Lewis and Clark Fund for Exploration and Field Research in Astrobiology
Final Report—Grant recipient: Caroline Hung, University of California, Riverside

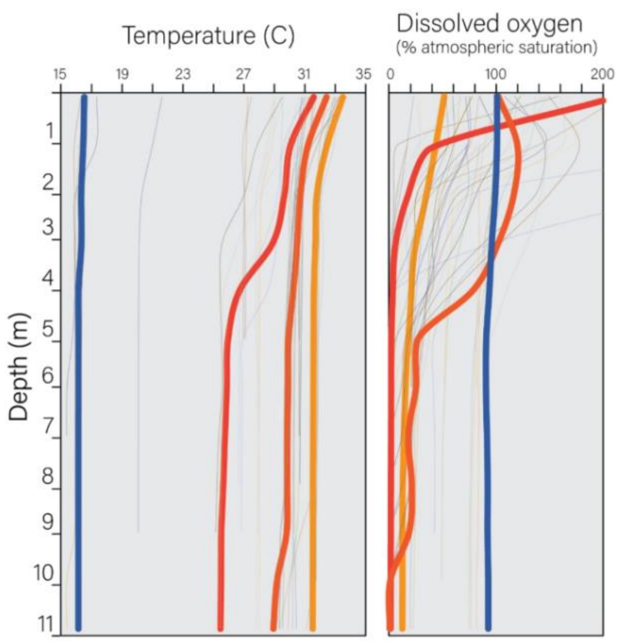
A Novel Interdisciplinary Study of Microbial Habitats and Community Structure in a Non-Desiccated, Redox Stratified Evaporite-Forming Mars Analog

Award Amount: $5,950
Award Period: June 2020 – July 2022

For this award, I conducted a field and lab study at the Salton Sea (SS), California's largest lake, to explore the extremophile-mediated processes involved in a saline, closed-basin with its aqueous geochemistry dominated by the evaporative crystallization sequence. Due to the continuous input of untreated irrigation waters loaded with nutrients and ammonium sulfate fertilizers, sulfate levels in the SS are around 10 times higher than those of the open ocean, despite salinity that is only twice as high. At such, the high level of sulfate presents a unique scenario that allows for 1) gypsum crystals to precipitate upon certain conditions when it reach its saturation state, and 2) unlimited sulfates for microbial sulfate reduction in anoxic bottom waters, prevalent in the summer months as a result of eutrophication spurred on by excess nutrients. In this anthropogenically modified system that is rapidly drying, approaches in geochemistry and microbiology are combined to investigate the cycling of nutrients, pathways for mineral formation, and the role of microbes in different niches (i.e., across thermal regimes, high salinity, anoxia) at the SS. Ultimately, the interplay of mineral precipitation and microbial activity in the natural laboratory at the SS have implications for the role of microbes to mediate the wet-to-dry transitions of the past Martian water cycle. Further, the extremophiles and their halophilic environmental niches I document at the SS may illuminate hints toward focal targets in which traces of life may be discovered since Mar's rich water history. In this final report, I outline the main goals proposed, as well as actionable tasks, results and short discussion/interpretation to accompany each figure.

Goal 1: To explore the mechanism(s) of evaporite crust formation, recognizing that it may be more complicated than simple desiccation of the analog basin (i.e., instantaneous gypsum crystal precipitation may be a result of rapid sulfur cycling mediated by sulfate reducers and sulfide oxidizers)

Task 1: Time-series monitoring of temperature, pH, oxygen levels, salinity, and concentrations of sulfide and major /minor elements through the seasons of a calendar year



Findings:

Figure 1. Temperature and dissolved oxygen depth profiles. Each line represents these two parameters measured at a single location at the deepest locale of the S. basin of the lake (33.26265, -115.739). All water column depth profiles collected to date are shown on this figure. Bolded lines show representative seasonal profiles: Blue represents winter when the water column is well-mixed while red, orange, and yellow represent various mixing states in the summer months.

