Narrative Progress Report:
1. PRF: 57209-DNI2; Institutional Account: 268887
2. Project Title: The inner lives of Archaea: the hydrogen isotopic composition of Archaeal lipids as a proxy of metabolic state.
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4. Collaborator: Pearson, A. (Harvard University)
5. Reporting Period: 01 July 2018 to 31 August 2019, and re-budget request in Nov 2019

Summary and Impact

Archaea are ubiquitous microbes in Earth's ocean sediments, extreme environments and oil/gas reservoirs. Prominent members, including methanogens, anaerobic methanotrophs, aerobic ammonium oxidizers, as well as the more well-studied thermoacidophiles. Different archaea perform critical biogeochemical reactions with global economic consequences. *Sulfolobus* strains are essential for high-temperature sulfur cycling in hydrothermal vents, hot springs and hydrocarbon reservoirs. Critically, the lipids from thermoacidophilic archaea are frequently used to understand environmental conditions, which are heretofore poorly calibrated due to our severely limited knowledge of how archaeal physiology is recorded in lipids – the result of few lab calibrations. Here we are working to develop a stable isotope-based organic geochemical tool to disentangle different archaeal metabolic phenotypes. It was recently shown that H-isotopic compositions in bacterial lipids (δD_{lipid}) are significantly offset from ambient water, where δD_{lipid} values vary strongly in response to changing environment. Because archaea in general tend to occupy systems characterized by extremes, including energy limitation, we anticipate that archaeal δD_{lipid} values also are particularly sensitive to variations in energy flux. In this study we are cultivating model archaeal thermoacidophiles in batch and continuous cultures under different electron donor supply regimes in order to quantify the response of δD_{lipid} to 'energy rich' versus 'energy poor' conditions. This approach will lend insight into the physiological response of archaea to energy stress and will directly impact our understanding of archaeal lipids, perhaps recalibrating them as in situ proxies for energy stress. The results from this work will fundamentally improve our knowledge of archaeal metabolic plasticity, and can be extended to archaea critical in to oil and gas-production, and reservoir preservations.

We met a number of milestones in in FY2019 (July 2018 to end June 2019) of the ACS-PRF-DNI proposal (now under no cost extension). We have now published our first peer reviewed manuscript [https://doi.org/10.1111/1462-2920.14851], led by graduate student A. Zhou (MS defended at Dartmouth in May 2019, now a PhD student at Unvi. Michigan). In December of 2019, postdoc Yuki Weber will present the initial archaeal lipid H-isotope finings at the AGU national meeting, and the most preliminary unit of this data was presented by PI Leavitt at the International Geobiology Conference in June 2019.

In the second year we also completed (i) successfully completed chemostatic growth rate experiments with the model mesophilic archaeon, *Nitrosopumulus maritiumus* and analyzed the GDGT abundances and H-isotope abundances on a subset; (ii) analyzed the H-isotopic composition of all prior *Sulfolobus* and *Acidianus* experiments; (iii) manuscript preparation of the experimental H-isotope manuscript (led by postdoc Weber) has commenced; second field-season in YNP completed by PI Leavitt in October 2019 (first in Sept 2018) – samples transferred to Dartmouth, awaiting analysis by visiting student Y. Zhang in 2020. To-date, we have sampled 20 hot springs, and archived both sediment and water samples for H-isotope analyses.

Re-budget request & no cost extension: In the coming final time unit (current, thru August 2020, requested extension through August 2021) of this project we will hire postdoc (Y. Zhang, starting June 2020) to finish out this work on the remaining analytical lipid and H-isotope work from cultur3es and the analysis of YNP field samples (remaining salary: $41,463.98). We kindly request to utilize the remaining materials and field work funds (combine: 5,226.74) to complete these analysis, and for travel to/from Harvard University and CU Boulder for sample analysis by Leavitt lab members.