

**ORGANIC BIOSIGNATURES AND HABITAT FEATURES OF NEAR-SURFACE GLACIAL ICE IN SVALBARD.** J. L. Eigenbrode<sup>1</sup>, L. G. Benning<sup>2</sup>, D. J. Tobler<sup>1,2</sup>, J.-D. Rodriguez Blanco<sup>2</sup>, M. L. Fogel<sup>3</sup>, M. Callahan<sup>1</sup>, J. Dworkin<sup>1</sup>, M. Glamoclija<sup>3</sup>, D. Glavin<sup>1</sup>, L. Kerr<sup>3</sup>, A. Kish<sup>3</sup>, P. Mahaffy<sup>1</sup>, A. McAdam<sup>1</sup>, A. Steele<sup>3</sup>, M. Voytek<sup>4</sup>, H. E. F. Amundsen<sup>5</sup>, and the AMASE 2008/09 teams, <sup>1</sup>Solar System Exploration Division and the Goddard Center for Astrobiology, NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA; Jennifer.Eigenbrode@nasa.gov, <sup>2</sup>Earth and Biosphere Institute, School of Earth and Environment, University of Leeds, Leeds, LS29JT, UK. <sup>3</sup>Geophysical Laboratory, Carnegie Institution of Washington, Washington, DC 20015, USA, <sup>4</sup>Science Mission Directorate, NASA Headquarters, Washington, DC 20546, USA, <sup>5</sup>Earth and Planetary Exploration Services, Jac Aals Gt 44b, 0364 Oslo, Norway

**Introduction.** Glacial ice is characterized by low water activities, chemically dilute conditions, and low temperatures, which are all factors that dramatically slow chemical reactions (i.e., mineral dissolution, hydrolysis and oxidation of organic molecules). These conditions, combined with the presence of external nutrient sources (e.g., dust, rock debris, etc.) nevertheless allow ice-dwelling life to persist in such environments. These conditions also enable the long-term preservation of biomolecules and other organics trapped within the ice. As an extraterrestrial analog to water-ice environments, such as the polar regions of Mars, glacial ice may not only be a harbor for extant life, but may also be a repository for aeolian materials containing molecular evidence of past life, impacts, or other planetary processes. As such, modern terrestrial glacial ice environments are key field study sites to distinguish allochthonous (i.e., formed in the ice) from autochthonous (i.e., of foreign sources) organic records. To this end, the Signatures of Life in Ice (SLIce) project aims to detect and decipher organic biosignatures in near-surface (0-1.25 m depth) glacial ice and to develop sampling strategies to search for biosignatures in extraterrestrial icy environments (e.g., polar regions of Mars, Europa, Titan, Enceladus).

**Sampling.** During the 2008 and 2009 field seasons of the Arctic Mars Analog Svalbard Expedition (AMASE), six sets of ice cores were collected and processed on five glaciers that differed in glacial type, local/regional aeolian and rock sources, hydrology, and geography. Ice cores consisted of blue ice cores, reworked surface ice, and firn (frozen compacted snow). At each site 5 cores were collected, 4 of which were sliced into sections while the 5<sup>th</sup> one was preserved for trace metal analyses. In addition, on one of the glaciers a duplicate set was collected just above a prior coring site. This duplicate set served as a duplicate for *in situ* biological tests and provided additional material for lipid and hydrocarbon analyses. Finally, at each site a suite of environmental samples (clean snow, snow algae, cryoconites, surface runoff, subglacial runoff and sediments) were collected to determine likely inputs into the glacial ice cores.

**Analyses.** For each core and environmental sample site a set of biomolecular tests for (a) adenosine-5'-

triphosphate (ATP; a marker for metabolic activity), (b) gram-negative bacterial lipopolysaccharides (LPS; used as a proxy for total bacterial loads), (c) DNA extraction/PCR and (d) live/dead cell counts were conducted immediately upon melting and filtering of the ice in the field laboratory (i.e., within ~48 hours) on both the cores and the melted and filtered materials. In addition, in all cases dissolved ammonia was also immediately analyzed in each sample. Sub-samples for other dissolved nutrients, trace metals, molecular analyses of lipids, hydrocarbons, and amino acids as well as sub-samples for high-resolution imaging and mineralogy were collected. These analyses are being conducted in various SLIce related institutional laboratories.

