NASA Early Career Collaboration Award Report: Characterizing the lipid profile of a Mars-analog chaotropic brine environment

Award recipient:

William Wallentine - Ph.D. candidate at the University of Tennessee-Knoxville

Collaborators:

Dr. Jennifer Eigenbrode - organic biogeochemist at NASA Goddard

Sarina Wahl - previously a postbac at NASA Goddard

Dr. Erin Gibbons - Postdoc at NASA Goddard

In the search for life in the geologic record of extraterrestrial environments, lipid biosignatures have stood out as a prime target due to their geologic recalcitrance, structural variability, and phenotypic specificity. For example, hot environments like hydrothermal vents select for more geologically stable lipids (hopanoids, saturated fatty acids), and cold environments less stable lipids (unsaturated fatty acids). Polyextreme environments, characterized by extremes of multiple physicochemical parameters, have more complex and often unpredictable lipid profiles. Cold and chaotropic brines, which are expected to be abundant across the solar system, are one such enigma where the study of expected lipids is limited by the rarity of such environments on Earth. Furthermore, as cold and chaotropicity have been observed to negate the effects of each other, it is unclear what lipid membrane strategy would prevail and what lipids would ultimately be preserved in the geologic record.

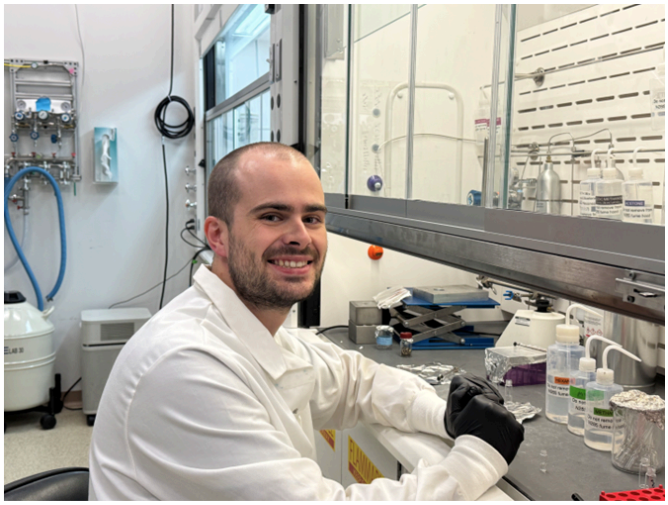

Figure 1: Will working on lipid extracts in the Planetary Environments Laboratory, NASA Goddard

The NASA Early Career Collaboration Award afforded me the opportunity to approach this problem by working with Dr. Jen Eigenbrode at NASA Goddard to study the lipids found in sediments and bacterial isolates from Don Juan Pond (DJP), an Antarctic cold and chaotropic $CaCl_2$-dominated brine. In the first two-week long visit in the summer of 2024, I worked with Dr. Eigenbrode and her post-bac Sarinah Wahl to identify changes in the lipid profile of two DJP bacterial isolates when grown in a range of $CaCl_2$ concentrations and temperatures. The goal with this experiment was to see what lipids each pressure selected for independently and when combined together. During the first visit, I was able to quickly learn how to operate complex instruments, identify problems and troubleshoot, as well as performing lipid extractions independently. I also learned some of the potential limitations of my proposed project. Bacteria, especially uncharacterized environmental isolates, have incredibly complex lipid regulation mechanisms that can cause rapid changes in lipid profiles after small changes in temperature, differences in growth phase, or even from handling. This highlighted the importance of understanding basic membrane physiology to better design experiments that interrogate complex molecular processes. For my next visit I would characterize the lipid profile of sediments from the

environment. With such a profile, I could begin answering questions about what lipids are ultimately selected for and preserved in this environment, providing more astrobiological relevance.

