"FOLLOW THE SALT": PATTERNS OF BIOSIGNATURE PRESERVATION AND EVAPOCONCENTRATION IN HYPERSALINE MAGNESIUM BRINES. C.I. Pozarycki[1], K. M. Seaton[1], N. Nuñez, E. Ingall[2], A. Pontefract[3], J. B. Glass[2], P. Doran[4], J. Bowman[5], B. E. Schmidt[6], A. M. Stockton[1], and the OAST team [1]School of Chemistry and Biochemistry, Georgia Institute of Technology; chadpoz@gatech.edu, [2]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, [3]Johns Hopkins University Applied Physics Laboratory, [4]Department of Geology & Geophysics, Louisiana State University, [5]Scripps Institution of Oceanography, [6]Department of Astronomy, Cornell University

Introduction:

The detection of life in our solar system may be facilitated by the evapoconcentration and preservation of organic biosignatures [1,2]. Brines are expected to be ubiquitous on hydrated, rocky solar system bodies, and it is presumed that magnesium ion-containing (Mg^{2+}) brines may exist in the ice crust of Europa and within the ice caps of Mars at low water activities (a_w) [2, 3]. While no location on Earth exactly duplicates the conditions of these extraterrestrial brines, some locations on Earth have conditions analogous to some of those seen elsewhere. This work focuses on analyses of ionically complex brines which evapoconcentrate and preserve biosignature molecules.

Study Sites:

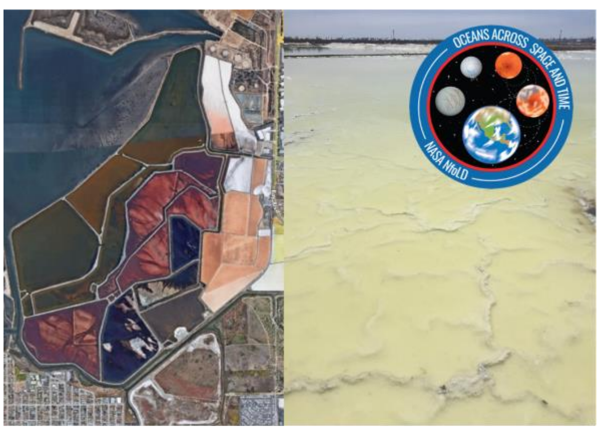

Figure 1: South Bay Salt Works in San Diego and a Mg^{2+} brine pond (Site 4) at this location studied by OAST.

Two areas, the South Bay Salt Works (Fig. 1) and Western Australia's transient lakes were selected by the Oceans Across Space and Time (OAST) consortium, a NASA NAI CAN8 project with a goal of learning "how ocean worlds and their biospheres co-evolve to produce detectable signals of a past or present living world."

The South Bay Salt Works (SBSW): is a salt harvesting facility in San Diego, CA which concentrates ocean water through a series of lochs, evaporating it until it reaches saturation [4]. Each of the eight sites sampled in this work has a complex ionic composition and has been analyzed for amine composition and for ATP concentration. This area was sampled in 2019 and 2020.

The Western Australian Transient Lakes (WATL): are a series of lake systems in southern Western Australia which are known to have a wide range of pH and salinity and include hypersaline, highly acidic lakes [5]. The WATL may be Earth's best analogs for ancient Martian lacustrine environments [6]. A number of these sites have been previously studied or cataloged and preservation of microbes and organic compounds has been shown in WATL halite crystals [5]. Analyses of samples collected by OAST in 2022 are underway.

Analytical Methods:

Ion concentrations were determined via Inductively Coupled Plasma-Mass Spectrometry by ACZ Labs. Water activity was determined via the AquaLab 4TE.

Mg^{2+} is known to inhibit some chemical analyses and damage instruments, often making analyses of these high concentration brines and the organics within challenging or impossible [7]. The methods below were optimized to analyze these highly saline samples.

CE-LIF: Capillary Electrophoresis (CE) separation paired with Laser induced fluorescence (LIF) detection is a high-speed method that uses low volumes of reagent and sample. This non-destructive technique can achieve high resolution separations and detect attomoles of molecules. A capillary zone electrophoresis method developed by Seaton et al. (manuscript submitted and in review) was used here for analyses of amine compounds. SBSW samples of non-turbid liquid brine were separated from salt crystals via centrifugation immediately before labeling and analysis by CE-LIF.

ATP Assay: Adenosine Triphosphate (ATP) is a key energy transferring molecule in terran biology, a potential biosignature molecule, and a marker of microbial activity thanks to its rapid degradation [8]. It is measured using a luciferase assay (Roche ATP Assay Kit) with a luminescence detector (HY-LiTE 2). Filter-extracted and unfiltered liquid ATP were measured on the day of sample collection to account for the rapid degradation of ATP.

Results:

ATP Assay: In 2019 SBSW data, those sites with higher concentrations of Mg^{2+} ions correspond to those samples which have high levels of ATP, nearing saturation, while no ATP was detected in samples with the lowest Mg^{2+} levels (<1 M). The ATP assay fails at Mg^{2+} concentrations >3 M.

CE-LIF for Primary Amines: Samples taken at Sites 4 and 5, brines with Mg^{2+} levels of >4 M, have orders of magnitude higher amino acid concentrations that other sites and have electropherograms that indicate a far higher diversity of amine features. Amino acids are also present in samples from Sites 1, 3, 6, 7, and 8, each with moderate Mg^{2+} levels, and are markedly lower in the lowest Mg^{2+} concentration sample, Site 2.

