SNOW ALGAE CONSORTIA AS INDICATORS OF POTENTIAL HABITABILITY C. M. Phillips-Lander[1], Z. R. Harrold[1,2], A. Sanchez[1], A. Garcia[1] P. Sbraccia[1], J. Raymond[3], and E. M. Hausrath[1] [1]Department of Geoscience, University of Nevada-Las Vegas, Las Vegas, NV, USA, (charity.lander@unlv.edu) [2]Desert Research Institute, Reno, NV, USA [3]School of Life Sciences, University of Nevada-Las Vegas, Las Vegas NV, USA

Introduction: Life present in icy and snowy Earth environments suggests icy planetary bodies like Europa and Enceladus and snowy or glaciated regions on rocky planetary bodies like Mars may be habitable [1-5]. Ice on Earth [6,7] and Enceladus [8] contains a mixture of ice and organics, and Earth and Mars' polar regions are rich in Fe-minerals [9,10]. Moreover, snow/ice grain sizes in these planetary environments (0.1-5 mm) [11-13] are similar to glacial surfaces on Earth (0.1- 2.5 mm) [14] where snow algae blooms occur. However, life in these environments faces significant challenges including high UV fluxes, low temperatures, and liquid water and nutrient (including Fe and P) limitations.

Snow algae can form very high concentration (10^6 cells ml^{-1}) blooms. While some blooms cover vast areas of snowy surfaces, other blooms are patchy, suggesting not all snow/ice is equally habitable. Snow algae may be nutrient-limited in some environments [15-17]; however the delivery of dust to snow/ice surfaces likely reduces nutrient limitation [18]. Previous work in our laboratory [19, 20] demonstrated snow algae co-cultures colonize mineral surfaces and enhance mineral dissolution under Fe-limited conditions. Because Earth-based lifeforms can help inform our understanding of habitability, we use snow algae as an indicator of how changes in availability of mineral surfaces, nutrient (Fe, P) availability, snow grain size, and light availability influence the habitability of snow/ice environments.

Methods: We conducted three sets of experiments to evaluate the following habitability questions: (1) Do snow algae co-cultures require contact with mineral surfaces to acquire limiting nutrients? (2) Can snow algae co-cultures use phosphate in basaltic glasses to supplement P-limited conditions? and (3) How does snow/ice grain size influence habitability?

Chloromonas brevispina cells were collected and isolated by Ronald Hoham from Lac Laflamme, Quebec, Canada [21] and deposited in the Culture Collection of Algae, University of Texas, Austin (UTEX). This strain (B SNO96) hosts a number of bacteria and is accordingly called a co-culture. Cultures were maintained on M1 growth medium [22].

Algae cell counts were measured by direct counts of formaldehyde-fixed samples collected during experiments. These samples were re-suspended by vortexing and 10 µL aliquots were loaded into disposable hemocytometer chambers (Incyto C-Chip, Neubauer Improved, Model # DHC-N01). Cells were counted at 45× magnification (Olympus BH microscope). Algae cell concentration (cell mL^{-1}) was calculated as:

$$C_{algae} = \frac{N \times D}{n \times V_{grid}}$$

where n is the number of grid blocks counted, N is the total cell count in n blocks, D is the dilution factor due to formaldehyde fixation, and V_{grid} is the volume of grid used for enumeration.

Dialysis Experiments Fe-limited and M1 full nutrient media were autoclave sterilized, chilled to 4°C, and supplemented with filter-sterilized vitamin solution. For Fe-limited media, the Fe-EDTA solution was omitted and the standard trace metal solution was replaced with a Fe-free trace element solution. Initial pHs of the media were ~5.3.

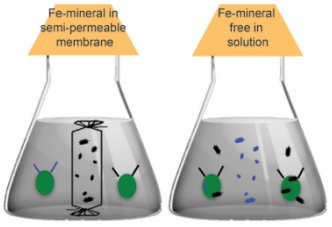

Figure 1: Minerals were incubated either in dialysis tubing (left), a semi-permeable membrane, or freely (right) in the Fe-limited media.