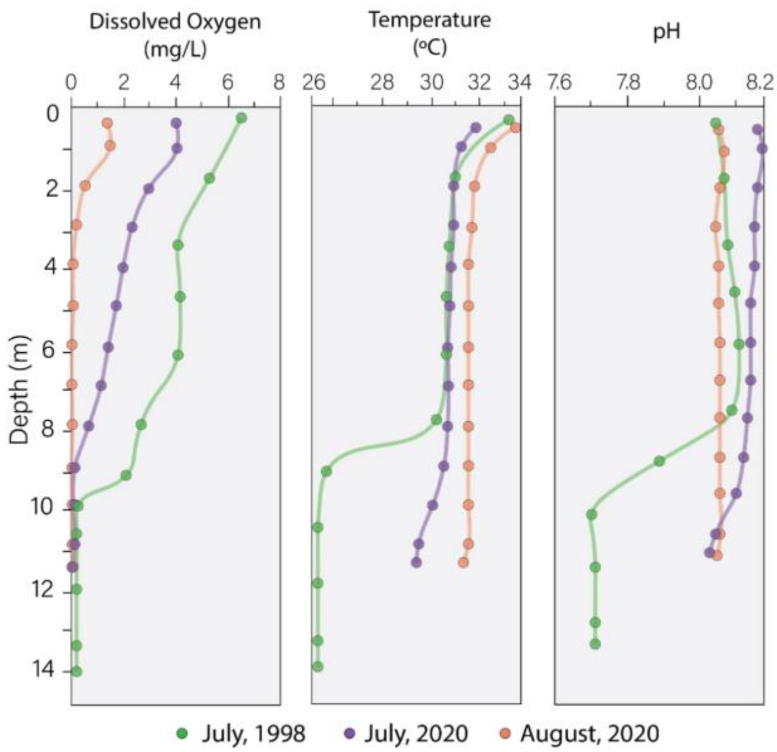


Figure 2. Temperature, dissolved oxygen, and pH of the water column from July 1998 (Schroeder et al., 2002, Hydrobiologia), July 2020, and August 2020 (this study). As the lake shallows over the last two decades, the thermal regime of stratification has changed, appearing less intense such that anoxic conditions expand towards the surface waters.

