Nevada Space Grant: Geobiology Summer Short Course

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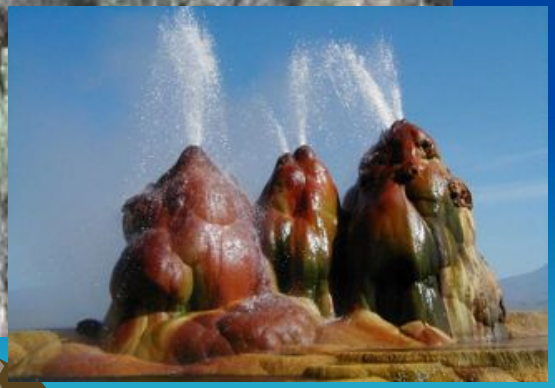
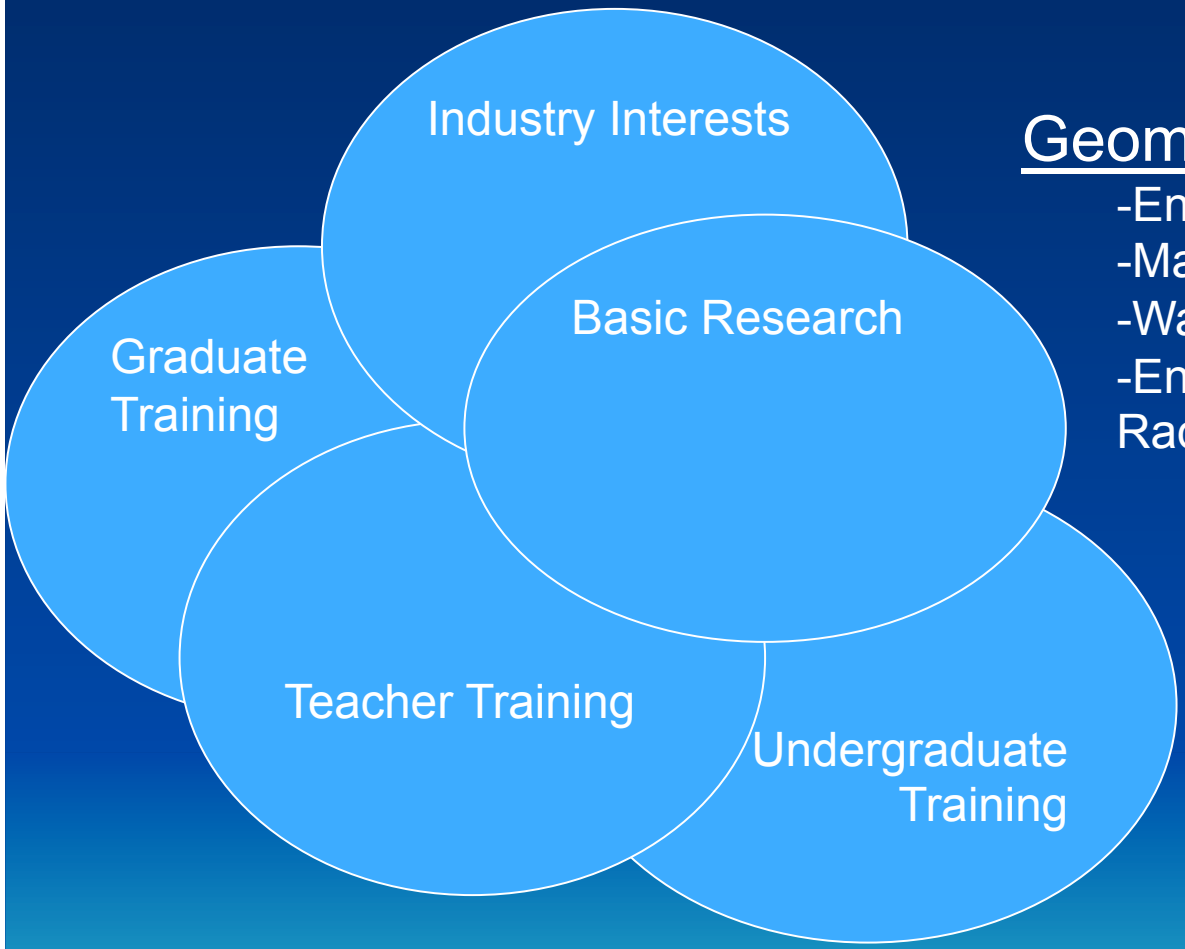
The Genesis:

- BallonSat, RockOn
- TEA- Teachers Experiencing Antarctica
- Spaceward Bound
- Antarctica- Biology Class
- Nevada Earth and Space Science Initiative (NESSI)

Nevada





Geomicrobiological Principles:

- -Energy Sources
- -Materials- life building blocks
- -Water Activity
- -Environmental Constraints- e.g. pH, Radiation,

Overall Aims: (Win:Win:Win?)

