Project Report:

The fieldwork supported by this grant from the Lewis and Clark Fund in Astrobiology was carried out in the Little Sippewissett salt marsh, which we accessed from Woodneck Beach in Falmouth, MA. The grant supported my travel and research costs to organize and join a team of geochemists and microbiologists all working together on questions related directly to my dissertation research. In total our research team included myself, and my advisor Dr. Victoria Orphan from Caltech, sulfur geochemistry specialists Dr. Greg Druschel and Fotios Kalfenatras from University of Indiana-Purdue University Indianapolis, and sulfur geochemistry specialists Dr. David Fike and Dr. Jennifer Houghton. Greg Drushel's team was responsible for making microvoltammetic sulfur species measurements to determine the form of sulfur present in the environment (polysulfides, elemental sulfur, sulfide, thiosulfate, etc…). In tandem, David Fike's group made parallel measurements with Unisense microelectrode for oxygen, sulfide and pH, which possessed a finer spatial resolution. The microelectrode setups required some serious field-gear, including large marine batteries, laptops and micromanipulator stands. To transport and keep this equipment dry in the field, I constructed a home-built research raft which we affectionately dubbed the R/V Berry Explorer!

(A)  **(B)** 

(A) Construction of research raft R/V Berry Explorer from foam dock pontoons and plywood.

(C) 

(B) Raft just fit inside our research vehichle minivan! (C) Taking its maiden voyage!

After a few days of prep-work, I retrieved our team in Boston and we began work in the marsh. As you can see, the level of field-attire ranged from beach-goer to serious field waders!

(Left to right, Fotios Kalfenatras, David Fike, Greg Druschel, Elizabeth Wilbanks)

After transporting our equipment to the field on the R/V Berry Explorer, we began setting up the microelectrode equipment to take profiles through the marsh pond sediments.

(Left) Transporting equipment up the tidal channel to reach the pool where the berry aggregates are found. (Right) Microelectrode set up by the berry pond in Sippewissett salt marsh, ready to begin collecting data!

After length instrument calibration, we collected matched microvoltametrric and Unisense microelectrode O_2/H_2S profiles through the marsh water and sediment at dusk and peak sun. Pink berry tissue samples were also collected in tandem with these measurements, were preserved immediately and then saved for RNA extraction after the finish of the fieldwork.

Water samples for HPLC and Ion Chromatography were collected and will be analyzed at Caltech later this fall (to provide a better characterization of the chemical environment at the time of sampling). Due to time constraints and difficulty with the microelectrode systems, we were unfortunately not able to collect *in situ* profiles during the night – however we did collect samples for RNA and water chemistry. All samples for RNA are being extracted and currently prepared for Illumina sequencing this winter. The data from microelectrode profiles is currently being run against lab standards and quantified by Greg Druschel and David Fike's groups.

Another goal of our fieldwork was to characterize the isotopic composition of the sulfide evolved from these microbial aggregates as the result of bacterial sulfate reduction (BSR). This fractionation of sulfur isotopes (from sulfate to sulfide) is used as an indicator of BSR, though this parameter is only poorly constrained by single species laboratory studies. One technical challenge we faced, is that these aggregates evolve very little sulfide, due to the tight association between sulfate reducing and sulfide oxidizing microbes. To overcome this, we impaled very large aggregates on silver wire to capture sulfide (as precipitated silver sulfide) from ***within*** the aggregate. This experimental setup, which looked much like a shish-kebab of microbial aggregates, was then tied to a long piece of fishing line and left in the marsh pond over night.

(Left) Elizabeth Wilbanks proudly holding the berry-skewer (Right) Measuring the silver wire-berry experiment.

After removing the wire from the pond, berries were gently slid off the wire, revealing that sulfide had precipitated as silver sulfide overnight, leaving a black tarnish mark where the aggregate had been sitting (see photo below). The berries were sectioned, with part of the material being embedded for microscopy and another portion saved for sulfur isotopic analysis of the biomass. The isotopic composition of the sulfide deposited on the wire will be analyzed by using secondary ion mass spectrometry (SIMS-7f) at Caltech during the first week in December (by Wilbanks, Fike and Orphan).

Removal of berry aggregates from the wire, revealing the black tarnish-marks left by sulfide precipitation.