microbial Observatory. It is a tractable, well defined ecosystem for which a long-term ecological and limnological data base exist and there is a high probability that these time series will be continued well into the future. The lake is located close to a major field station (the Sierra Nevada Aquatic Research Laboratory, administered by the University of California, Santa Barbara). As an alkaline, hypersaline, meromictic lake, Mono Lake is an interesting system, both ecologically and microbiologically. It is an ecologically simple system, which facilitates modeling. It is currently undergoing a human-induced (and thus predictable) limnological transition that imparts a predictable temporal trajectory to physical, biogeochemical and ecological processes. This change mirrors past, natural events in Great Basin lakes resulting from climate oscillations (Stine 1994; Benson et al. 1996, 1998) and similar, if less extreme, changes in physical limnology might be expected in other lakes. Finally, as evidenced by NASA's investigations there and a review of the literature (omitted from this proposal), Mono Lake is a good analogue for conditions under which life might have evolved during the early history of Mars or Earth.

**Long-term study site.** Limnological research on the plankton dynamics of Mono Lake has been actively pursued since 1979. From 1979 to 1981, research focused on the sole macrozooplankton, *Artemia monica*. The research focus was expanded to include nutrient cycling and primary productivity in 1982 and routine monthly limnological surveys were initiated. Routine measurements collected on each survey include depth profiles of conductivity and temperature at 3 (1982 – 1993) to 8 stations (1993 – present); oxygen, light, and chlorophyll at 3 stations (1982 – 1993) or one central station (1993 – present); Secchi depth and a full suite of zooplankton measurements (stage abundance, sex, fecundity, reproductive status, etc.) at 10 (1982 – 1993) or 20 stations (1993 – present). Beginning in 1993, 9-m integrated samples of chlorophyll and dissolved inorganic nitrogen in the upper water column at five stations were also collected. This long-term (20-yr) suite of limnological measurements provides a detailed description of spatial and temporal plankton dynamics and vertical density stratification in Mono Lake and is unique among research conducted at hypersaline lakes. While current support from NSF (DEB 95-08733) and the City of Los Angeles Department of Water and Power (LADWP) only provides for the continuation of this sampling through the year 2000, further support from Los Angeles is expected and it is the intention of Drs. Melack and Jellison to continue this program well into the future.

**Preliminary microbial diversity studies.** DNA samples were collected from throughout the Mono Lake water column in July 1994, April 1995 and July 1995. This DNA was used for PCR/DGGE analysis of the microbial assemblage as described in Murray et al. (1996). We sampled DNA from 18 dominant bands and used it directly as template for cycle sequencing. The sequences we obtained were then compared to sequences in databases using the BLAST program (Altschul et al. 1990). In addition, Drs. Samantha Joye, Bess Ward and co-workers used a nested PCR procedure with DGGE and oligonucleotide probes to analyze the distribution of ammonia oxidizing bacteria (Ward et al. in prep.).

PCR/DGGE of Mono Lake samples collected during both July field trips revealed highly stratified assemblages whose vertical distributions were coherent with temperature, salinity, irradiance, sulfide and dissolved oxygen distributions. In April 1995, Mono Lake had just re-stratified a period of winter

holomixis (see Figure 1). Water column gradients were weak and oxygen had just been depleted at depth. The microbial assemblage was uniform throughout the water column, except at 20m where a nepheloid layer observed by an ROV was accompanied by a distinctly different microbial assemblage.

The number of bands (operational taxonomic units, OTU) per sample (lane) ranged from 8 to 20, which is low compared to other locations we have examined where the number of OTU per sample is typically 20 to 40. Analysis of the readable sequences of the fragments (134 to 161 bp except for one sequence of 79 bp) revealed affinities (best-match similarity 94%) to known organisms, but no exact matches were found with bands from samples. In the two controls, matches to the expected sequences were 99% and 100% for 161 and 135 bp sequences, respectively. A number of sequences were encountered more than once, both at multiple depths on a given sampling day and on different sampling days. A band with a partial 16S rDNA sequence (159 bp) having 91% similarity to a *Thiomicrospira* species was found in the region of the oxic/anoxic interface. We have not yet examined these samples for the presence of Archaea; however, halophilic Archaea might be expected to be important in this hypersaline environment (Duckworth et al. 1996, Grant et al. 1998) and would add to the diversity of the microbial community.

The sequence from a band that was ubiquitous in samples from the oxycline and monimolimnion (water column beneath the persistent chemocline) in July of both years was distributed throughout the water column in April. Based on the correlation of these distributions with direct counts, this sequence was most likely from an unusual phytoplankton found in the lake, tentatively identified as a *Nannochloris* species (Jellison and Melack 1988). This organism is identical to isolates (Dr. R. Lewin, UCSD) from a South San Francisco Bay saltern and a hypersaline, alkaline lake in Inner Mongolia. We have obtained a full (1786 bp) 18S rDNA sequence (GeneBank accession number AF125167) of the SSFB isolate and partial sequences of the Mono Lake and Inner Mongolia isolates (which align without any mismatches to the SSFB sequence). Phylogenetic analysis (Hepperle et al. submitted) indicates that this organism represents a new class of algae (proposed name: *Picocystis salinarum* of the class Picocystophyceae; Hepperle et al. submitted), the last common precursor of the Ulvophytes, Trebouxiophytes, and Chlorophytes. The organism has an unusual composition featuring arabinose as an important component of the cell wall and an unusual suite of pigments (Roesler et al. 1999; Hepperle et al. submitted). Physiological properties of the organism (described by Roesler et al. 1999) indicate that it is halo- and alkalitolerant and capable of growth at low light ( $4 \mu\text{E}/\text{m}^2/\text{sec}$ ), well suited for survival at the base of the pycnocline in lakes like Mono Lake.