- Develop Research (exploratory or otherwise) Beneficial to PI-based Research
- Develop Graduate Research- Train graduate students on analytical skills in environmental and microbial ecology
- Develop Participant's Research Skills- experimental approaches as well as inferential approaches
- Embed Elementary Teachers into Field Research
  - so that they could experience the first-hand collection of data through actually doing real science (increase depth-of knowledge).
  - see if having a teacher on a research project would help scientists develop skills enabling more effective instruction and delivery of content
  - see if this partnership would have any effect on classroom science instruction by the teachers

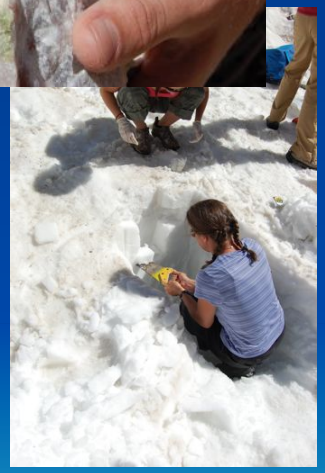
“5 E” Learning Cycle of teaching (Educator/Teacher Infused)

- Engagement – Hook – Introduces concept and activates prior knowledge
- Exploration – hands-on activity where experience allows construction of knowledge
- Explanation – Through careful questioning, content is conveyed, *Vocabulary reinforced*.
- Elaboration – A second hands-on learning experience where the knowledge is used to extend new knowledge or practice new knowledge
- Evaluation – both Formative and Summative

Week 1:

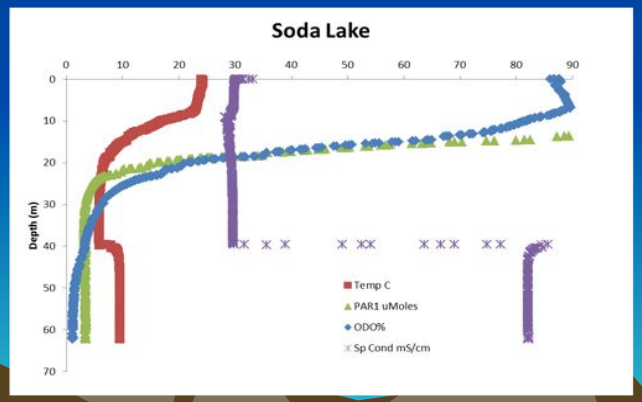
- Orientation (NASA Branding):
- Field Trip to Eastern Nevada
  - Leehman Caves,
  - Snow Fields,
  - Rock outcrops
  - Hot Springs
  - Industry Engagement

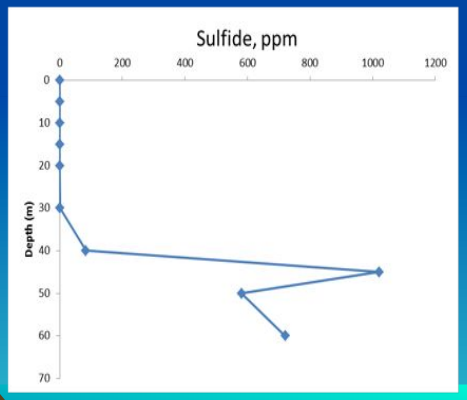
- Engaged, Explored, Explained Concepts Central to Geomicrobiology and Astrobiology and the search for life and signs of life.



Soda Lake: Terminal- Amictic Lake

- Freshwater over top of Saline Water
- Does not Mix
- Bottom Waters Anoxic
- Analogs for terminal lakes elsewhere