Andradite ($Ca_{3.0}(Fe_{0.6},Al_{0.4})_2(SiO_4)_3$) from Garnet Hill, CA and San Carlos olivine ($Mg_{0.9}Fe_{0.1}SiO_4$) from Alfa Aesar were crushed to a 35-75 µm size fraction, sonicated for 1 min. three times in 100% ethanol and air-dried. We separated Fe-minerals from snow algae consortia using dialysis tubing in half of the experimental trials (Figure 1).

Phosphate-Limitation Experiments P-limited and M1 media were sterilized, chilled to 4°C, and supplemented with filter-sterilized Fe-EDTA and vitamin solutions. Initial pHs of the media were ~5.5.

We crushed Stapafjell basaltic glass (0.22 wt% P) [23] and McKinney basaltic glass (0.75 wt% P) [24] to a 75-150 µm size fraction, sonicated for 1 min. three times in 100% ethanol and air-dried in a sterile petri dish. To each P-limited batch reactor we aseptically added 0.5 g of basalt.

Snow Grain Sizes Quartz beads (end member grain sizes of 0.25 and 2.0 mm) were used as proxies for snow grains in batch reactors. The beads and 400 ml reactors were acid washed and autoclaved. The beads were aseptically added to the 50 ml mark in the reactor. Then 2.5 ml of snow algae co-cultures were aseptically added to the base of the beads. More beads were slowly added to the 300 ml line, followed by addition of 250 ml of M1. The beakers were covered with transparent, sterile petri lids. The sides and bottom of the beakers were wrapped in black paper and aluminum foil to ensure that light was only available at the top of the beaker. Beakers were incubated at 4°C for 45

days. Snow algae growth was measured at 48 h intervals.

Results and Discussion: *Snow algae grow better in contact with mineral surfaces under Fe-limited conditions.* Snow algae in contact with forsterite surfaces had 1.3x higher cell counts than snow algae separated from forsterite by dialysis tubing (Figure 2). Snow algae cell counts were similar (~4.92x10^5 cells ml^{-1}) in full nutrient media and in Fe-limited medium with andradite with and without dialysis tubing.

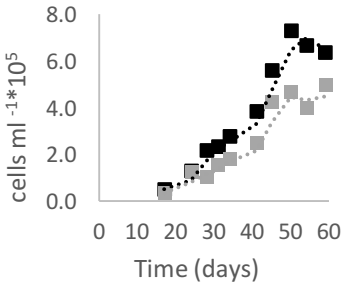

Figure 2: Snow algae growth in forsterite experiments with (■) and without (■) dialysis tubing.

P-availability has profound effects on habitability. Snow algae cultures grown under P-limited conditions changed color from green to orange (Figure 3). Leya et al. [25] showed algae experience chlorosis in response to N-limitation, which can influence the cell pigmentation (i.e. color). Cultures supplemented with Stapafjell basalt glass had 5x higher cell counts than those supplemented with McKinney Basalt glass, likely because the latter contains some feldspar, making it more crystalline, which reduces dissolution rates [24]. This indicates dissolution rates profoundly influence nutrient availability and habitability.

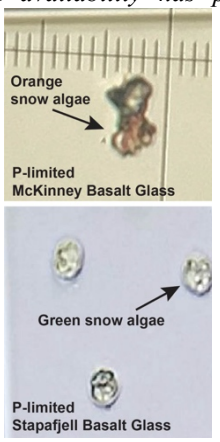

Figure 2: Snow algae change color from green to orange under P-limited conditions with McKinney basalt glass.

Grain size influences the time required for snow algae blooms to form. Low temperatures and the presence of the beads limit the rate of mixing and diffusion of snow algae in solution. Our experiments show snow algae swim upward through the beaker with time, to form blooms at the surface, similar to field-based blooms in which growth is linked to light and nutrient availability [18]. Snow algae blooms formed in both large (2 mm) and small (0.25 mm) bead experiments. Blooms formed 7 days faster in the large beads, probably because of their larger porosity, permeability, and light flux (550 v. 280 lux). Additional experiments are required to differentiate these variables.