**Minimizing and tracking of procedural contaminants introduced during sampling, handling and analysis.** Tracking the addition of compounds from sampling, handling, and analysis procedures (i.e., procedural contaminants) is critical for establishing confidence in all astrobiology studies (on Earth and in space) that involve molecular detection (Eigenbrode *et al.*, 2009). On Earth, where biology is ubiquitous, lower detection limits are used to establish acceptable background values, particularly in the field. The SLIce team made an exceptional effort to limit and monitor for procedural contaminants that may alter the molecular signatures of interest. To do this, an established cleaning protocol (modified version of Eigenbrode, *et al.*, 2009) was applied. *In situ* tests for ATP and LPS concentrations on surfaces of the coring equipment before sampling were near detection limits and generally higher than ice surfaces. Thus, we deemed our cleaning technique efficient at removing most biological material (and presumably, other organic molecules) from our equipment. However, our measurements also revealed that the glacier ice core tops had, in most cases, noticeably greater ATP and LPS concentrations than deeper core sections indicating the presence of glacier surface biosignatures (see below). Finally, our measurements also revealed that biomolecules from the ice transferred to the coring equipment demonstrating that forward contamination is a major issue for terrestrial ice studies, and thus of similar significance for extraterrestrial studies, unless

for extraterrestrial studies, unless kept in check by standard control and monitoring procedures.

**Results.** Live and dead cells were observed on filters and in most cases again the top sections of the cores showed a higher abundance of live materials and an increased contribution from eukaryotic cells. In most cases prokaryotic cells were clustered and associated with mineral grains, which most likely acted as their metabolic source. This close microbial-mineral association likely represents a preferred habitat. Semi-quantitative estimates of cellular material concentrations on the filters were estimated using LPS abundance while metabolic activity was quantified using ATP measurements. General populations of living (or recently dead and preserved) life were reflected in genetic material recovered from 0.2- $\mu\text{m}$  filters in DNA extractions, which were further probed using primers specific to bacteria, archaea, and eukarya. Bacteria and archaea were detected in the top sections of all ice cores as well as in our suite of environmental samples, except clean snow, which had an insufficient abundance of DNA for amplification. Eukarya were detected in most surface samples but they highly varied in relative abundance. Interestingly, one sample of reworked surface ice from 1-m depth also showed a weak eukarya presence confirming that surface biosignatures can be entombed and preserved in blue ice.

Fatty acids extracted from filters show significant variations in molecular weight and degree of saturation. Fatty acid abundances provide a second estimate of cellular biomass concentrations. Lipid profiles for both the glacial ice core sections and the glacial environmental samples (i.e., cryoconites, snow algae, subglacial sediments) are being used to distinguish different sources of organic matter (allochthonous and autochthonous). In addition, other hydrocarbon signatures help constrain the levels of combustion-product aerosols or wind-blown ancient organic matter from neighboring rocks (where applicable).

Finally, the near-surface ice habitat was characterized by very low dissolved ammonia (<2  $\mu\text{M}$ ) regardless of whether the ice was reworked surface material or deeper blue ice, and only slightly higher for some environmental samples (below 15  $\mu\text{M}$ ). Dissolved ammonia in ice was comparable to local seawater (0.3-1.0  $\mu\text{M}$ ), which is strongly diluted by glacial ice meltwater during the arctic summer field season. Dissolved nitrate ( $\text{NO}_3^- + \text{NO}_2^-$ ) concentrations were also low (<10  $\mu\text{M}$ ) for ice and environmental samples, except for snow algae (~17  $\mu\text{M}$ ) and local seawater (10-16  $\mu\text{M}$ ). Dissolved phosphate also varied (0-0.55  $\mu\text{M}$ ) but was generally near zero for blue ice. X-ray diffraction analyses of the mineral grains retrieved from the melted core sections revealed that the prime inorganic nutrient sources in the ice cores were mainly sheet

silicates (mica, chlorite), feldspars (albite, microcline), other interlayer clays and sometimes carbonates. In particular, unexpectedly high amount of sulfate was detected by evolved gas analysis in filtered ice particulates from the northeastern most site.

A suite of complementary analyses of the nutrients, dissolved organic carbon, trace metals and other organic molecules are in progress in order to get a better handle on the relationships between observed live biological materials and the present nutrients.

**Conclusions.** Our understanding of the habitat and life signatures locked into surface glacial ice is just beginning to qualitatively and quantitatively take shape. SLIce results demonstrate strong variability in the conditions of near surface glacial ice in Svalbard. The range of biological, nutrient, and other habitat parameters for the ice cores provide a favorable dataset for evaluating organic signatures associated with ice-life and distinguishing sources for the overall complex mixture of molecules. Analysis of the comprehensive data package will help constrain key variables that are important to supporting ice-life habitats or the markers for their ecology. For instance, microscopic observations provide direct support for a favored habitat associated with minerals. The blue ice samples impose exceptional challenges to most of our analyses since concentrations of target analytes are near detection limits. However, current results have revealed evidence for strong organic molecular record stored in this ice and some data supports detectable ice-life activity. Further clarity on the limits to and context of these data will provide guidance for astrobiological investigations to other planets or moons in our Solar System.

**References:** Eigenbrode, J., Benning, L., Maule, J., et al., 2009, An In-field organic decontamination protocol for sampling devices used in life-detection studies, *Astrobiology*, v.9, DOI: 10.1089=ast.2008.0275

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