UNDERSTANDING HABITABILITY AND BIOSIGNATURE PRESERVATION IN A HYPERSALINE MARS ANALOG ENIVRONMENT: LESSONS FROM SPOTTED LAKE. A. Pontefract[1,2], J. Hachey[1], A. Mojarro[1,2], V. K. Walker[3], H. Rowedder[2], T. F. Zhu[4], C. Lui[1], M. T. Zuber[1], G. Ruvkun[2], and C. E. Carr[1,2]. [1]Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139 (apontefr@mit.edu), [2]Deparment of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, [3]Department of Biology, Queens University, Kingston, Ontario, Canada K7L 3N6, [4]School of Life Sciences, Tsinghua University, Beijing 100084, China.

Introduction: The transition of Mars from wet to dry during the Hesperian (~3.7 Ga), resulted in widespread deposition of sulfate and some chloride salts that are observed on the surface today [1,2]. Moreover, the discovery of polyhydrated sulfates on Mars, suh as those found inside Columbus Crater in Terra Sirenum [3], has provided compelling evidence for the presence of paleolakes: some of which may represent remnants of evaporitic brine pools. On Earth, hypersaline lakes impose severe osmotic stress on microorganisms, including chaotrophic effects due to high levels of membrane destabilizing cations (Mg^{2+}, Ca^{2+}) [4]. Yet, these environments host an astonishing diversity of microorganisms and are capable of preserving evidence of life [5]. For example, amino acids can be preserved for 4-40 Ma in salt crystals [6], and ATP (adenosine triphosphate) can remain "indefinitely" unhydrolyzed in hypersaline settings [7]. If life existed on Mars, it is feasible that brine pools could have hosted a wide array of microorganisms, and under increasingly dehydrating conditions, salts may have provided a last refuge for surface or near-surface organisms [5].

Here we provide a first look at the subsurface microbial community of Spotted Lake, a sulfate-rich hypersaline lake. Biosignatures of this environment, including sulfur isotope fractionation, may prove useful to future life detection missions on Mars.

Field Site: Spotted Lake (Fig. 1) lies within a closed basin approximately 10 km northwest of Osoyoos, BC (N49.078018˚, W119.567502˚). In spring the lake is one connected body of water, but in summer much of this water evaporates, giving the lake its characteristic "spotted" appearance. The salinity of the lake varies, but can reach upwards of 37%, with a pH ranging from 8.0-8.3. The local geology causes a large influx of dissolved Mg^{2+}, Na^{+}, and SO_4^{2-} into Spotted Lake resulting in a diverse suite of evaporite minerals that have been studied since the early 1900's [e.g., 8], due to transient commercial interest, and more recently, because of the similarity to the deposits within Columbus Crater on Mars [9]. This latter study revealed two phases of mineral formation (evaporation and freezing), the combination of which created a mineral assemblage largely dominated by bloëdite and epsomite, but also including mirabilite, gypsum and halite (as a result of freezing processes).

Fig. 1. *(A) Spotted Lake (black arrow) is located in the rain shield of the Cascade and Coast ranges at the southern part of the Thompson Plateau (red line), British Columbia, Canada. (B) Brine pools at the time of sampling. Photo: V. K. Walker.*

Methods and Results: *Physiochemistry* – Salinity (371 g/L), hardness (212 g/L), and major ions (2.1M Mg^{2+}, 1.8M Na^{2+}, and 2.8M SO_4^{2-}) were consistent with prior studies. An oxygen meter was not available at the time of sampling, but the presence of obligate anaerobes in all sediment samples allowed us to infer the presence of an anoxic subsurface environment.

Geochemistry – Soil samples were collected for ICP-MS (Inductively Coupled Plasma – Mass Spectrometry) and XRD (X-Ray Diffraction). Soil chemistry was found to be similar to that of surface mineralogy conducted by Cannon et al. [9]; we detected the presence of gypsum, bloëdite, and epsomite. In addition, hexahydrite was present in all samples, and some samples contained the clay phyrophillite.

Sulfur Isotope Analysis – Sulfur isotope analysis ($\delta^{34}S$) was conducted on soil and water column samples, and analyzed by the IsoLab, University of Washington. Sulfate was extracted from the water and soil samples, and sulfide extracted from the gas phase (trapped within the soils) and from the solid phase. Results showed a large negative fractionation between the sulfate and sulfide, indicating strong microbial processing of sulfur in the system (Fig. 2).