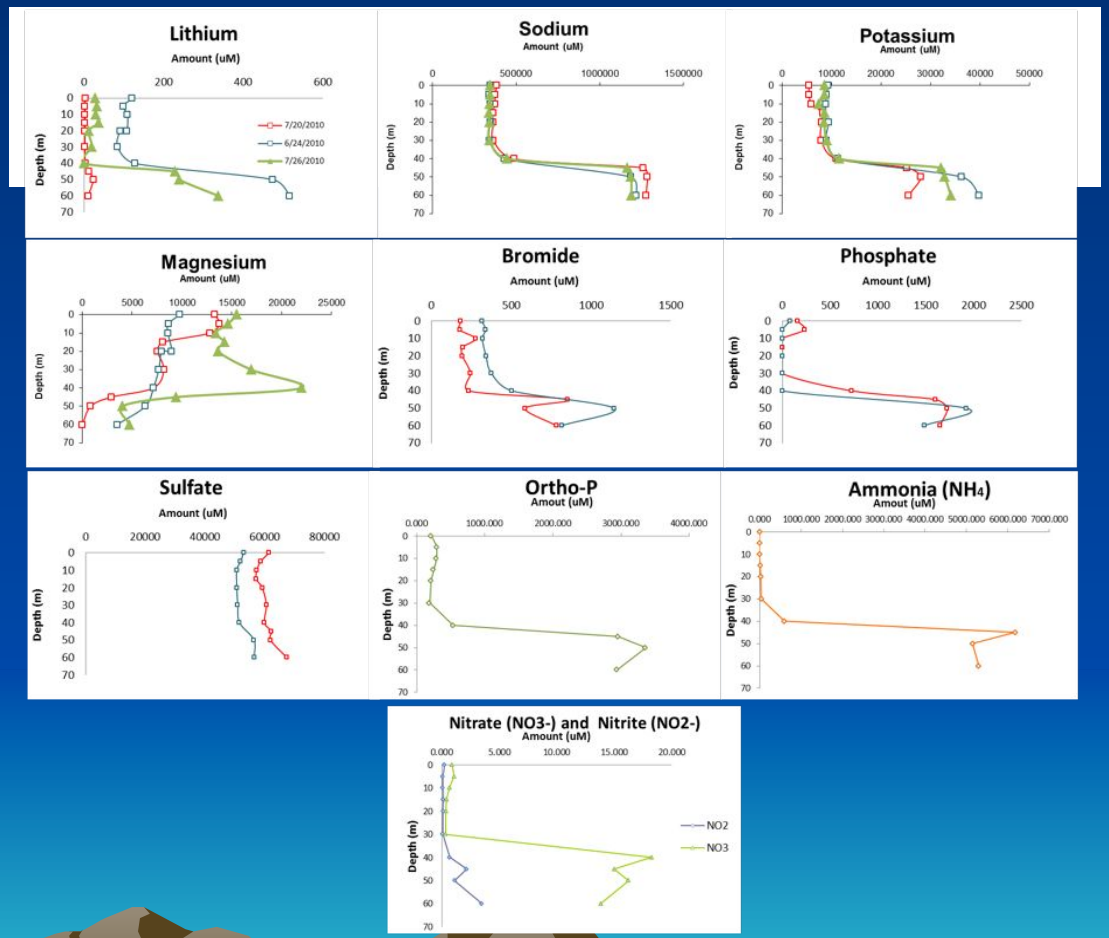


Soda Lake: Terminal- Amictic Lake

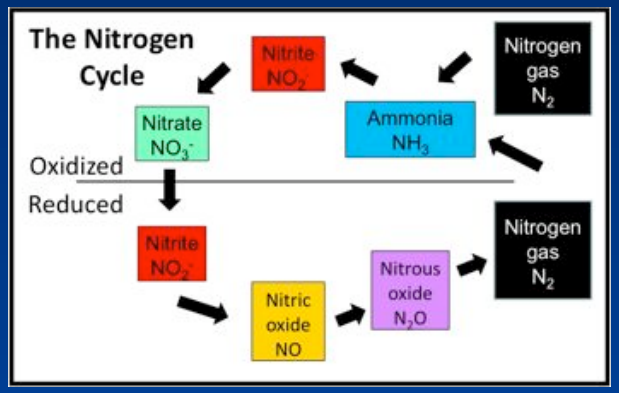
Analytical Skills

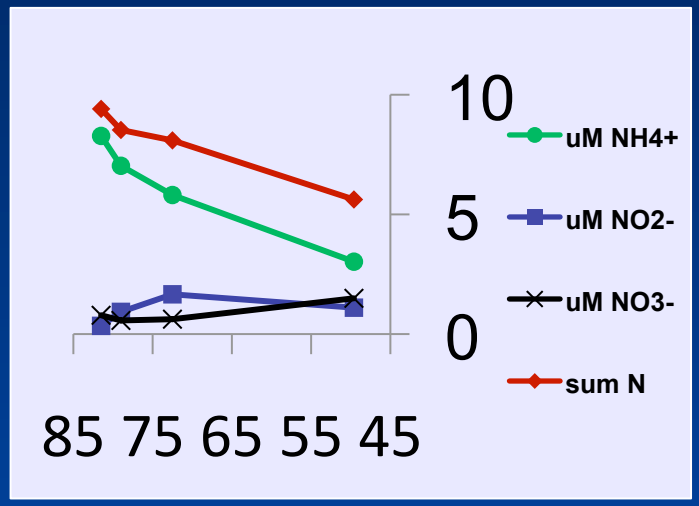
- Spectrophotometry
- Fluorometry
- Microscopy
- Chromotography
  - Ion and gas

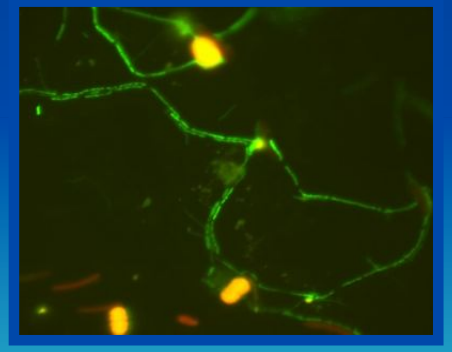
- Concepts
  - Buoyancy-Mixing
  - Ecosystem Energy
  - Oxidation Reduction
  - Respiration of other things besides Oxygen!



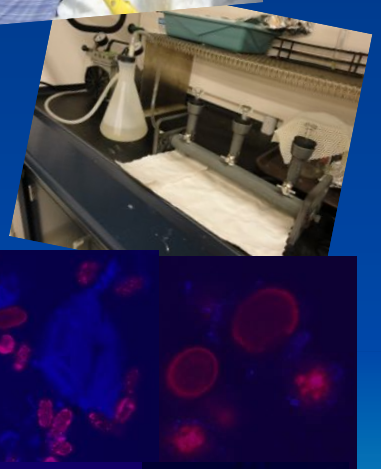
Hot Springs

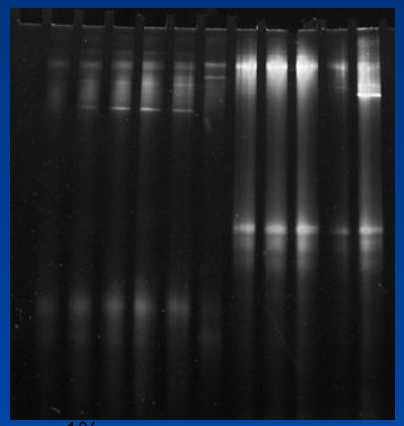

The Nitrogen Cycle
Nitrite NO_2
Nitrogen gas N_2
Nitrate NO_3^-
Ammonia NH_3
Oxidized
Reduced
Nitrite NO_2^-
Nitrogen gas N_2
Nitric oxide NO
Nitrous oxide N_2O