Discussion:

SBSW ATP Preservation:

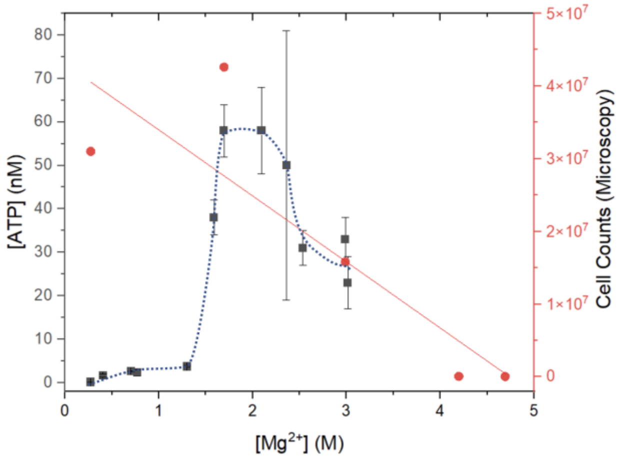

Figure 2: ATP concentration (black squares) and cell counts determined by microscopy (red squares, Klempay et al. 2021 [4]) of samples from SBSW. The red line represents a decrease in cell counts and known cellular activity. The drawn dotted line is intended guide the eye along a general trend in ATP concentration. Error bars on ATP measurements represent heterogeneity in sample triplicates.

Results show a spike in ATP concentration with Mg^{2+} concentration greater than ~1.5 M despite a decline in cell count (Fig. 2). Other analyses by the OAST team support minimal or absent cellular activity in higher concentration Mg^{2+} SBSW samples. This, along with rapid hydrolysis of ATP in hydrated systems suggests preservation of ATP may be occuring in these low a_w brines, since observed ATP increases despite an expected decrease from a lack of active cells. Evapoconcentration may also be playing a role in this trend but cannot explain the persistence of large quantities of ATP in regions with low or no cellular activity. These data support previous observations of ATP preservation in low a_w regions, and the spike in concentration near 2 M supports a hypothesis of osmoregulatory ejection of ATP [9].

SBSW Amine Concentration:

The order of magnitude increase in amino acid concentrations with increases in $[Mg^{2+}]$ (and $[Br^-]$, an evaporite tracer) supports the evaporitic concentration of these biomolecules and also the increased expulsion of these compounds as an osmoregulatory response. The osmoregulatory expulsion of amino acids and other primary amine osmolytes may explain the increased diversity of unidentified peaks in the highest $[Mg^{2+}]$ samples (Fig. 3).

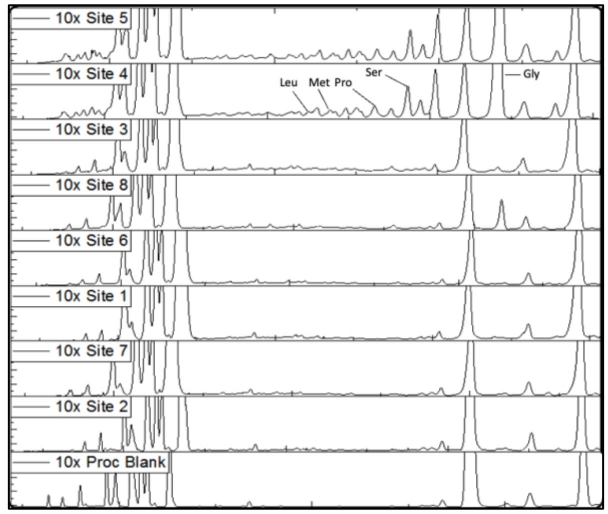

Figure 3: 2019 SBSW electropherograms sorted from lowest $[Mg^{2+}]$ (Site 2) to highest (Site 5) including a procedural blank. Selected amino acids are labeled.

Conclusions:

We show that low a_w, predominantly Mg^{2+} brines appear to preserve short-lived biosignature molecules even in brines with a_w below the known limit to life. Evidence supports that these regions may also concentrate amino acids and amine osmolytes. This is promising for the biosignature potential of evaporitic systems and could indicate that a “follow the salt” approach is warranted in the search for preserved biosignatures.

Analytical techniques which can overcome high Mg^{2+} concentrations, such as CE-LIF, may be required for the analysis of salt rich samples from Earth systems and in the search for life in our solar system. The employment of salt-tolerant analytical methods for biosignature detection in spaceflight may be crucial. We encourage further study of Mg^{2+} brine ponds and other low a_w systems using salt-tolerant analytical techniques.

Acknowledgments:

This work was funded as part of the Oceans Across Space and Time program of the NASA Astrobiology program, grant 80NSSC22K1409, PI B.E. Schmidt. More information about OAST and its investigators can be found at: https://oast.eas.gatech.edu/ and additional funding from the NASA FINESST award to K.M.S.

References:

[1] Mathies R.A. (2022) Astrobiology, Vol 17, Issue 9 [2] Buffo J.J. (2022) Astrobiology, Vol 22, Issue 8. [3] Reghunadhan Nair C. P. and Unnikrishnan V. (2020) ACS Omega, 5, 9391-9397. [4] Klempay B. (2021) Environ. Microb. 23(7); 3825-3839 [5] Benison K. C. and Bowen B. B. (2006) Icarus, 183, 225-229 [6] Conner A.J. and Benison K. C. (2013) Astrobiology, Vol 13, Issue 9 [7] Ohta Y. (2014) Mass Spec., Vol. 3, A0031 [8] Parnell J. (2007) Astrobiology, Vol 7, 578–604 [9] Tuovila B.J. (1987) Appl & Env. Microb., Vol 53, No. 12; 2749-2753