### **Background Information on Mono Lake**

Mono Lake is located in eastern California approximately 100 miles south of Lake Tahoe. It is located in a region of active volcanism (Lajoie, 1968), which strongly influences the lake's character and setting. At the current surface elevation of 1946 m, the lake has a surface area of  $186 \text{ km}^2$  with a maximum depth of 48m. The lake is currently meromictic, a condition of persistent chemical stratification. The current salinity of the mixolimnion and monimolimnion are 73 g/kg and 86 g/kg, respectively (Jellison unpublished). The lake's pH is 9.7 with carbonate (400 mM), sulfate (130 mM) and sodium (1400 mM) as the dominant ions Oremland and Miller (1993). Sulfide concentrations can exceed 1.5 mM in the monimolimnion. With both biogenic and thermogenic sources, methane concentrations in the lake are also high ( $55 \mu\text{M}$  in the hypolimnion) and there are extensive areas of methane seeps in the lake bed. Earlier studies (Domagalski et al. 1989) and our own unpublished observations have shown that Mono Lake supports elevated DOC concentrations ( $>80 \text{ mg/L}$ ,  $>7 \text{ mM}$ ).

Chemical and geochemical measurements performed in Mono Lake include brine composition (Domagalski et al., 1989; Johanneson and Lyons, 1994), sources and abundance of gaseous hydrocarbons (Oremland et al., 1987), methanogenesis, methane oxidation, and sulfate reduction (Oremland and King, 1989; Oremland et al., 1993; Oremland and Miller, 1993), and bacterial oxidation of methyl bromide (Connell et al., 1997). Bacteria isolated from the lake's surface waters grow best at pH 9.7 and are able to use organic osmolytes as carbon sources (Diaz and Taylor, 1996). Mono Lake waters contain very high

concentrations of fallout-derived actinides (Anderson et al., 1982) and hydrothermally-derived arsenic (Maest et al., 1992).

The lake has a simple food web, with the brine shrimp *Artemia monica* as the sole macrozooplankter (Lenz 1984; Dana and Lenz, 1986; Lenz et al., 1986). Phytoplankton productivity is relatively high (269-1060 gC/m<sup>2</sup>/yr, Jellison and Melack, 1993a), consistent with reports from other soda lakes (Cloern et al. 1983a; Duckworth et al. 1996). Primary production decreases during prolonged meromixis due to lower vertical nutrient fluxes and depletion of nutrients stored in mixolimnion lake bottom sediments (Jellison and Melack 1993a). The phytoplankton community contains an unusual phytoplankter, recently named *Picocystis salinarum*, as a dominant. During summer, this organism is found in a dense layer or plate at the base of the photic zone and top of the pycnocline where it replaces the photosynthetic bacteria found at similar locations in other lakes (for example Big Soda Lake; Cloern et al. 1983 a, b). Its production is highest during spring when it is distributed throughout the water column and before *A. monica* populations have begun to increase (*A. monica* grazes on *Picocystis* but avoids the pycnocline) suggesting that plate formation is at least partially a response to grazing pressure. Bacterioplankton abundance is >10<sup>10</sup> cells/L. Bacterioplankton cells tend to be large (>1 um), with many chain-forming organisms. This is consistent with reports from other soda lakes (Zehr et al. 1987, Duckworth et al. 1996).

A 5-yr period of meromixis occurred during the mid-1980s as a consequence of record high runoff from the Sierra. The effect of meromixis and its breakdown on lake physics and its influence on nitrogen fluxes, organic matter deposition and biogeochemistry were described in a series of papers (Jellison and Melack 1993b; Jellison et al. 1993; Miller et al. 1993; Jellison et al. 1996). State-mandated curtailment of diversions and above-normal runoff in 1995 initiated the current episode of meromixis, which is predicted to last several decades (Jellison et al. 1998). The current meromixis has already had a profound effect on phytoplankton standing crops and ammonia distributions (Figure 1a, b). Another significant response of the lake's biogeochemistry is the disappearance of ammonium oxidizing bacteria and ammonium oxidation from the water column (Hovanec 1998, Ward et al. in prep.), possibly in response to elevated sulfide or polysulfide concentrations (Joye and Hollibaugh 1995), or decreased ammonia fluxes (Jellison et al. 1993).

Nitrogen fixation (diazotrophy) has not been thoroughly investigated in Mono Lake. Oremland (1990) measured nitrogen fixation in free-floating microbial tufts found at the edge of the lake during the last episode of meromixis (reported rates are too high due to a calculation error); however, he did not detect water column nitrogen fixation. A recent report documents the role of benthic nitrogen fixation in supporting production of algal mats grazed by brine fly larvae (Herbst et al. 1998). Since the Mono Lake epilimnion is often nitrogen-limited, and will become increasingly so as meromixis progresses (Figure 1a, b and Jellison et al. 1998), nitrogen-fixing microorganisms could play an important role in ecosystem nitrogen dynamics.

The activities of diazotrophs may vary as a function of distributions of ammonia, oxygen, light and temperature and other physical and chemical variables. Diazotrophs are taxonomically diverse so that taxonomic or phylogenetic identity cannot be used to predict diazotrophy. Nitrogen fixation is a function of the presence of diazotrophs, expression of nitrogenase (the enzyme responsible for nitrogen fixation) and conditions conducive to nitrogen fixation (generally, low ammonium and low oxygen). Given the large gradient of ammonium in Mono Lake (Figure 1d), it may be anticipated that nitrogenase would only be expressed in surface waters. On the other hand, oxygen concentrations are high in surface waters. The vertical profiles of oxygen and nitrogen would thus be expected to have complex effects on the distribution and activity of nitrogen-fixing microorganisms. Because these gradients are strong and well defined in Mono Lake, it is a particularly interesting site for studying the factors controlling the growth and activity of nitrogen-fixing microorganisms.