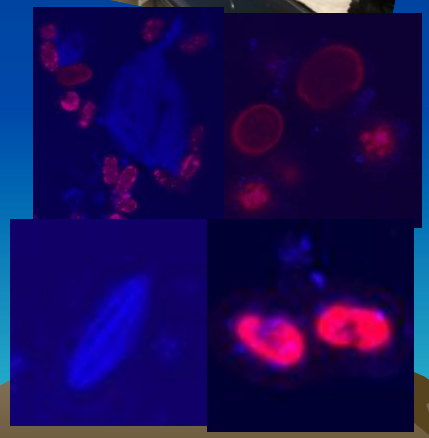

10
5
0
uM NH4+
uM NO2-
uM NO3-
sum N
85 75 65 55 45

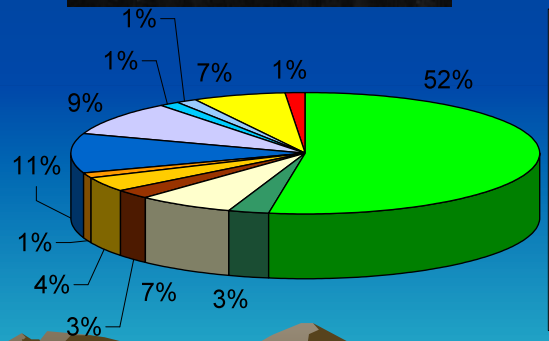


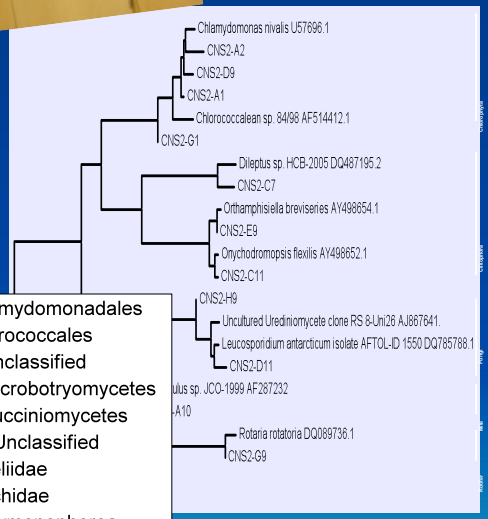
Snowfields





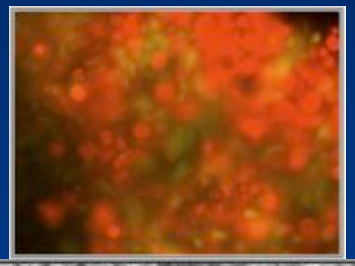


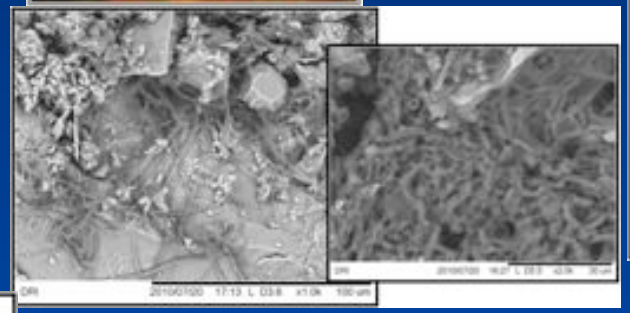

52%
7%
3%
7%
4%
1%
11%
9%
1%
1%
1%
Chlorophyta, Chlamydomonadales
Chlorophyta, Chlorococcales
Basidiomycota, Unclassified
Basidiomycota, Microbotryomycetes
Basidiomycota, Pucciniomycetes
Chytridiomycota, Unclassified
Ciliophora, Tracheliidae
Ciliophora, Oxytrichidae
Ciliophora, Oligohymenophorea
Ciliophora, Unclassified
Arthropoda, Microcaeculus
Rotifera, Rotaria

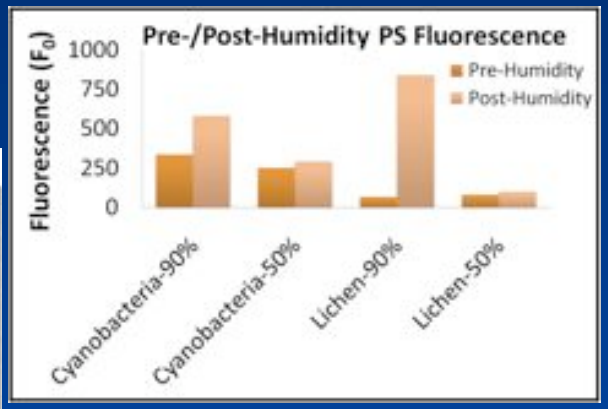

Chlamydomonas nivalis U57696.1
CNS2-A2
CNS2-D9
CNS2-A1
Chlorococcalean sp. 84/98 AF514412.1
CNS2-G1
Dileptus sp. HCB-2005 DQ487195.2
CNS2-C7
Orthamphisiella breviseries AY498654.1
CNS2-E9
Onychodromopsis flexilis AY498652.1
CNS2-C11
CNS2-H9
Uncultured Urediniomycete clone RS 8-Uni26 AJ667641.
Leucosporidium antarcticum isolate AFTOL-ID 1550 DQ785788.1
CNS2-D11
Rotaria rotatoria DQ089736.1
CNS2-G9