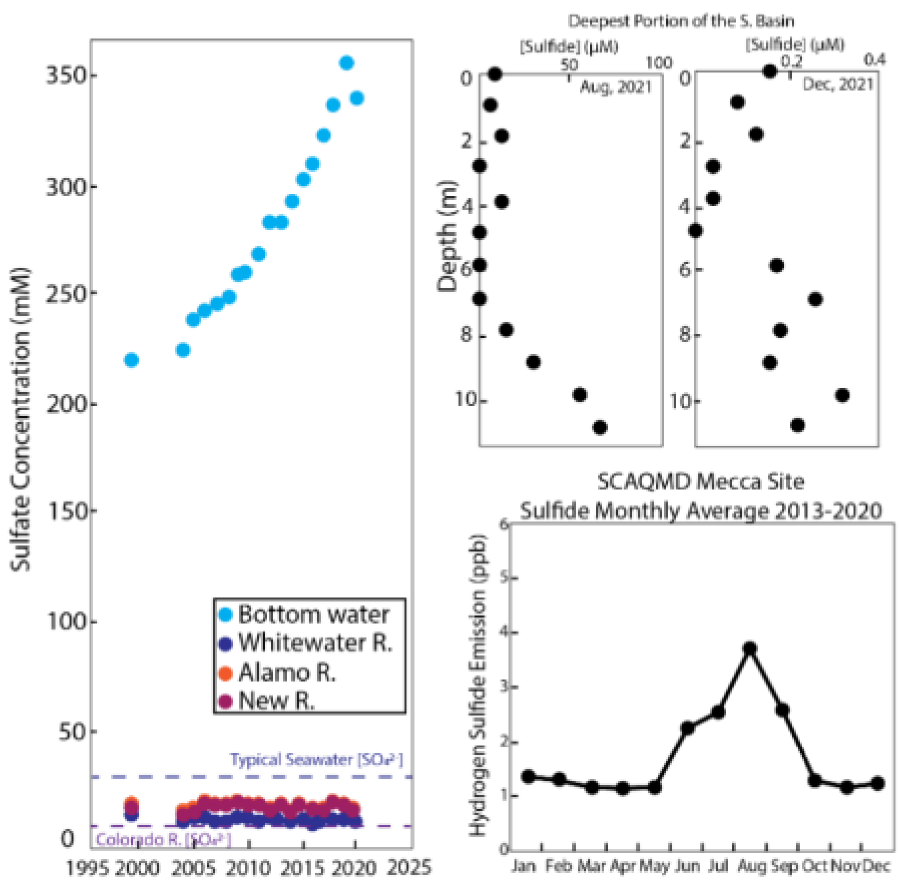


Figure 3. Left: Sulfate concentrations in the Salton Sea and three main riverine tributaries over the last two decades. Data publicly available from Bureau of Reclamation. Top right: Sulfide concentrations on a representative day from August and December of 2021, note the difference in the scale of the x-axis. Bottom right: Sulfide emission data publicly available from SCAQMD Mecca Site. Sulfidic gas emission from the SS is related to the upward release of euxinia during water column mixing.

Figure 4. Left: "Greentide" patches of precipitated gypsum crystals in the surface water can be identified by the naked eye when on the lake on August 30th, 2020. Right: Satellite image of a greentide phenomenon on August 30, 2020, by the NASA Modis satellite image confirmed the field sighting of a greentide patch.

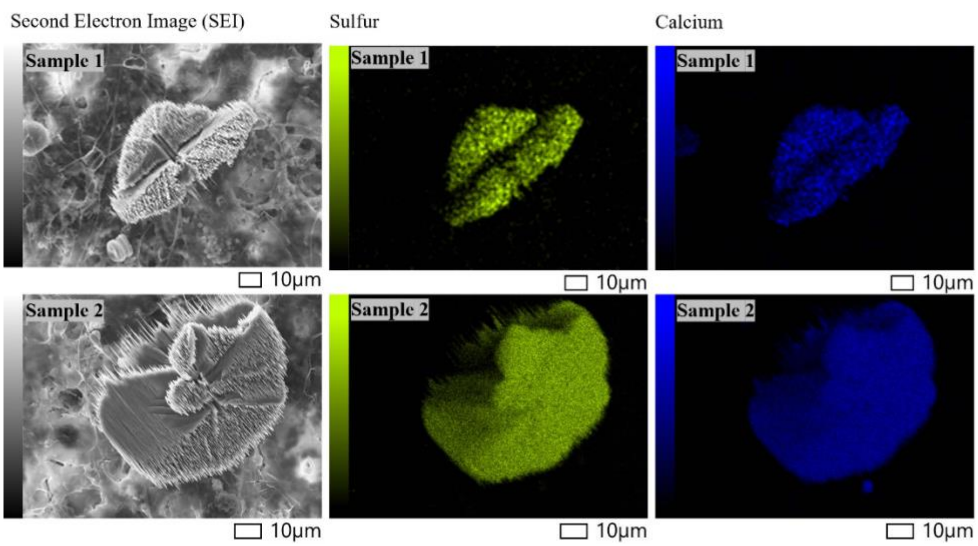


Figure 5. Secondary electron image and elemental maps of gypsum (hydrated calcium sulfate mineral; $CaSO_4 \bullet H_2O$) crystals collected form the water column during the greentide event in August 2020. Spiny features of such crystals may suggest the instantaneous nature of such crystallizations.

Interpretation for Task 1:

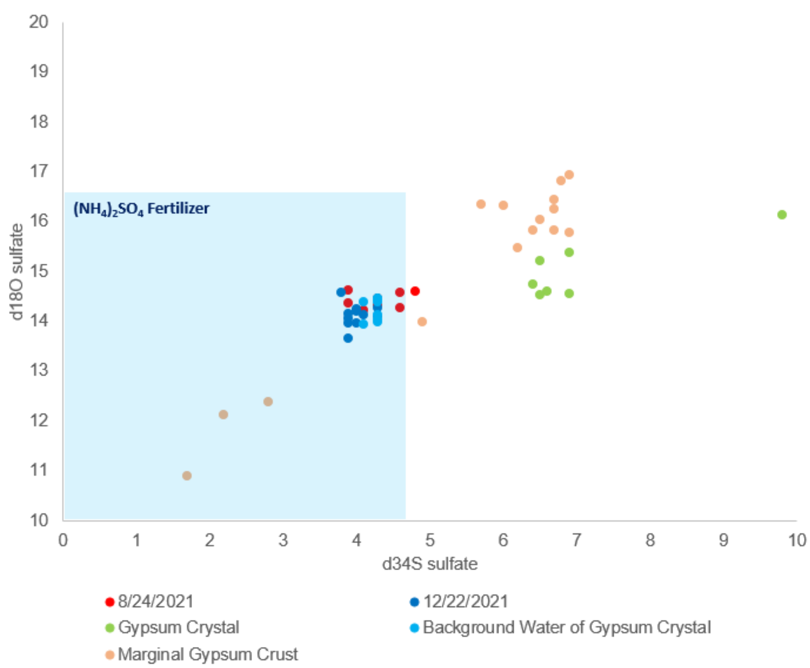
Observed geochemistry of the SS water column