The physical limnology of Mono Lake has been the focus of recent studies (Romero et al. 1996, 1998; Jellison et al. 1998; MacIntyre et al. in press, see Jellison Prior Support). December profiles indicate increasing stratification (sharper pycnocline, Figure 1c) as the water level in the lake rises in response to high snowmelt runoff and restricted diversions. The lake level has risen 3 m since diversions were reduced in 1995 (current lake level is 1946 m). Stratification is expected to continue to increase as the

lake level rises to the target lake level of 1948 m. One consequence of the increased stratification is that vertical fluxes of ammonium and sulfide are reduced and thus their concentrations will continue to increase beneath the chemocline

As the lake level rises, the chemocline becomes deeper. The top of the chemocline is now at 15 m where at the onset of meromixis in 1995 it was at 11-12 m depth. Thus, the light climate at the top of the chemocline will decline over time and the thickness of the region between the seasonal thermocline (& chemocline) and the permanent chemocline will increase. The seasonal oxycline most closely follows the seasonal thermocline and is thus now above the permanent chemocline. Another consequence of the rising lake level is that less of the lake is meromictic each year (i.e. more of the lake bottom is exposed to oxygenated water and mixing). Currently half of the bottom and a third of the lake volume is beneath the permanent chemocline.

Finally, MacIntyre et al. (in press) report that mixing across the chemocline is driven largely by atmospheric forcing which generates internal waves on the pycnocline. These waves break where the pycnocline intersects the bottom (within 3.5 m of the bottom). This increases vertical eddy diffusion by 2-3 orders of magnitude and creates an annular zone of vertical mixing where the pycnocline broadens. Heat and nutrients then diffuse horizontally from the edges to the center of the lake where diffusion across the pycnocline approaches molecular diffusion.

## HYPOTHESES

Relatively little is known about the types of microorganisms dwelling in Mono Lake, their phylogenetic diversity, taxonomy, ecology or ecophysiology. For example, recent phylogenetic analysis of a dominant phytoplankter reveals it to represent a new *Class* of algae with some unusual biochemical properties. **One goal of this Microbial Observatory is to assess the phylogenetic diversity of Mono Lake microorganisms with the objective of cataloguing new or unusual organisms.** We have not stated “we will find new organisms” as a formal hypothesis because it is essentially a truism (we’ve already found new organisms) and it will be addressed automatically by a study plan, like the one we are proposing, that relies heavily field sampling and molecular techniques. We hope to go beyond simply finding new organisms; however, to define the factors contributing to their distribution and to learn how they fit into the functioning ecosystem of Mono Lake. This leads us to formulate a second goal. **The second goal is to define the niches of Mono Lake microorganisms in time and space and to understand their responses to changes in the lake’s hydrodynamics.** This goal can be expressed in 3 objectives from which follow a series of hypotheses to be tested during this project:

- 1) Understand the response of the microbial community to short-term and small-scale variation in mixing (for example spatial variation in vertical diffusion as a result boundary mixing or localized gravitational circulation; temporal variation in response to wind mixing events). Hypotheses to be tested:
  - 1a. Enhanced vertical mixing where the pycnocline intersects the lake bottom will result in enhanced microbial production, elevated standing crops and broader vertical distributions of microorganisms at these locations relative to the center of the lake.
  - 1b. Representatives of a given guild (sulfide oxidizers, nitrogen fixers, methanotrophs) will be phylogenetically different in regions of boundary mixing than away from them. We expect this because vertical fluxes are lower and more constant and are controlled by other processes in the center of the lake.
  - 1c. Anisotropic, isopycnal mixing will result in a gradient of productivity and community composition between the center of the lake and the lake edge.
  - 1d. Organisms capable of nitrogen fixation are not uniformly distributed through the vertical profile of the lake: the distribution of nitrogen fixing microorganisms does not necessarily reflect the distribution of active nitrogen fixation.

- 1e. As a corollary of 1d, expression of nitrogenase genes and nitrogen fixation reflects vertical distributions of nitrogen-fixing microorganisms AND distributions of the controlling environmental factors; e.g., ammonium, oxygen and light.
- 2) Understand their response to the long-term (multi-year) trajectories of average properties of the lake (for example surface salinity; monimolimnion H<sub>2</sub>S, NH<sub>4</sub>; pycnocline depth and sharpness; primary production; nutrient limitation in the mixolimnion, etc.). Hypotheses to be tested:
- 2a. Long term changes in system primary productivity affects the delivery of labile OM through the chemocline to bottom water microbes and thus modulates bacterial production and the buildup of reduced metabolites in the monimolimnion.
  - 2b. Composition of the monimolimnion microbial community will change through time in response to increasing toxicity of NH<sub>3</sub> and H<sub>2</sub>S accumulating in bottom waters. Mixolimnion community composition will also change due to decreased primary production, changes in the phytoplankton assemblage and reduced diffusion of metabolites and organic from the monimolimnion.
  - 2c. Evolving separation of the halocline (permanent chemocline) and the oxycline will result in the formation of new niches which will be reflected in microbial diversity and the vertical distribution of species.
  - 2d. Mixolimnion nitrogen fixation will become increasingly important as meromixis persists.
  - 2e. Toxicity of polysulfides (which last longer in oxygenated water at high pH, thus may be abundant in alkaline lakes), aminated fermentation products of osmolytes (for example methylamine) or other reduced metabolites diffusing into the mixolimnion from the monimolimnion are responsible for eliminating nitrifying bacteria from surface waters.
  - 2f. As a corollary, the relative magnitude of the flux to the mixolimnion of reduced metabolites, including sulfide and polysulfides, should decrease as the lake as a whole becomes more “oxic” in response to filling. If the disappearance of nitrifiers was in fact due to fluxes of toxic metabolites from the monimolimnion, nitrifiers should return to the lake at some point.
- 3) Elucidate the characteristics of ecological interactions among microbes and between microbes and other organisms in the lake. Hypotheses to be tested:
- 3a. Light limitation at the chemocline due to increased depth of the overlying water column will eventually prevent formation of the typical summer plate of *Picocystis*, eliminating bacteria that are closely coupled to *Picocystis* production.
  - 3b. The unusual biochemical composition of *Picocystis* will affect microbial processing of this carbon.