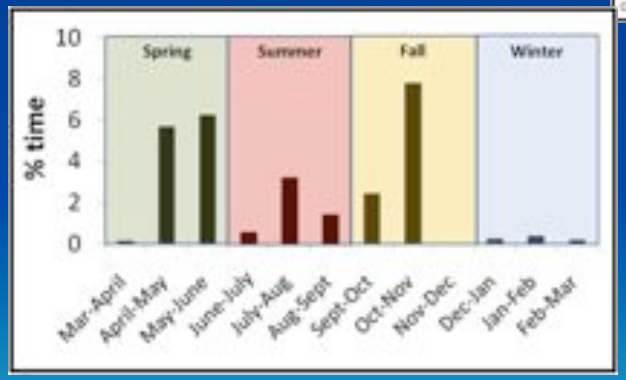
Cryptoendoliths

Cryptoendoliths



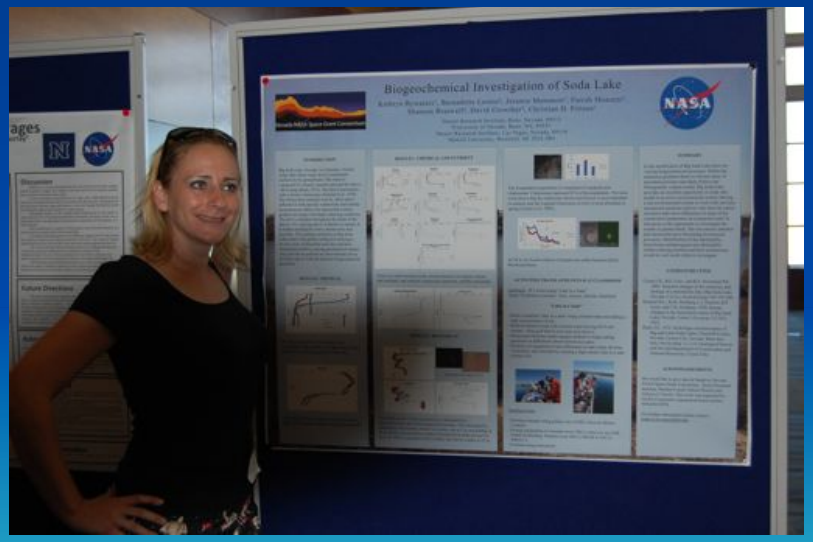






Results

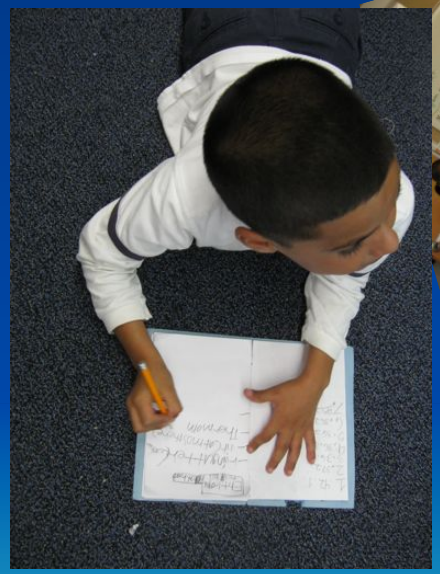
- Presentations
  - 8 student presentations and counting...
  - 2 faculty presentations and counting...
- K-12 Class Activities
- Graduate Thesis?
- Papers?
- Proposals?



- Anecdotal Confessions
- Ongoing Requests from in-service Teachers and graduate students

Transferring Knowledge to Classroom

Last Week:
- Roger Corbett Elementary
- Tier 1 School, 90% ELL,



Outlook

- More Evaluation
    - -Feedback
    - -Effectiveness
    - -pre/post, Students faculty etc..
- Future Offerings
    - -in South?
    - -Regional?
    - -National?
- Additional Partnerships
- More NASA personnel

Biogeochemical Investigation of Soda Lake

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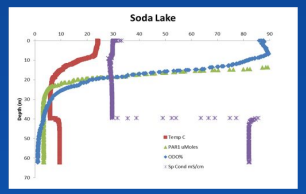
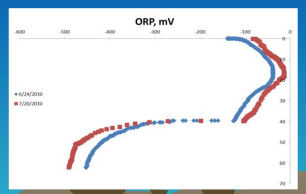
INTRODUCTION

Big Soda Lake, Nevada, is a terminal, volcanic crater lake whose water level is maintained exclusively by groundwater. The crater is composed of volcanic, basaltic sand and the lake is ~60 m deep (Rush, 1972). The lake is meromictic with a distinct chemocline (Kimmel et al. 1978). The chemocline currently rests at ~40 m and is reflected in both specific conductivity and salinity measurements. Below the chemocline a redox gradient develops with highly reducing conditions. The pH is consistent throughout the depth of the lake at ~9.5, proving that it is alkaline in nature. It is further stratified by both a thermocline and oxycline. The existing conditions at Big Soda Lake make it the perfect setting for studying a diverse array of microbial activities and their interactions within a varying geochemical regime. Our goal was to perform an observational survey of Soda Lake to infer the inherent biogeochemical processes.

RESULTS - PHYSICAL

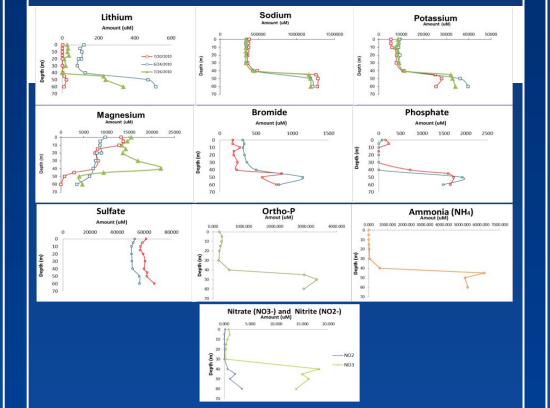


The data collected by YSI displays how temperature, light, dissolved oxygen and specific conductive strong stratification.