Implications for Habitability Constraints on Icy Planetary Environments: Light, temperature and nutrient availability are key factors influencing the distribution of habitable extraterrestrial snow environments. The similarity in grain size between bead experiments and planetary ices makes these experiments ideal places to examine the interplay of these factors in a controlled setting and examine the habitability of icy environments. These factors also have the potential to influence biosignature formation if habitable ice environments are colonized. Future work will examine how these factors interact to control the distribution of snow algae blooms.

References: [1] Sagan & Lederberg, 1976, *Icarus*, 28, 291-300. [2] Rothschild, 1990, *Icarus*, 88, 246-260. [3] Chyba & Phillips, 2001, *PNAS*, 98, 801-804. [4] Cockell & Raven, 2004, *Icarus*, 169, 300-31. [5] McKay et al., 2008, *Astrobio.*, 8, 909-919. [6]Reynolds et al., 2010, *Aeolian Rsh*, 15, 73-90. [7]Yallop et al., 2012, *ISME J.*, 6, 2302-13. [8] Steel et al., 2017, *Astrobio.*, 17, 862-875.[9] Horgan and Bell, 2012, *Geology*, 40, 391-394. [10] Phillips-Lander et al., 2015, *GSA Annual Mtg*. [11] Cassidy et al., 2013, *Planet. & Space Sci*., 77, 64-73. [12]Brown et al., 2006, *Science*, 311, 1425-1428. [13] Kieffer, 1990, *JGR Solid Earth*, 95, 1481-1493. [14] Singh et al., 2011, *Encyclopedia of Snow, Ice, and Glaciers*. [15] Hamilton & Havig, 2017, *Geobio. J.*, 15, 280-295. [16] Tazaki et al., 1994, *Clay and Clay Min.*, 42, 402-408. [17] Lutz-Meindl, U., and Lutz, C. (2006) Micron, 37, 452-458. [18] Hoham, R. (2000) In J. Seckbach, Ed. Journey to diverse microbial worlds: Adapt. to exotic environs. [19] Harrold et al., in review, *Appl. Env. Micro*. [20] Phillips-Lander et al., 2018, LPSC Biosignature abstract [21] Austin UoTa, 2017, https://utex.org/products/utex-b-sno-0096. [22] Hoham et al., 1979, *Phycologia*, 18, 55-70.[23] Hausrath and Brantley, 2010, *JGR Planets* 115, E12. [24] Phillips-Lander et al., 2017, *LPSC Abs. #1667*. [25] Leya et al., 2009, *FEMS Microb. Ecol*. 67,432-443.

Acknowledgements: NASA grant # NNX14AN24A funded this work. We thank Toluwalope Bamisile for her assistance in the laboratory, and appreciate helpful feedback from Arya Udry.

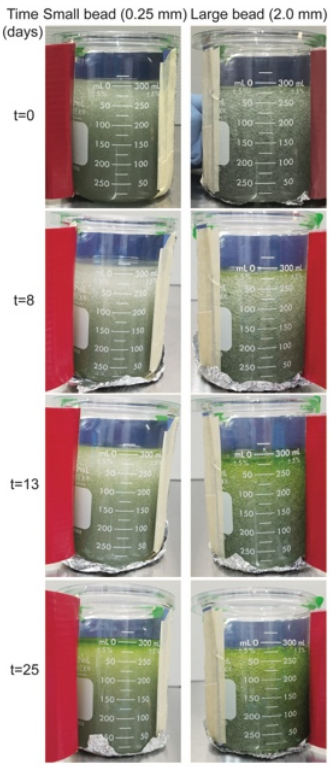

Figure 3: Snow algae blooms form faster in 2.0 mm than 0.25 mm grain size experiments. Growth is evident by day 7 in 2 mm and by day 13 in 0.25 mm experiments, with blooms evident 5-8 days later.