Prior to the second, month-long visit in July 2025, Dr. Eigenbrode and her postdoc Dr. Erin Gibbons zoomed with me multiple times to develop a concrete plan for the sediment analyses using two sets of samples from a sediment core collected from DJP. The first set contained ~20mg from each of the ten depths, and we analyzed the lipids in these samples using pyro-GCMS with TMAH or TMSH to methylate and visualize the bulk lipid profile. This technique involves the least amount of sample preparation, having lowered potential contamination, but also limited ability to resolve certain classes of lipids such as hopanoids or those in low abundance. The second set of samples contained ~20g of sediment from three different depths and underwent a total lipid extraction, allowing for the concentration and detection of lower abundance lipids. While I was working on the extractions, we were simultaneously running the smaller samples using pyro-GCMS. Much of the first week involved troubleshooting the instrument. This involved taking apart the pyrolyzer device multiple times and replacing parts, running blanks, checking for minute leaks, etc. Once we got to our samples, we found fairly low signals from using TMSH, and had to switch to using the more powerful TMAH with a larger amount of sediment. As we were going through the samples, we found that there was some carryover between runs, discovering that at some point a sample cup got lodged into the pyrolyzer device and was releasing steady amounts of lipids each time we ran a sample. At this point, it was too late to redo the experiments, as my total lipid extracts were ready. Despite some level of carryover between process blanks and samples, there are still strong lipid signals that will take a decent amount of time to process (Figure 2). I plan on working through this dataset more in the future to determine the lipid distribution within these sediments and how that relates to the evolution of the ecosystem. Once I am able to generate lipid profiles for my isolates, I hope to find connections between the bacterial and sediment lipid profiles as well.

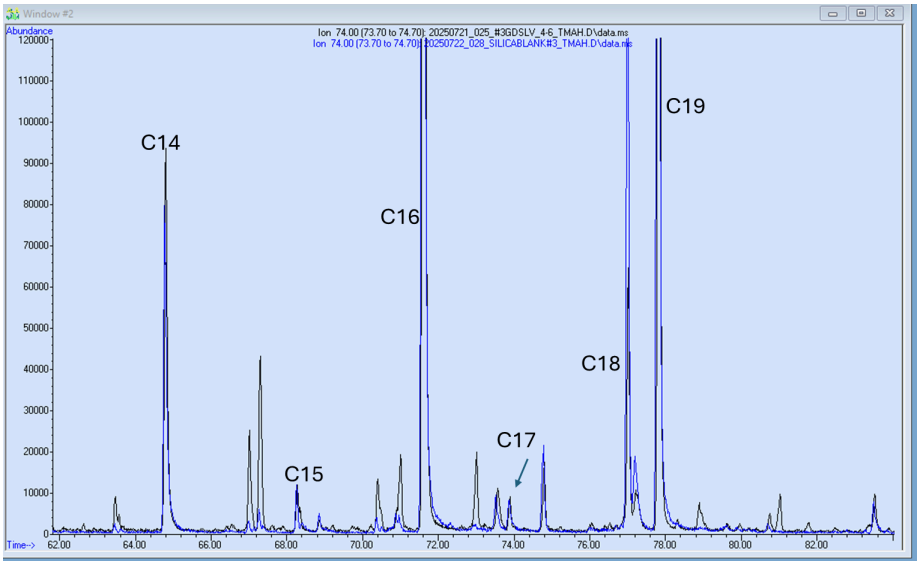

Figure 2: Example select ion chromatogram of DJP sediment sample (black) and process blank (blue), with specific peaks of common fatty acids indicated.

Acknowledgements
I am extremely grateful for the NASA Astrobiology Program for providing me with the opportunity to pursue this project and to work with NASA Scientists. The scientific and personal learning experiences from this award are invaluable to me. Thank you Melissa-Kirven Brooks for your patience with me and coordination of the award, Dr. Eigenbrode for giving me your valuable time and attention, Sarinah Wahl and Dr. Gibbons for walking me through each step of the process, and Dr. Mikucki for your advisorship and valuable samples.