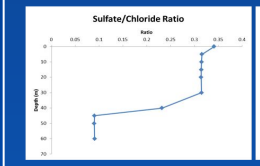


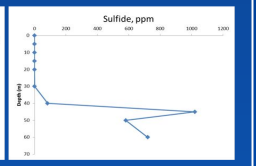
Coincident with the distinct chemocline at 40 m there is a dramatic increase in reducing potential.

RESULTS - CHEMICAL AND NUTRIENT

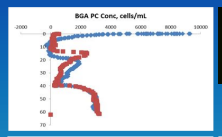


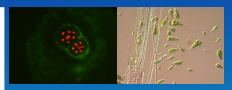
There is a rapid increase at the monimolimnion of cations, anions and nutrients. And reduced compounds ammonia, sulfides and nitrite.



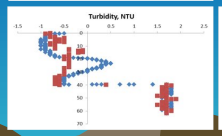


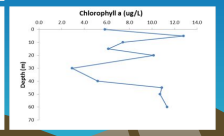
RESULTS - BIOLOGICAL



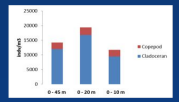


Algae obtain from a depth of 10 m

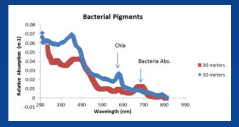


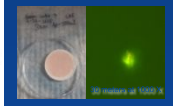


The upper region of the mixolimnion is dominated photoautotrophs and chemoorganoheterotrophs. The chlorophyll a, obtained by fluorometry, shows two peaks; one at 5 m and another at 20 m. BGA concentrations (relative fluorescence) peaks around 20 to 25 m. This is consistent with turbidity that shows a spike at 25 m.



The Zooplankton population is comprised of copepods and cladocerans. Cladocerans represent 81% of the population. Previous work shows that the cladoceran *Moina hutchinsoni* is most abundant in summer and the copepod *Diaptomus sicilisis* most abundant in spring (Cloern et al. 1983).





At 30 m we found evidence of purple non sulfur bacteria (likely Rhodospirillum).

ACTIVITIES TRANSLATED INTO K-12 CLASSROOM

Approach: 5E Lesson using "Lake in a Tank"
Target Vocabulary/concepts: Oxic, Anoxic, Salinity, Stratified

"Lake in a Tank"

-Build a stratified "lake in a tank" using colored water and adding a high concentration of salt.
- Build an identical tank with colored water leaving OUT salt content. Drop golf ball in each tank and observe.
- Observation between tanks engages students to begin asking questions on differences observed between tanks.
- Students use equipment to test differences in tank water, develop vocabulary, and conclude by creating a high salinity lake in a tank of their own.

Teaching Goals:

- Develop concepts using golden rule of ABC (Activity Before Content)
- During explanation of concepts never TELL when you can ASK
- Verbal Scaffolding: Students must SEE it, HEAR it, SAY it, WRITE it
- Evaluate using assessment.

SUMMARY

In the stratification of Big Soda Lake there are varying biogeochemical processes. Within the numerous gradients there is a diverse array of microbial activities that likely follow the Winogradsky column model. Big Soda Lake provides an excellent opportunity to study this model in an active environmental system. Having a real environmental system to work with can raise unexpected questions such as; why does the cation and anion data show differences in some of the conservative parameters on a temporal scale? It also gives us the opportunity to investigate the system in greater detail. The oxic-anoxic interface and chemocline have fluctuating biochemical processes. Identification of the alkalophilic, halotolerant methanogenes and alkalophilic sulfate-reducing bacteria and their mechanisms would be well worth while to investigate.

LITERATURE CITED

Cloern J.E., B.E. Cole , and R.S. Orwmland RS. 1983. Seasonal changes in the chemistry and biology of a meromictic lake (Big Soda Lake, Nevada, U.S.A.). Hydrobiologia 105:195-206.

Kimmel B.L., R.M. Gersberg, L.I. Paulson, R.P. Axler, and C.R. Goldman. 1978. Recent changes in the meromictic status of Big Soda Lake, Nevada. Limnol. Oceanogr. 23:1021-1025.

Rush, F.E. 1972. Hydrologic reconnaissance of Big and Little Soda Lakes, Churchill County, Nevada: Carson City, Nevada. Water Res. Info. Service Rep. 11, U.S. Geological Survey and Nevada Department of Conservation and National Resources, Carson City.

ACKNOWLEDGMENTS

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Evaluating Snow Microbial Assemblages

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Abstract

Psychrophiles are organisms that grow optimally below 20°C (1). The Ice Cream Basin is home to many in-certain peaks with an abundance of alpine snow environments perfect for psychrophilic habitation. We analyzed samples from three different locations, Wheeler Peak, Pacific Crest Trail, and Mount Conness, characterizing and comparing the psychrophilic communities at varying depth intervals in the snow. Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) showed no notable difference in community structure with depth, but there was a distinct difference when comparing different snow environments (i.e. shaded vs. full sun exposure). The chlorophyll concentration decreased as the depth of the snow increased. By creating a clone library and utilizing DNA sequencing technology we were able to obtain 16S and 18S rRNA gene sequences from samples collected from Mount Conness, which allowed us to identify microbes living in the ecosystem. This information enabled us to produce bacterial and eukaryl phylogenetic trees, giving us a clear look into the diversity of this psychrophilic community. Out of seventy bacterial results there were fifty-three β-Proteobacteria, thirteen Sphingobacteria, and only three Actinobacteria, with one unclassified bacteria as well. These results will guide us in our future plans for experimentation.

