National Aeronautics and Space Administration

Exploring abiotic constraints on microbial habitability in subsurface hypersaline brines

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Strategic Focus Area: Habitable Worlds

I. Introduction and Motivation

The terrestrial subsurface boasts substantial heterogeneity in its environmental abiotic composition which is perhaps best exemplified by deep fracture fluid environments of Precambrian Shields.

- Microbial adaptations to high salinity and a biofilm vs. planktonic lifestyle would always form and operate "faster" than changing geochemical conditions, and could provide ample adaptation strategies to climate, mineralogical, and salinity changes from late Noachian to modern Mars subsurface aquafers.
- Microbial-fluidic and later microbial-mineral interfaces could show evidence of these adaptations over geologic time and we have spatially detected low-biomass microorganism preservation in brines and solid evaporite minerals. Due to the low biomass settings these detections, when possible, bode well for understanding the preservation potential of Mars analogue mineralogy **(Fig. 1)**, specific lithologies in former aqueous settings, and extant life measurements in saline brines.
- Our project will help constrain microbial habitability in Moab Khtosong brines and rock substrate, by (1) evaluating the biogeochemical support of the abiotic environment and (2) investigating community metabolic networks under these conditions.

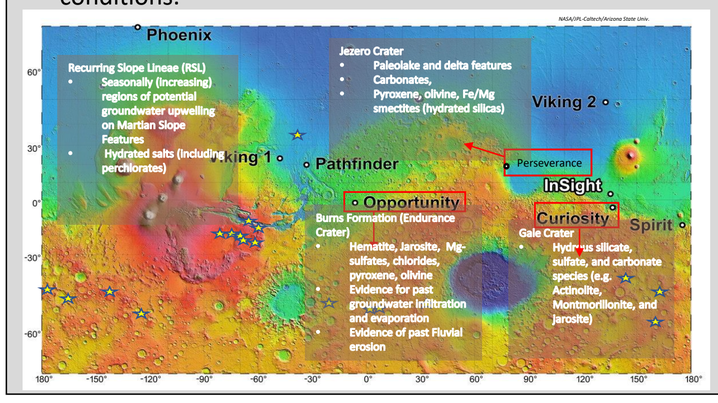


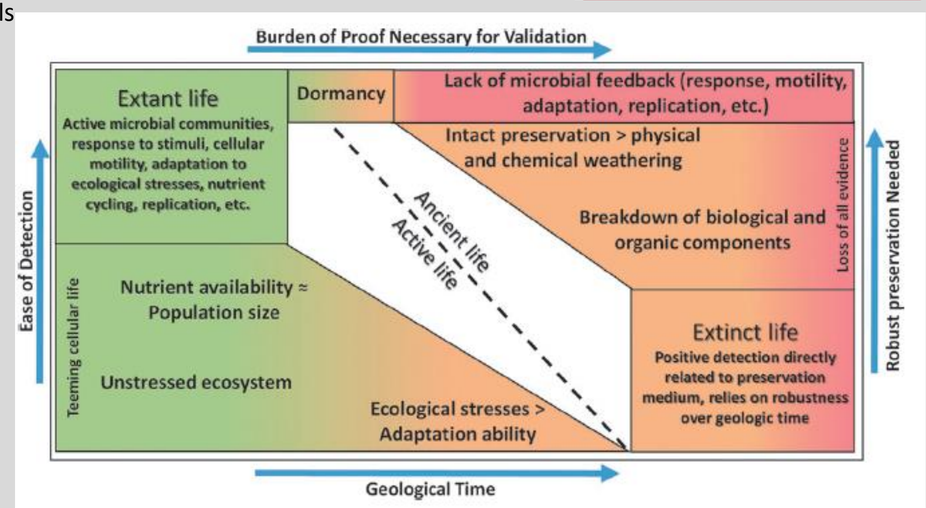
Fig. 1. Habitable ancient lacustrine environments on Mars. Features accessible on the Martian surface that are a product of groundwater upwelling allow us to model microbial maintenance energies that biology requires for sustainable inhabitation within subsurface brines.

II. Objectives and Approach: Measurements of Extant Biology and Biosignature Gradients

Biosignature detection for ancient/extinct signs of life relies on the preservation medium whereas extant/active life requires an agnostic approach to withstand the high burden of proof for biological validation **(Fig. 2, Bottom)**.

- Spatial detections of microorganism preservation in brines and solid evaporite minerals has been observed. Due to the low biomass settings these detections, when possible, bode well for understanding the preservation potential of Mars analogue mineralogy, specific lithologies in former aqueous settings, and extant life measurements in saline brines.
- Our unique microbiology instrumentation to increase and test the quantity of microbial communities within in-situ brines to determine microorganism community features, biogenic signatures, abiotic features that can be falsely determine as a positive read for life.
- For positive enrichments cellular abundances will be estimated, cells will be imaged microscopically, DNA/RNA will be extracted from positive enrichments for taxonomy, and expression activity will be evaluated via fluorescent molecular probing and transcriptomic analysis. For positive enrichments of the rock amended media, the rock surfaces **(Fig. 2, Top)** will be imaged with fluorescent stains and SEM to determine the distribution of attached cells and how they relate to the underlying mineral substrate.