While we are interested in other aspects of microbial ecology, for example studies aimed at elucidating the microbial loop trophic structure of Mono Lake, the level of support available through the Microbial Observatories program constrains what we can accomplish. We are confident that the work plan set out below is achievable, if ambitious, as most of the methods proposed are currently in use in one or another of the PIs laboratories: while they may be tedious in some cases, they are routine. But, we have intentionally limited the scope of project we are proposing to make certain that we can fully address the questions raised above. The relationship between the hypotheses and the field program will be identified in the descriptions of the proposed studies below.

## RESEARCH PLAN

### *Field Techniques*

**Sampling locations and schedule.** The field program will consist of two elements: regular sample collection in conjunction with ongoing LTREB and LADWP studies; and group field trips focused on processes or other special topics. Regular sampling will be conducted on approximately monthly intervals by Jellison and co-workers at SNARL. Water samples will be collected primarily from a central

deep station in the lake southwest of Paoha Island that has been sampled repeatedly during previous studies (Station 30 in the LTREB database). Sampling depths will be varied to obtain maximal vertical resolution of the clines that develop in the lake. Ten to twenty depths will be sampled depending on the complexity of the lake's physical structure (obtained from CTD cast). The following table summarizes the samples to be collected or measurements to be made routinely and gives references to the methods we will use.

Table 1. Routine measurements

Water level (LADWP gauge, weekly)  
 Meteorology (met station, recorded at 10 minute intervals)  
 Runoff (LADWP gauge, weekly cumulative)  
 Conductivity, temperature, transmissometry, fluorometry, PAR, O<sub>2</sub> (coarse) profiles (Seabird Seacat CTD, YSI O<sub>2</sub> meter)  
 Nutrient profiles (Miller et al. 1993, Joye et al. 1999).  
 H<sub>2</sub>S, methane profiles (Miller et al. 1993, Joye et al. 1999).  
 Chlorophyll profiles (Jellison and Melack 1993a)  
 Primary Production (Jellison and Melack 1993a)  
 Primary Production (<sup>14</sup>C)  
 Phytoplankton community composition (inverted microscope)  
 Nitrogen fixation (<sup>15</sup>N, see below)  
 Particulate organic C and N (Carlo-Erba CHN analyzer)  
 Bacterial biomass (epifluorescence, Porter & Feig 1980)  
 Bacteria pdn (<sup>3</sup>H L—leu, Kirchner et al 1995, Simon & Azam 1989)  
 Bacterial and Archae community composition (DGGE - Murray et al. 1996, Murray 1998) or TRFLP - Liu et al 1997)  
 DOC/DON other organic compounds profiles (Shimadzu TOC 5000 coupled to an Antek N analyzer, primary amines, total carbohydrates)  
 Zooplankton biomass, age structure, etc. (Lenz 1984; Dana and Lenz 1986; Lenz et al. 1986)

All of these measurements will be made monthly throughout the duration of the project except for nitrogen fixation, <sup>14</sup>C primary production, bacterioplankton production and sulfate reduction. These variables will be measured every summer during one of the group field efforts (profiles at multiple sites and replicate profiles at the routine sampling station) and monthly for one year (the same year that quarterly measurements of other properties are being made, Year 2, see below) by a UGA student in residence at SNARL.

This suite of measurements will allow us to address most of the hypotheses concerning the response of the microbial community to continued meromixis (**Hypotheses 2a-d, 2f and 3a**). Mono Lake mixed last during in the winter of 1994/1995. As described above, we have a set of microbial community DNA samples that record changes taking place during this mixing event and establish community composition during the early phases of restratification for the current meromictic episode. Sampling over the period for which we are requesting funding and comparison with these archive samples will allow us to document the response of the microbial community over an 11-year time span.

We have budgeted for one group field trip per year of 2 weeks duration in which all of the personnel involved in the project would participate. In practice, some of the focused experiments will require sampling more frequently than this, so this schedule will be modified as necessary by staying longer, going more frequently but staying for a shorter period of time, or rotating the focused field effort between PIs. The schedule of these events is given in the table below. These field efforts will be during the summer unless otherwise noted. While we would like to do all of them all of the time, the level of effort that can be supported by this program simply will not allow it. We have intentionally reduced the field

commitment during Year 5 to provide time for follow-up or repeat studies and to give the PIs time to focus on publication of results. The studies and their relation to the hypotheses are described below.