Aims and Methods

1) Collect surface samples and depth profiles from Wheeler Peak (WP) and Pacific Crest Trail (PCT) on the back side of Mount Lincoln, and process previously collected samples from Mount Conness (MC).
2) Use confocal microscopy and fluorescent nucleotide staining to visualize the microbial life in our samples.
3) Measure chlorophyll concentrations at different depths of the snow.
4) Characterize the psychrophilic community by performing denaturing gradient gel electrophoresis (DGGE) on samples from all three sites.
5) Identify microbial members of the Mt. Conness psychrophilic community by generating a 16S and 18S rRNA gene clone library and sequencing representative genes.

Results

Snow sampling

We hiked up to an elevation of 3,292 m to Rock Glacier on Wheeler Peak and to an elevation of 2,406 m on Pacific Crest Trail. Mt. Conness samples were obtained on a previous expedition in 2009 (Fig 3). Sampling sites were identified visually by the presence of pigmentation in the snow (Fig. 4). At least one depth profile and multiple surface samples were collected during both PCT and WP trips. Depth profiles were taken in intervals of 10 cm to a depth of 1 m. Surface sample depths varied depending upon the depth of the pigment present (2-10 cm). The snow samples were transported back to the laboratory in insulated coolers and allowed to melt naturally overnight at ambient temperature to avoid cell deterioration.

Fig. 1. WP depth profile sampling

Fig. 2. PCT depth profile sampling

Fig. 3. Map showing sampling sites

Snow filtering

The snow samples were processed for DNA extraction using a peristaltic pump to pump the melted snow through a 0.1 mm mesh screen and then through 0.2 µm polyethersulfone filters (Fig. 5a).

Samples for pigment analysis were filtered through 0.45 µm glass fiber filters and the filters were extracted in acetone and analyzed by Jeremy Mammott, in the laboratory of Dr. Chris Francis in another lab.

Fig. 4. Pigmented snow surface sample seen in PCT1B 8/30/09

Fig. 5. Snow filtering: a) isolating cells for DNA extraction, b) DAPI slide preparation

Microscopy

Samples for microscopy were fixed with formalin (3.7%), stained with 4',6-diamidino-2-phenylindole (DAPI) and filtered through 0.2 µm polycarbonate membrane filters using a vacuum pump (Fig. 5b). The filters were then attached to glass microscope slides with immersion oil and covered with a cover slip.

Slides were examined using a confocal microscope (600X). Optical sections were taken in two sets, under ultraviolet conditions to fluoresce the DAPI and CY5 conditions to fluoresce any chlorophyll-pigmented cells in order to create 3D-renderings.

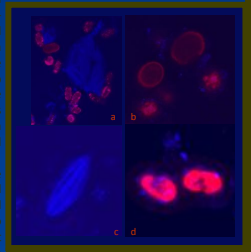

Fig. 6. Confocal microscope micrographs; a) PCT 1B surface; b, c, d) WP2 surface.

When examining the PCT 1B surface sample an abundance of elliptical cells were observed (Fig. 6 a) compared to the WP2 surface sample which revealed an abundance of more spherical cells surrounded by a halo of DAPI-stained bacteria (Fig. 6 b, d). We also viewed what appeared to be the skeleton of a diatom, which was likely deposited on the snow from some other location (Fig. 6 a).

Chlorophyll measurements

Pigment analysis was performed to assess the amount of photosynthetic life in snow samples using standard methods of pigment extraction and fluorometric determination (2).

Site Description	Starting Depth (cm)	Ending Depth (cm)	Chlorophyll (ug/L)
WP 2 Pond 1 Mat	0	0	0.10
WP 2 Pond 2	0	0	0.02
WP 2 Surface	0	0	5.85
WP 2 Sub surface 2	0	Nd	1.68
WP 2	0	8	2.15
WP 2	8	18	2.85
WP 2	18	28	1.98
WP 2	28	48	1.05
WP 2	48	68	0.31
WP 2	[illegible]	[illegible]	0.11
WP 2 Pond 3	[illegible]	[illegible]	[illegible]
WP 4 Grab Sample	0	3	400.53

Table 1. Chlorophyll concentration measurements of multiple WP sample sites.

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE is a technique that can be used to separate DNA fragments independent of size (3). Unique 16S and 18S rRNA gene sequence have different melting (denaturing) points that are sequence-dependent. A denaturing gradient of urea and formamide is produced, and allows for the separation of different gene sequences in a sample. 16S and 18S rRNA gene sequences were amplified from each snow sample using polymerase chain reaction (PCR). The DNA was then loaded onto a polyacrylamidegel and run overnight at 71 V at 70°C.

The 16S rRNA gene products displayed two banding patterns, lanes 1-5 had similar banding patterns but lane 6 had a distinct pattern. The 18S rRNA gene products showed at least two patterns, one in lanes 1-5 and another in lane 11. However, the quality of the gel made it difficult to [illegible] pattern was present in lane 10 (Fig. 7). This analysis will be repeated to see if sequence differences can be further resolved.

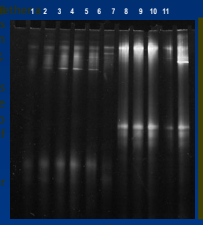

Fig. 7. DGGE
Lanes 1-5: 16S rRNA genes
Lanes 6-11: 18S rRNA genes
Lane 1: PCT 2A 0-10 cm
Lane 2: PCT 2A 20-30 cm
Lane 3: PCT 2A 40-50 cm
Lane 4: PCT 2A 60-70 cm
Lane 5: PCT 2A 90-98 cm
Lane 6: PCT 2B surface
Lane 7: PCT 2A 50-60 cm
Lane 8: PCT 2A 80-90cm
Lane 9: PCT 2A 98-98 cm
Lane 10: PCT 1B surface
Lane 11: PCT 2B surface

Gene Sequencing Results

Samples collected from Mount Conness were subjected to DNA extraction, PCR, and these products were cloned into *Escherichia coli* to create a clone library. The clone library was taken to the Nevada Genomics Center for sequencing.