After two years of continuous (monthly, bi-monthly) monitoring of the water column, the SS thermal regime and stratification is dynamic on the seasonal and diurnal scale. In winter months, the water column is vertically mixed throughout. There is no thermos-stratification and subsequently, no chemo-stratification. However, in the summer months, the upper and lower portions of the water column of the SS do not easily mix due to temperature-related stratification (Figs. 1 and 2), especially during the daytime when water temperature can reach 36 degrees Celsius. To set up anoxic conditions below surface waters, the process of algal decay consumes any dissolved oxygen that does mix downward, such that oxygen does not penetrate beneath 3-4 m. From there, microbial sulfate reduction takes over. As a product of one of these metabolisms, sulfide accumulates in the bottom water (Fig. 3). This sulfide can then mix upward when thermal stratification breaks down, influencing the chemistry of shallow waters. As the SS shallows, the water column will mix more often, leading to frequent (i.e., as often as daily) upward migration of this accumulated hydrogen sulfide to the surface. This observation suggests that physical factors, such as temperature, predominantly controls the biogeochemical cycling of the lake as much as the availability of nutrients to set up anoxia. The preexisting set-up of the geochemical conditions are required for the subsequent microbial cycling and mediation of the water column.

Observed microbial mediation of the SS system

High nutrient inputs (as discussed above) and associated sulfate levels have led to a novel sulfur cycle in the Salton Sea. The summertime water column shows supersaturated dissolved oxygen concentrations in surface waters due to extremely high rates of photosynthesis. Immediately below this surface layer, 3-4 m down, the water column is anoxic and sulfidic due to the consumption of O_2 through respiration. In these waters, bacterial sulfate reduction is then the most competitive metabolism. As a result, abundant organic matter is consumed. As a biproduct, sulfate is reduced into hydrogen sulfide, which can accumulate in deeper water that is isolated from the surface. When the water column mixes upon the removal of the thermos-layers, anoxic and euxinic layers mix with the surface, which allows for the emission of hydrogen sulfide to the atmosphere from the lake (Fig. 3). In fact, high sulfide release events in the summer produce ambient atmospheric levels above the 30 ppb threshold set by the State of California. Sulfide cycling in large quantity is also coupled to periodic blooms of gypsum (Fig. 4), which are marked by rapid precipitation of micro-crystalline gypsum (Fig. 5) in the surface water—at times over hundreds of square miles—when the lake finally overturns after prolonged stratification allowing for sulfide to accumulate in high concentrations. These events take place very rapidly, though their occurrence has diminished over recent years as the lake shallows and mixing happens more frequently. They are a subject of ongoing study, and one area of interest is the possibility that they contribute to the gypsum crusts found surrounding many of the shores of the lake, which have implication for microbial signatures detectable by isotopes (expanded in the next section) or microscopy.

Task 2: Analyze $\delta^{34}S$ and $\delta^{18}O$ isotopes on samples of the water column, underlying sediments, gypsum crust, and gypsum crystals from whiting events



Finding:

Figure 6. Mixing model of the $\delta^{34}S$ and $\delta^{18}O$ isotopes for sulfates of the seasonal water column, gypsum crystals and its background water as well as the marginal gypsum crusts. Note that the $\delta^{18}O_{SO42-}$ of atmospheric oxygen is 23.5‰ while that of the average Colorado River water is <2‰. In this depiction, water column samples all fall within the boundary of the ammonium sulfate fertilizer literature values (Syznkiewicz et al. 2015). However, gypsum crystal from greentide phenomenon and marginal crusts appear to go

beyond the sulfate isotopic value set by human influence.