Table 2. Focused Activities

	Project Year				
	1	2	3	4	5
A. Calibrate/optimize methods for bacterial production, etc.	X				
B. Survey Lake – “biogeography” (summer and winter)	X				X
C. Seasonal Cycle (quarterly sampling)		X			
D. Monthly: bacterial production, primary production calibration		X			
E. Algal decomposition (quarterly)		X			
F. Role of boundary mixing/microstructure			X		
G. Organism specific incorporation of substrates (autoradiography and <i>in situ</i> PCR or probes)			X		
H. Special chemical analyses (VFA, polysulfide, methylamine, HPLC AA, etc)			X		
I. Ammonium/methane/sulfide oxidation		X	X		
J. Annual routine measurements and samples (N fixation, bacterial pdn, sulfate reduction, isolations, etc.)	X	X	X	X	X

*A. Calibrate/optimize methods for bacterial production, etc.* This is relatively self explanatory. The unusual composition of Mono Lake water means that standard methods often have to be modified (for example acidification of bacterial productivity samples to precipitate macromolecules). This would be done in Year 1 to prepare for the rest of the study and during year 5 to assess changes as a result of prolonged meromixis and changes in the lake’s physical structure.

*B. Survey Lake.* The purpose of this exercise is to evaluate spatial variation in bacterial community composition so that data from the routine monitoring station can be placed in the context of processes in the whole lake. Since this will also allow us to evaluate the representativeness of the chosen monitoring site, we will do it during the first field trips. We are proposing 2 samplings, one at maximum and one at minimum stratification, to capture the likely endmembers of the distributions. This activity addresses **Hypotheses 1b and 1d.**

*C. Seasonal cycle.* This will involve quarterly trips of the PIs to Mono Lake to conduct measurements (for example N fixation, sulfide or methane oxidation) that are not done on the routine monthly sampling trips. These measurements will let us put the long term change (if any) in variables measured primarily at one time of the year into the context of seasonal variation. The activity is scheduled for Year 2 to gain an understanding of the seasonal variation in activity and distributions early in the study so that we can modify the sampling plan if necessary. This activity also addresses **Hypotheses 1b and 1d and Hypothesis 2b and 2d.**

*D. Monthly measurements.* This is listed as distinct from routine monitoring (and C) as it will involve measurements (bacterial production, <sup>14</sup>C fixation, etc. that are not routinely measured by the monitoring program. A UGA student-in-residence will conduct these measurements which will let us put the long term change (if any) in variables measured primarily at one time of the year over into the context of seasonal variation. This activity is scheduled for Year 2 to coincide with activities D and E. and also addresses **Hypotheses 1b, 1d and 2b.**

*E. Algal decomposition.* *Picocystis salinarum*, which we have in culture will be labeled with <sup>13</sup>C and used in experiments to follow the metabolism of its biomass by microbial populations representing different regions of the water column (mixolimnion, oxycline, chemocline, monimolimnion, etc.). As an initial step, we will thoroughly characterize the Mono Lake strain of the organism grown in Mono Lake water. Hepperle et al. (submitted) report substantial variation in the carbohydrate composition between strains and we have not yet characterized its osmolytes. Distribution of the <sup>13</sup>C label will be followed by GC-MS. Hollibaugh and Joye are currently funded by NSF for an analogous study of phytoplankton decomposition in sediments (see Current and Pending support: NSF OCE 98-07588). This experiment

directly addresses Hypothesis 3b and indirectly addresses **Hypothesis 2b** will be conducted quarterly during Year 2 in conjunction with activities C and D.

*F. Role of boundary mixing.* This study will directly address **Hypotheses 1a-e**. We will collect samples from the vicinity of the pycnocline along a transect from a region of enhanced vertical mixing near the shore to the center of the lake. Phylogenetic composition; rates of bacterial production; chemoautotrophic production; sulfide and methane oxidation; nitrogen fixation and expression of nitrogenase; and substrate (acetate, selected amino acids, glucose) concentrations and turnover rates will be compared. This will be a major effort for all PIs and has been scheduled for Year 3 to minimize competition with other project activities.

*G. Organism-specific activities.* These experiments will allow us to evaluate the distribution of microbial activity (protein synthesis, carbon fixation, acetate amino acid or glucose incorporation, expression of *nifH* or *pMMO* genes, etc., see below) among the cells in a sample and to associate them with specific groups of organisms. For example, we could compare the relative abundance of chemoautotrophs versus heterotrophs versus photoautotrophs in a sample from the pycnocline. This activity is scheduled for Year 3 to coincide with the boundary mixing studies because it will be very useful in assessing short term and small scale responses of the microbial community to mixing events. It addresses **Hypotheses 1a-e, 2b, 2d**.

*H. Special chemical analyses.* This study will expand the suite of chemical measurements made on routine sampling trips to include compounds not normally measured such as volatile fatty acids, polysulfide, methylamine, HPLC amino acids or GC/MS carbohydrates. It will be done in Years 2 and 3 in conjunction with the boundary mixing study and contributes to resolving **Hypotheses 1a, 1b, 2a-c**

*I. Ammonium/methane/sulfide/oxidation.* This is listed as a special activity because it will not be measured during routine monitoring and because special effort will be devoted to it as one of the suite of measurements to be made during the boundary mixing studies in Year 3 to address **Hypothesis 1a**. No ammonium oxidation was detected in 1997 (it was present in 1995), but we will keep assaying it to test **Hypothesis 2f**. When (and if) ammonium oxidizing bacteria are detected in Mono Lake, we will conduct inhibitor experiments to test **Hypothesis 2e**.

*J. Annual routine measurements and samples.* This is self explanatory. Once per year, every year, at the same time every year (currently scheduled for summer but see C above), we will measure a suite of variables (for example nitrogen fixation or bacterial production) at the routine sampling site that are not measured during routine monitoring. These measurements will be made with samples from throughout the water column and will be repeated at least 3 times per field trip to assess short term variation. In conjunction with measurements made during the seasonal cycle study (C above) this will allow us evaluate the variance and trends in properties at time scales ranging from days to years; providing the data to test **Hypotheses 2a-f and 3a**. We recognize that we are proposing a fairly ambitious program, but if we can find time or if additional support for this project becomes available, we will also assess diurnal and shorter time scale variation in some properties (for example bacterial production).