Metagenomics – Soil samples were collected from four brine pools in the lake. To complement traditional short read sequencing from all samples (MoBio PowerSoil extraction, Ion Torrent PGM sequencing, libraries S1-S4, 583k reads, 96 Mb, average length 165 bp), we performed single molecule sequencing on one sample (M2). Genomic DNA was extracted using phenol

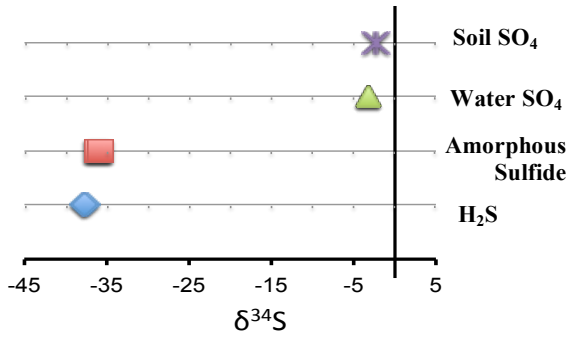

Fig. 2. *Plot of sulfur isotope data from the water column and soil showing large fractionation values between the sulfates (top) and sulfides (bottom).*

chloroform isoamyl (25:24:1) with only vortexing to limit DNA shearing, was purified using paramagnetic beads, and analysed for read length (BioAnalyzer, Agilent). Sequencing (library M2) was performed using a MinION Mk-1b from Oxford Nanopore Technologies (ONT; R9 flowcell, SQK-NSK007 kit). Over 5 flow cells, of 325,731 reads, 25,383 passed (Q>9), likely reflecting DNA damage, with 72.4 Mb of average length 2813 bp.

Taxonomy was assigned using inexact k-mer matching at the protein level (Kaiju; db: nr+euk). A much higher fraction (70%) of single molecule reads could be classified in comparison to short reads (37%), despite the higher error rate. The community was dominated by Bacteria (Fig. 3A), and at the Phylum level (Fig. 3B) comprised largely of organisms from the FCB group (Bacteroidetes), Proteobacteria (Fig 3C), and Terrabacteria (dominated by Firmicutes). Also observed were sulfate reducers (Desulfovibrio) and a small contribution (~7%) from the Archaea (Halobacteria).

Discussion:. The community of Spotted Lake is highly reflective of the geochemical setting, where anaerobic organisms dominate. The smaller number of

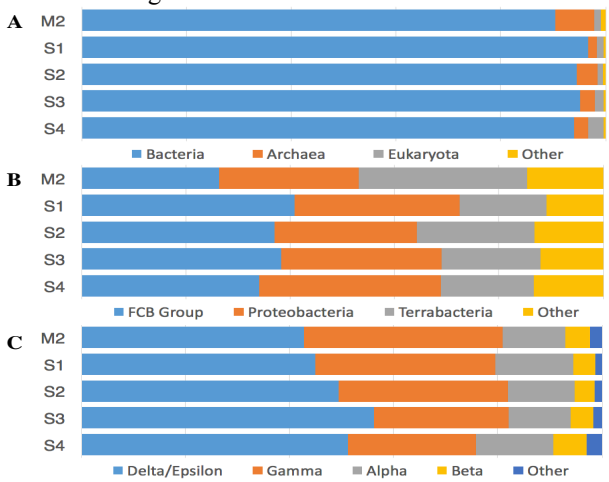

Fig. 3. *Taxonomic abundances estimated using Kaiju protein-space comparisons at the level of (A) Domain, (B) Bacterial Phyla, (C) Within the Proteobacteria.*

Archaea observed was surprising, though since the species discovered were aerobic, it is likely that they live primarily in the water column or the salt layer, which was not included in this first study, and the DNA sequenced was detrital. In comparing the metagenome from Spotted Lake against those from other studies, we found that the community clustered well with other halotolerant communities, and had metagenomic similarities with that of Ace Lake, Antarctica.

The presence of sulfate reducers in the system, along with the blackened color the mud and smell of rotten eggs (hydrogen sulfide) pointed to a biomarker that may be well preserved in this environment. The soil and gas phase sulfides were depleted in $\delta^{34}S$ by ~34‰, showing a strong microbial fractionation effect. The strongly negative value also indicates slow metabolic processes, where the highest rates of sulfur isotope fractionation occur at the lower limits of metabolic rate, within conditions of high sulfate. This causes the kinetic isotopic effect to become more pronounced [10, 11], and provides a very strong signature of life in the rock record.

Conclusions: Salt is an excellent medium for preserving organic molecules [6,7] and potentially serves as a genetic reservoir [12]. Moreover, sulfidic-salt systems can provide a robust biosignature over geologic time. Given this, Spotted Lake provides an excellent analog site for future life detection experiments, and may help to provide a better understanding of past and/or present life on Mars.

Acknowledgements: We thank the Okanagan Nation Alliance (ONA) and the Osoyoos band office for access to Kɬ'lilwɯ (Spotted Lake), which is considered sacred to the ONA. Thank you to Shuhei Ono for providing lab facilities, as well as valuable insight on methodology and interpretation. Financial support provided by NASA ASTID (NNX08AX15G) and MatISSE (NNX15AF85G).

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