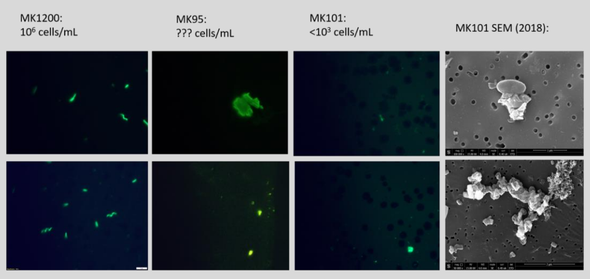
Fig. 2 (Top). Images from the first field campaign in 2018-2019. The MK95 borehole was the first to be accessed and is the focus of this investigation with MK101 acting as a 2nd authigenic series and MK1200 as our positive control. **(Bottom)** Geobiological distinction between extinct and extant life within microbial brine systems and evaporite salt minerals. Can be applied to all lacustrine systems depending on lithology, a_w, geochemistry, organic content, and mineral kinetics.



III. Implications for Life Detection Measurements in Low Biomass Brine Settings

Multiple measurements for biogenicity are critical in any biosignature and biological regime (extinct and extant, respectively) however a combination of agnostic approaches alongside microbial techniques has allowed us to confine cell counts per borehole.

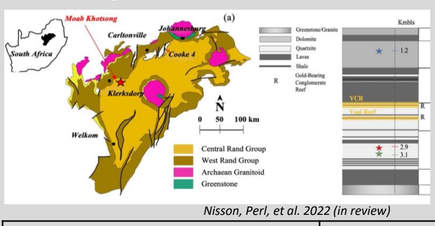
- Ongoing work is using enhanced fluid techniques to increase our biological yield such that 16S rRNA gene sequencing can be completed. Following this, our plans are to measure the functional genes present in these brine systems to match the geological, fluvial, and geobiological values **(Figs. 3,4)**.



Very Low Biomass in Brine Systems Relative to Meteoric Fracture Fluid

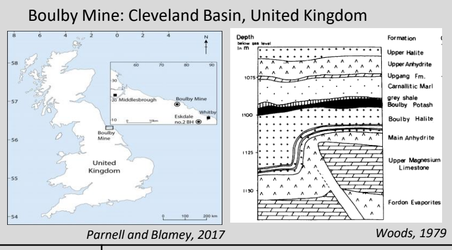
Figs 3,4. (Top) Microbial Cell Staining. Varied cell concentrations within natural/in-situ brines clearly show cellular communities among hypersaline non-biological features. **(Bottom) Mars Analog Field Site Comparisons.** Permian reference brine sites show higher cell concentrations with two different evolutionary paths due to brine formation differences and surface residence times.

Moab Khotsong Mine: Witwatersrand Basin, South Africa



Nisson, Perl, et al. 2022 (in review)

Boulby Mine: Cleveland Basin, United Kingdom



Parnell and Blamey, 2017 *Woods, 1979*

	Moab Khotsong Brine	*Boulby Brines*
Depth Sampled (mbls)	3,100-3,200	800 – 1,300
Total Dissolved Solutes (g/L)	215 – 246	260 – 306
Water Activity (a_w)	0.839	0.566 – 0.742
Brine Formation Mechanism	Radiolysis and Silicate Hydration	Evaporite Dissolution
Subsurface Residence Time (Ma)	~1200	Permian-Triassic Age (~300-200)
Cell Counts (cells/mL)	~10^2-10^4	~10^{4-7} (surface driven); ~10^2-10^3 (artisan depths)

Nisson, Perl, et al. 2022 (in review) *Bottrell et al., 1996; Payler et al., 2019; Perl et al. (in-prep)*

IV. Moving from Life Detection to Future Investigations for Biological Validation

Future work and spinoff investigations will focus on microbial maintenance energy as a chemical biomarker and habitability index assessment **(Figs 5,6)**. We will model microbial maintenance/ metabolic potential as it (1) might exist at different depths or (2) through time and at different subsurface depths. Based on our laboratory experiments we will utilize one of two models of reactive transport or no transport.