### **Analytical techniques**

**Rate Measurements and pool sizes:** Methane ( $^{14}\text{CH}_4$ ),  $\text{NH}_3$  (dark  $^{14}\text{CO}_2$  fixation) and  $\text{S}^{2-}$  ( $^{35}\text{S}^{2-}$ ) oxidation rates will be determined using radiotracers [ $\text{CH}_4$  and  $\text{NH}_3$  oxidation via Joye et al., 1999;  $\text{S}^{2-}$  oxidation via Jørgensen et al., 1991]. Details of these methods are published and will not be presented here. Rates will be determined at ~20 depths in the water column (sampling carried out as outlined above), with high (0.25 – 0.5 m) resolution sampling within the oxy- and chemo- clines. After injecting radiotracers, samples will be attached to a mooring and incubated at the appropriate depth in the lake for 12-36 hours (Joye et al. 1999). Uptake of selected amino acids (in addition to L-leucine), carbohydrates and acetate will also be measured using standard techniques.

Total chemoautotrophic production rates will be estimated by following the fixation of  $^{14}\text{CO}_2$  into organic biomass (Karl, 1980, 1987).  $^{14}\text{C}$ -bicarbonate will be added to water column samples and they will be incubated for 12-36 hours. These data can be used in conjunction with the oxidation rate data to assess chemosynthetic growth efficiencies. The total bacterial production rates (see below) can be compared to

chemoautotrophic production data to assess autotrophic vs. heterotrophic bacterial activity. Phytoplankton primary production rates will be determined using accepted  $^{14}\text{C}$  tracer techniques. Sulfate reduction rates will be determined using  $^{35}\text{S-SO}_4^{2-}$  tracer techniques (Fossing and Jørgensen, 1989).

Nitrogen fixation will be measured using the  $^{15}\text{N}_2$  method (Montoya et al. 1996). The  $^{15}\text{N}_2$  method provides a direct measurement of net  $\text{N}_2$ -fixation, typically with greater sensitivity than the acetylene reduction assay when applied to dilute solutions (Montoya et al. 1996), albeit at the cost of requiring a mass spectrometric analysis, which typically prevents rapid turnaround of the experimental samples. All experiments will be carried out as time-series incubations under simulated in situ conditions. Time series measurements will be made early in Year 1 to determine optimal incubation times, and incubation volumes for different depths. All experiments will be carried out at least in duplicate and in triplicate when incubator space and sample volumes are sufficient.

Isotopic analyses will be carried out by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using instrumentation that will be in place at RPI before the start of this project. J. Montoya (Georgia Tech.) and Dr. Jun Abrajano of RPI have coordinated purchase of mass spectrometer instrumentation, with the result that Georgia Tech and RPI have purchased identical instrument packages with the goal of facilitating collaborative work between the institutions. J. Montoya is a collaborator with J. P. Zehr (RPI, co-PI of this project) on another project and has promised his assistance in getting  $^{15}\text{N}_2$  fixation assay techniques up and running at RPI.

**Chemical analysis.** The availability of labile organic compounds influences rates of bacterial activity. Therefore, in addition to quantifying concentrations of bulk DOC and DON, we will also quantify pool sizes of specific components of both pools. Concentrations of volatile fatty acids will be determined using HPLC methods (Albert and Martens, 1997). Urea, primary amines (OPA fluorescence), amino acids (OPA/HPLC) and carbohydrates (MBTH, GC/MS at the Complex Carbohydrate Research Facility at UGA). Additionally, compounds that could inhibit or alter the activity of certain bacterial groups (e.g., S inhibition of nitrification) will also be examined. Concentrations of polysulfide ( $\text{S}^0$ ) will be determined using colorimetry (Kelly et al., 1969; Visscher et al. 1990) and/or differential pulse mode polarography (Overmann et al. 1996). Concentrations of methylamine and trimethylamine will be determined using gas chromatography. Concentrations of dimethyl sulfide and methane thiol will be determined using gas chromatography (Bremner and Banwart, 1974; Visscher and Taylor, 1994).

### *Molecular Techniques.*

**Phylogenetic analysis of bacteria.** We will examine the phylogenetic composition of microbial assemblages as follows. DNA will be extracted from the organisms collected on Sterivex cartridge filters and purified as described previously (Murray et al. 1996). The purified DNA will be split into 3 aliquots. Two of the aliquots will be stored in TE buffer at  $-80^\circ\text{C}$  for future reference or sharing with other investigators and the third will be used for the work described below

We will use PCR/DGGE (Murray et al. 1996, Ferrari and Hollibaugh in press) or terminal restriction length polymorphism (T-RFLP, Liu et al 1997) as a survey tool to analyze the spatial and temporal variation of the microbial community in Mono Lake. The primer set most commonly used with PCR/DGGE (Muyyzer et al. 1993, Murray et al. 1996) is specific for Bacteria; however, Murray (1998) has reported a set of primers that can be used with Archaea. Moeseneder et al. (submitted) have compared PCR/DGGE and T-RFLP in marine samples and found that the dendrograms of sample similarity were the same for both techniques. Significant bands in these samples will be excised and sequenced ((Hollibaugh Prior Support, T-RFLPs will be run on sequencing gels, we have an Hitachi FM-BIO laser fluorescence imager with adequate sensitivity for this task).