Interpretation for Task 2:

Marginal crusts are present in many shoreline regions of the lake today. Crust formation is one of the quantitatively important ways sulfate is removed from the water column, predominantly through precipitation tied to lake water evaporation and possibly through flash gypsum precipitation events. Over the past 24 months, we have sampled water, gypsum precipitates, and marginal crusts to analyze isotopic properties and concentrations of sulfur present as sulfate and sulfide phases to describe and quantify the sulfur cycle in the lake. Preliminary isotopic data for sulfate suggest the basin sulfur mass balance is primarily influenced by those values of the ammonium sulfate fertilizer (Fig. 6; 200 more data points for the $\delta^{34}S$ for more sulfide and sulfate samples of water column and sediment will be available soon). However, the $\delta^{34}S$ and $\delta^{18}O$ fractionation for described gypsum crystals and marginal crusts are rather similar and heavier. In fact, the gypsum crystals have heavier fractionation than the background water it precipitated from. This suggests there are fractionation pathways (likely microbially associated) not yet explained. By producing the complete mass balance of the fractionations associated with the SS sulfur cycle, we hope to illuminate the biotic (e.g., microbial sulfide oxidation) and abiotic (e.g., precipitation upon supersaturation) pathways and associated isotopic signatures related to the greentide phenomenon and marginal crust formation.

<u>**Goal 2: To assess the related niches for life and the associated microbial communities and ecologies**</u>

Task: Analyze microbial communities within collected samples from the field to cross-correlate diversity and geochemical environment

Finding:

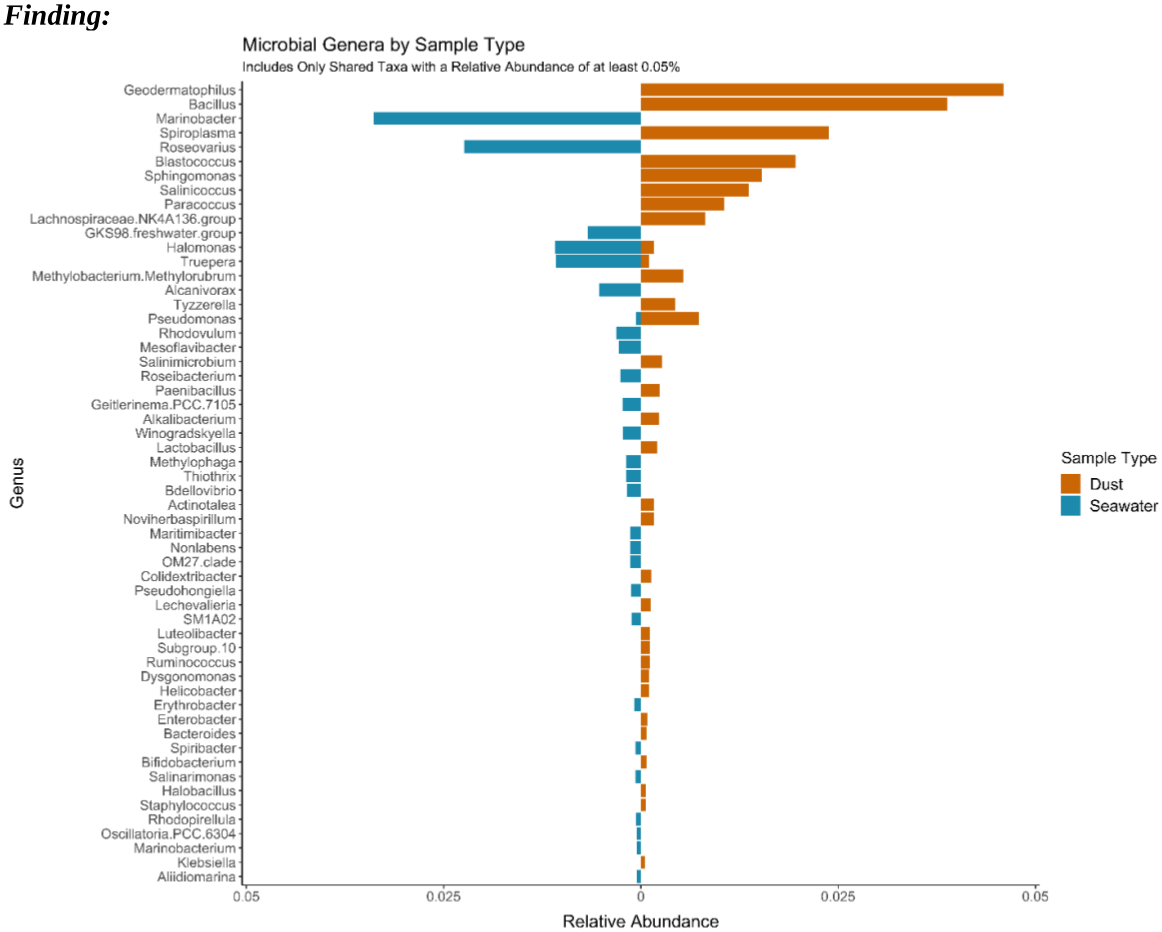


Figure 7. Result of 16s rRNA analyses of SS water column (water samples over depth collected August 2021) and dust samples produced by the Aronson Lab (graduate student, Hannah Freund) from the samples provided by the Lyons lab and partially funded by this award. Notably, the water column community was dominated by Gamma-proteobacteria (prokaryotes usually found in extreme environments). As expected, halophilic bacteria are predominately present, along with other extremophiles such as thiothrix (i.e., Beggiatoa, sulfide oxidizing bacteria) and methylophaga (methylotrophs/methanotroph).

Interpretation:

To go past just the answers to: who's there? We will analyze the microbial diversity along with the geochemical data produced in this study. The same sampling scheme for microbes was repeated two more times in Dec 2021 and April 2022 to appreciate the full seasonal dynamic of the water column geochemistry. Once all the data are compiled, community richness and diversity will be compared in the context of detailed geochemical analyses of the water column over the sampling events. Further, porewater and sediment samples were also collected for microbial analyses.

Future planned publications from data generated via this grant award:

1) *Scientific Reports Special Issue for Anthropogenic Modifications*: Outline how historical and modern water policy for the agricultural region have negatively impacted the SS basin, imposing issues of water quality upon the dire water supply as a reflection of perpetual drought through lens of both geochemistry and microbiology.
2) *Scientific Reports Special Issue for Ecology of Extreme Events*: Outline the demise of the "greentides" —a unique phenomenon juxtaposing the interplay of extreme lake chemistry and microbial mediation— and how this phenomenon becomes less common as lake shallows and thermal regime disappear. This article would have the most implication for astrobiology to document how the ecology of a system changes drastically as it becomes more unfit for life such that "extreme" events come and then cease to exist with evolving environments.
3) *Geochimica et Cosmochimica Acta*: Outline the mass balance of the sulfur cycle at the SS, mainly looking at $\delta^{34}S$ and $\delta^{18}O$ isotopes of sulfides and sulfates from samples of seasonal water column, gypsum crystals from greentide events, marginal gypsum crusts, acid-volatized sulfur (FeS; iron monosulfide) and chromium-reducible sulfur (FeS_2; pyrite) and porewater of lake sediment cores. Only a portion of this dataset (originated from this project) is presented here in the final report.
4) *Frontiers*: Document the microbial communities and ecology in the niches influenced by dynamic changes in water and sediment geochemistry at the SS. The extremophiles (halophiles, sulfate reducers/sulfide oxidizers) characterized in the different wet-to-dry components (sediment → water column → playa → dust) of the SS basin have implications for what to be expect in terms of microbiology in 1) drying lake influenced by climate change, or 2) dried basin in outer space.

Financial report:

Category	**Amount Spent**	**Notes**
Field Travel	$3000	Gas and subsistence as well as lodging for consecutive field days
Field Equipment	$2635	Supplies for the field in boat operation, safety, and sample collection
Lab Equipment/Analyses	$315	Most lab analyses were done in-house in the Lyons Biogeochemistry lab at UCR, which eliminates the need to use funds from this grant as general lab operations are funded by PI. 16s rRNA surveys were covered by and completed with Aronson Lab at UCR's Dept. of Microbiology. Remaining cost applies for travel to IUPUI to run d18O on sulfates.

Total Funds spent: $5950 ($4950+ $1000 Covid relief fund)