Bacterial 16S rRNA gene sequences

Taxonomic classification of the bacterial 16S rRNA gene sequences was assigned using the Ribosomal Database Project II (4). The sequences assigned to different taxa are shown in a hierarchical order (Fig. 8). An abundance of β-Proteobacteria, 53 of 70 total sequences, was revealed, including 24 unclassified Oxalobacteraceae.

- > > domain Bacteria (70)
- > > > phylum "Bacteroidetes" (13)
- > > > > class "Sphingobacteria" (12)
- > > > > > order "Sphingobacteriales" (12)
- > > > > > > family Cytophagaceae (1)
- > > > > > > > genus Hymenobacter (1)
- > > > > > > family Sphingobacteriaceae (6)
- > > > > > > > genus Solitalea (6)
- > > > > > > > unclassified_Sphingobacteriaceae (2)
- > > > > > > family "Chitinophagaceae" (5)
- > > > > > > > genus Ferroglobacter (4)
- > > > > > > > unclassified_"Chitinophagaceae" (1)
- > > > > unclassified_"Bacteroidetes" (1)
- > > > phylum "Actinobacteria" (3)
- > > > > class Actinobacteria (3)
- > > > > > subclass Acthinobacteridae (3)
- > > > > > > order Actinomycetales (3)
- > > > > > > > suborder Frankineae (3)
- > > > phylum "Proteobacteria" (53)
- > > > > class Betaproteobacteria (53)
- > > > > > order Burkholderiales (42)
- > > > > > > family Comamonadaceae (9)
- > > > > > > > genus Polaromonas (8)
- > > > > > > > unclassified_Comamonadaceae (1)
- > > > > > > family Burkholderiales_Incertae_sedis (6)
- > > > > > > > genus Methylibium (6)
- > > > > > > family Oxalobacteraceae (27)
- > > > > > > > genus Massilia (3)
- > > > > > > > unclassified_Oxalobacteraceae (24)
- > > > > > > unclassified_Betaproteobacteria (11)
- > > > unclassified_Bacteria (1)

Fig. 8. Bacterial 16S rRNA gene hierarchy

Eukaryl 18S rRNA gene sequences

Chlamydomonas nivalis was the most dominant form of chlorophyte algae observed (Fig. 10). A variety of heterotrophic protozoans was observed. We also found a diverse array of fungi, and protists affiliated with the phylum Ciliophora. In addition we detected sequences affiliated with two kinds of multicellular organisms including mites and rotifers (Fig 9).

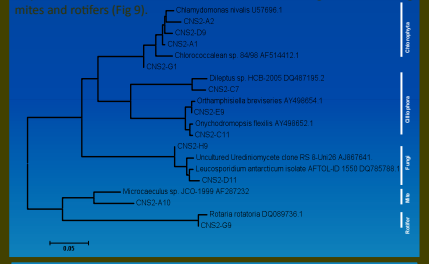

Fig. 9. Eukaryl 18S rRNA gene tree

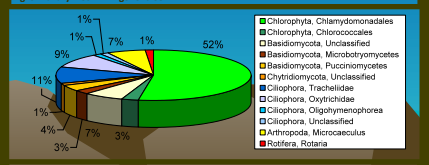

Fig. 10. Taxonomic affiliation of Mt. Conness snow eukaryal rRNA gene sequences

Discussion

Differences in cell morphology observed with confocal microscopy suggest different species of algae were present, and that with the PCT samples possibly new, undetected Chlamydomonas nivalis.

A halo of bacteria surrounding the PCT algal cells is likely bacteria being attracted to a polysaccharide matrix surrounding the algal cells (5). The purpose of the association between algae and bacteria is unknown though the bacteria could be utilizing the algal polysaccharide as a carbon source.

Chlorophyll analysis of WP 2-depth profile showed that as the depth of snow increased the chlorophyll concentration decreased. WP grab sample was a particularly dense red algal patch, that showed an abundance of chlorophyll, as is to be expected.

After amplification a number of PCR products were lost and therefore we were not able to compare WP to PCT on the DGGE.

The different banding patterns in the DGGE gel suggest that PCT 2B and 2A have different bacterial and eukaryl community structures. This could be visually represented by the fact that the 2B surface was more heavily pigmented than 2A.

Mt. Conness bacterial library showed a dominance of Betaproteobacteria, in particular, sequences affiliated with uncultivated members of the Oxalobacteraceae. The role of these organisms in snow fields is unknown, but related sequences have been detected in Antarctic snow algal assemblages (6).

16S rRNA results showed the dominance of the red-pigmented *C. nivalis* snow algae. In addition a diversity of other sequences detected appear to make sense because it is known that rotifers and ciliates prey on *C. nivalis* (7).

Future Directions

This work contributes to ongoing research in Dr. Murray's laboratory where she is studying microbial communities associated with snow fields as part of the NAI Icy Worlds research program. The snow samples discussed here will be further characterized and compared between all three snow field locations to generate a better understanding of the diversity and community structure of snow-associated microbial assemblages. This work is also part of a larger effort to develop life detection technologies in icy habitats and understand the requirements of life in such locations.

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