More complete sequence information will be obtained from selected samples by amplifying, cloning then sequencing cloned fragments generally as described by Fuhrman et al. (1993). Samples will be amplified with the universal primer set (520F-1392R) used by Fuhrman et al. (1993) or domain-specific primers for Bacteria (8F-1406R, Liu et al. 1997) or Archaea (1F-1100R, Munson et al. 1997). The significant advantage of using universal primers, as pointed out by Fuhrman et al. (1993), is that the

domain-specific primer sets (designed from sequences in the databases) could exclude unusual organisms that might be found in this unusual and phylogenetically poorly characterized environment. The clone library obtained will be screened using PCR/DGGE or RFLP, then the 16S rDNA fragment from clones that appear different will be excised, purified and sequenced in both directions. Sequences will be edited and checked for chimera formation. Sequence similarity will then be checked initially against sequence databases using BLAST. Novel sequences will be subjected to more thorough phylogenetic analysis and submitted to GeneBank

Harvesting large quantities of DNA for direct shot-gun cloning is impractical because the lake water is extremely difficult to filter. We are aware of the potential artifacts associated with mixed template PCR amplifications (Reysenbach et al. 1992; Farrelly et al. 1995; Suzuki and Giovannoni 1996; Wang and Wang 1997) and we will keep them in mind when interpreting our data.

**Diazotrophs.** Sample processing in the field (filtration of 2 liters of lake water through 0.2 um low nucleotide-binding filters) will be as described by Zehr et al. (1998) and DNA extraction procedures will follow the protocol described by Braun et al. (1999). Diazotroph distribution and diversity will be assessed by PCR amplification of *nif* genes. PCR will be performed as described in Zehr et al. (1998) and Zehr and McReynolds (1989) except we will use a new set of nested PCR primers soon to be reported in Zani et al. (in prep.). The amplified 359bp *nifH* fragment is purified (by agarose gel electrophoresis), cloned (pGEM T-vector, Promega) screened by RFLP to identify unique phylotypes which will then be sequenced. The nested PCR primer approach greatly enhances sensitivity and reduces effects of sample inhibition. Amplification products will be quantified using competitive PCR with a competitive template containing the primer sequences, but which results in a smaller product that can be distinguished from the target sequence.

Nitrogenase gene expression will be assessed by reverse transcriptase PCR of mRNA using AMV reverse transcriptase (Promega). After the reverse transcriptase amplification step, 1µl of the RT sample is used in a nested PCR (see above). Controls include RNAsed and DNAsed preparations, and by amplifying RNA preps without the RT step.

**Isolates.** We will also obtain sequence information from isolates collected each year during the major summer field trip. Bacteria will be isolated by streaking on plates of the medium described by Diaz and Taylor (1996). A glove bag and appropriate gases and apparatus will be set up at SNARL for anaerobic isolations. Cultures will be screened (16S rRNA gene) by PCR/DGGE or T-RFLP. Unique 16S genes will be sequenced. The DGGE and T-RFLP signatures obtained from cultures will be compared to banding patterns obtained from the mixed template amplifications (above) to obtain information on the distribution of the organism and its isolability.. Unique isolates will be characterized (BIOLOG), maintained for the duration of the project and deposited with ATCC. Unfortunately the funding level of this program will not support a thorough analysis of the physiological characteristics of all of these isolates; however, we will perform further characterization of organisms that stand out as being either phylogenetically or physiologically unique make and them available to others (advertised through meetings such as ASM and the new ASM members web site). We will pay particular attention to properties with biotechnological applications like osmolytes and enzymes. Grant et al. (1998) estimate that less than 20% of the patents involving extremophilic microorganisms are for halophiles, suggesting that opportunities remain for exploiting this group of organisms.

We will also examine the distribution and composition of the following guilds of bacteria: sulfide oxidizing (16S rDNA, Muyzer et al. 1995); ammonium oxidizing (16S rDNA, Ward et al. 1997), sulfate reducing, ([NiFe] hydrogenase, Wawer and Muyzer 1995, Wawer et al. 1997); methanotrophs (particulate methane monooxygenase, Cheng et al. in press) and nitrogen fixing organisms (*nifH*, Zehr and Capone 1996) by PCR/DGGE or T-RFLP. We expect changes in these assemblages with depth and time in response to the seasonal and interannual changes in mixing, stratification, organic matter fluxes and sulfide or ammonium accumulation. We also expect changes in distribution and activity in response to localized mixing

**Cell-specific analyses.** In order to further define the niches of individual strains or species of bacteria in these (and other) guilds, we will explore the use of autoradiography (Fuhrman and Ouverny

1999, Ouverny and Fuhrman 1999) or vital stains (Frischer et al. 1999) in conjunction with group-specific probes or *in situ* PCR (Hodson et al. 1995, Chen et al. 1997, Chen et al. in press) to examine changes in activity. For example, incorporation of  $^{14}\text{CO}_2$  by individual autotroph cells could be used to assess the relative abundance of autotroph populations, changes in activity with depth or mixing conditions, etc. This may be difficult with Mono Lake water because of its high DIC concentrations. Similarly,  $^3\text{H}$ -labeled substrates can be used to identify the cells responsible for uptake and incorporation of specific classes of molecules by heterotrophs. *In situ* rt-PCR of mRNA can be used to identify cells expressing genes (for example pMMO), providing another means of evaluating activity on an individual cell basis. Hodson and co-workers are in our department and have offered to assist us with *in situ* PCR.

**Other approaches.** The field of molecular microbial ecology is changing rapidly. For example, we have learned of an approach under development in Dr. G. Herndl's laboratory in the Netherlands (G. Herndl, pers. comm.) that uses capillary electrophoresis to separate live bacteria based on cell-surface properties with discrimination (at least with cultures) at the species level. If this approach can be applied to natural samples, it offers many opportunities for experimentation that are directly relevant to the work we are proposing here. While it is premature to claim that we will use this approach in our study, we will follow its development (and that of other emerging molecular techniques) and apply them to the problems outlined in this proposal, when and if they are appropriate.