- Taking the Martian Recurring Slope Lineae (RSL) into account we can understand the fluid kinetics and subsurface-to-surface groundwater transport
- Model the features of water aquifers as they might exist at different depths in the current day Martian subsurface under these craters
- Reactive transport flow where we start with surface water (in the Noachian, etc.) and model it moving to depth in the Martian crust. This can occur over millions of years depending on reactivity rates

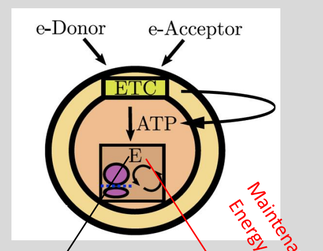


Fig. 5. Microbial Maintenance Energies as a Biomarker. Understanding the distribution of physiochemical and geochemical parameters in potential Martian subsurface fluid environments can help us constrain where microbial life might be, and what metabolic types we expect to find

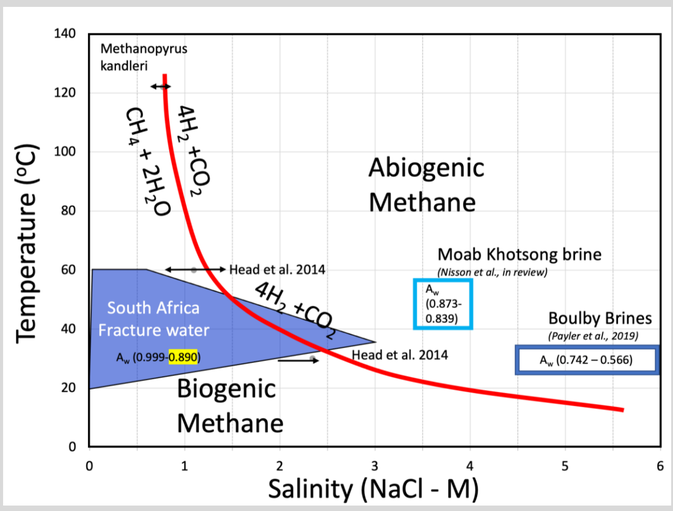


Fig. 6. Relationships between Salinity and T for Biogenic Methane. The associations between water activity, brine kinetics, salinity, and temperature are critical for understanding biogenic fluxes for metabolic processes.

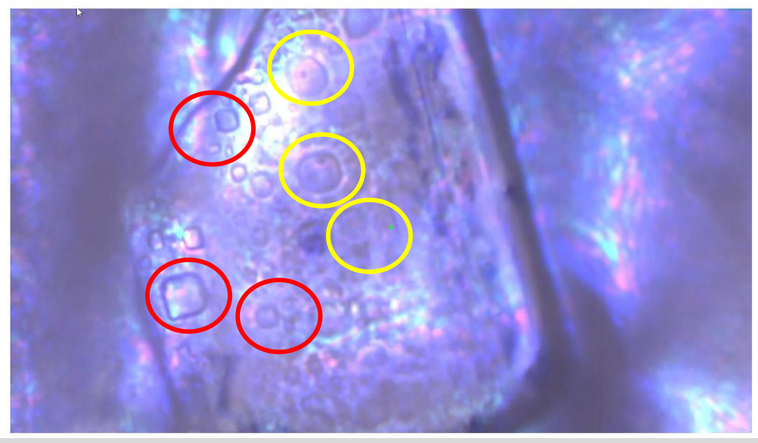


Fig. 7. Single cell Preservation per (3x) Fluid Inclusions. A significant milestone of this investigation has been the Raman-validated and spatially-confirmed presence of single cells per entombed fluids. Perl et al. (2020; 2023) has shown this type of preservation on the order of 10^3 – 10^8 in the Great Salt Lake, however this is one of the first occurrences of single cells

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Publications:

- Nisson, D.M., Kieft, T. L., Drake, H., Warr, O., Sherwood Lollar, B., Ogosawara, H., Perl, S.M., Friefeld, B.M., Onstott, T.C., (submitted) Hydrogeochemical and Isotopic Signatures Elucidate Deep Subsurface Hypersaline Brine Formation through Radiolysis Driven Water-Rock Interaction
- Perl, S.M. Nisson, D.M., Onstott, T.C. (in-prep) Detections of Cellular Material in Low Biomass Mars Analog Brine Systems
- Nisson, D. Kieft, T.L., Hernandez, J.C., Perl, S.M., Stepanauskas, R., Warr, O., Lollar, B.S., Yokochi, R., Chmiel, G., Caffee, M., Liebenberg, B., Onstott, T.C. (2021) Influence of Alpha Particle Radiolysis on the Formation and Microbial Metabolic Composition of a Deep Subsurface Hypersaline Brine in the Witwatersrand Basin, South Africa. AGU Fall Meeting 2021
- Perl, S.M., Nisson, D. M., Onstott, T.C. (2022) Detections and Validations of Single Cell Microorganism Preservation and Associated Biosignature Activity. Astrobiology Science Conference 2022, Atlanta, GA. in Detecting Life and Habitable Environments on Mars: A Synthesis of Applied, Analog, Experimental and Theoretical Approaches I Oral. Abstract #203-02

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