## MANAGEMENT PLAN

**Project responsibilities.** Jellison will be responsible for monthly routine sampling, though a UGA graduate student will assist him when bacterial production and other rate measurements are made over a seasonal cycle (Year 2). PIs will share responsibility for logistics, gear, sampling and measurements made on the major field trips. Many other project responsibilities will be shared between the PIs, but primary responsibilities for specific portions of the project will be assigned as follows. Dr. J.T. Hollibaugh's group will have primary responsibility for phylogenetic analysis of natural bacterial assemblages and isolates, bacterial counts and production estimates, and running the Picocystis decomposition experiments. Joye will be responsible for methane oxidation, ammonium oxidation, sulfate reduction and organic substrate rate measurements and for most analytical chemistry work. Zehr will measure  $\text{N}_2$  fixation rates and analyze diazotroph populations. Jellison will be responsible for compiling field data and establishing and maintaining database access via the internet.

**Permits:** The necessary permits for sampling in Mono Lake have been obtained by Jellison as part of ongoing studies.

**Data storage and dissemination.** Routine field data (CTD, fluorescence,  $\text{O}_2$ , PAR, etc.) will be made available to all PIs and to other interested investigators as soon as it has been processed (usually same day). Sequence data will be submitted to Genbank as soon as it has been checked and verified. DNA samples or isolates will be made available to interested investigators once the PIs have had an opportunity to analyze them and publish their findings, but no later than 1 year after the end of the project. Depending on the type of data, other data will either be stored as computer files or in laboratory notebooks. Data will be available as a relational data base with appropriate metadata. The database will be made accessible to the public through a web site maintained on the on the SNARL server. We envision this web page and the link structure to be similar to the one we created for the Tomales Bay LMER (see web address given in Results of Prior Support – Hollibaugh).

**Public Outreach.** The Mono Lake Committee (MLC) is a non-profit citizen's group dedicated to protecting and restoring the Mono Basin Ecosystem; educating the public about Mono Lake and the impacts on the environment of excessive water use; and promoting cooperative solutions that protect Mono Lake and meet real water needs without transferring environmental problems to other areas. The committee maintains an award-winning web-site containing a wealth of information on the Mono Basin ecosystem including scientific research status and reports (<http://www.monolake.org/index.html>). Their web-site averages 11,000 user-sessions per month. The MLC has agreed to maintain links to our own web pages at SNARL and provide pages describing our research and result at their web site.

Mono Lake lies within the Mono Basin National Scenic Area and the US Forest Service maintains a large visitor center with a large array of interpretive exhibits. Annual visitation rates are 120-150,000. We will provide research summaries to the Visitor Center and pursue creating a small exhibit of research results. In addition, SNARL runs a summer lecture series open to the public at which we will present talks.

**Under-represented groups.** All of the institutions collaborating on this proposal have strong programs to encourage the participation of underrepresented groups in science and engineering. For example, the faculty at the Department of Marine Sciences at UGA is 50% female and Marine Sciences faculty members have collaborative research projects with students and faculty from historically black colleges like Armstrong State University and Savannah State College. Last summer we (JTH) participate in the Howard Hughes Medical Institute program for Women in Biomedicine by hosting 2 high school seniors as interns. We will ensure that the positions to be filled if this proposal is funded are advertised openly and we will especially encourage members of under-represented groups to apply.

## **SIGNIFICANCE OF PROPOSED RESEARCH AND RELATIONSHIP TO OTHER PROJECTS.**

The proposed studies will address a number of aspects of the microbiology, microbial ecology and biogeochemistry of a habitat that is not only a good analog for postulated conditions on ancient Mars, but also for early conditions on the Earth. As such, our findings will help us to understand the forces that shaped the basic structure of life on the Earth and other planets. Coupled with findings from other studies, they will also help us interpret the fragmentary evidence that remains from the early periods of the Earth's history and the even rarer samples that might hold clues to life on Mars and other planets.

Furthermore, relatively little is known about the microbial ecology of soda lakes. They are anomalous environments in that they have high concentrations of DOC and high standing crops of bacteria. This suggests that the microbial loop processes that have been studied more thoroughly in marine and freshwater environments are somehow decoupled in soda lakes. Understanding the reasons for this will help improve our understanding of the consequences of the microbial loop to the ecology and biogeochemistry of "normal" environments and provide insight into the processes that contribute to the storage and deposition of organic carbon (e.g. Domagalski et al. 1989, Meyers 1994). As environments of extreme physiochemical conditions, organisms living in soda lakes may possess novel adaptations to this environment, for example, transport systems, osmoregulatory compounds or ectoenzymes. Some of these adaptations may involve enzymes or other aspects of physiological properties with potential commercial significance.

The proposed studies are related to ongoing studies of the physical limnology, ecology and nutrient cycling in Mono Lake by Drs Robert Jellison, John Melack, Sally MacIntyre and collaborators at UC Santa Barbara and elsewhere. Jellison expects to receive continuation funding for the LADWP monitoring program on Mono Lake that supports the monthly field trips. MacIntyre has requested continuation funding (NSF) for her work on the physical limnology of Mono Lake. We are also in communication with Dr. Oremland's group at the US Geological Survey in Menlo Park who are interested in the cycling of Se and As oxyanions in Mono Lake. We will collaborate with them on field logistics and share data with them. Dr. K. Nealson (JPL/CalTech) is studying bacteria from Mono Lake, presumably (we have been unable to contact him directly) to gain insights into life on other planets and to evaluate the possibility of preserving microbes in authigenic carbonates (tufas).