Sea-ice microbes in warming Arctic coastal lagoons: contributions to biodiversity and estuarine food webs

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Summary
Arctic coastal lagoons are important for the functioning of Arctic ecosystems and societies, but little is known about the ecological roles of microorganisms inhabiting the ice that covers these lagoons for nearly nine months of the year. During the tenure of this development grant from CICOES we succeeded in developing novel collaborations and conducted 1) the first laboratory and field experiments to optimize methods for collecting and preserving RNA from sea ice microbial communities and 2) obtained first data on gene expression of natural sea ice microbial communities. The project succeeded in producing proof-of-concept data for applying metatranscriptomics to the study of sea ice microbial communities, and in stimulating new research directions with potential relevance to larger scale ecological and societal processes in the region. The successful collaboration developed among the three PIs from three NOAA-CICOES-associated institutions led to the generation of a well-reviewed National Science Foundation (NSF) proposal. It also contributed to the training of two female junior researchers (one research associate, one masters student) and will be the basis for a master's thesis and publication. Results from the study were presented at several conferences. The PIs will revise and resubmit the proposal to NSF by summer 2024 and will continue to seek collaborations with NOAA scientists interested in polar research and sea ice ecology.

Background
Coastal lagoons are among the world's most productive habitats. In the Arctic, lagoons encompass more than half of the Beaufort Sea coast, providing food and habitat for large populations of migratory fish and waterfowl essential to both the Arctic food web and the culture of northern Alaska's Iñupiat communities (Ellanna and Wheeler 1989; Brewster et al. 2008; Dunton et al. 2012). Microbes form the basis for these food webs. The rapid ongoing warming of the Arctic is changing these lagoons by altering nearshore ice conditions, increasing coastal erosion, and thawing permafrost in lagoon watersheds. For most of the year sea ice is a dominant feature in these lagoons, comprising up to half of their volume in winter, and melting dramatically in spring for a brief open-water season; yet to date, the effects of sea-ice on the lagoons' ecosystems have not been examined. The Beaufort Lagoon Ecosystem Long Term Ecological Research project (BLE-LTER; Fig. 1; https://ble.lternet.edu/) was recently established to study how these changes may impact society, biota, and the fate and transport of carbon, water, and energy within the Arctic and beyond. Central to the BLE-LTER is a study of long-term changes in microbial ecology (led by PI Crump) and the role of microbes in lagoon element cycling and food webs. *This pilot project leveraged the BLE-LTER project, developed partnerships between OSU, UW, and UAF PIs, and gathered initial data to determine how sea-*

ice impacts microbial communities and organic matter cycling in coastal lagoons of the rapidly warming Arctic.

Microbes drive cycling of C and N in coastal ecosystems by carrying out key functions including primary production, organic matter remineralization, and transformations of inorganic compounds. Microbes also fuel detritus-based food webs by incorporating abundant terrestrial and marine detrital organic matter into biomass (Dunton et al. 2006; Harris et al. 2018). In the Beaufort lagoons, the composition and genomic functions of microbial communities vary substantially across seasons, and these seasonal shifts reoccur annually (Kellogg et al. 2019; Baker et al. 2021). This suggests that microbial communities are not resistant to seasonal conditions, but instead are resilient to strong seasonality in Arctic lagoons because they reassemble in a predictable fashion every year. *We hypothesized that the seasonal cycle in sea-ice formation and sea-ice melt structures microbial communities in these lagoons by preserving microbial diversity and ecosystem function through winter and by providing a large pulse of detritus organic matter to lagoon food webs during spring melt.*

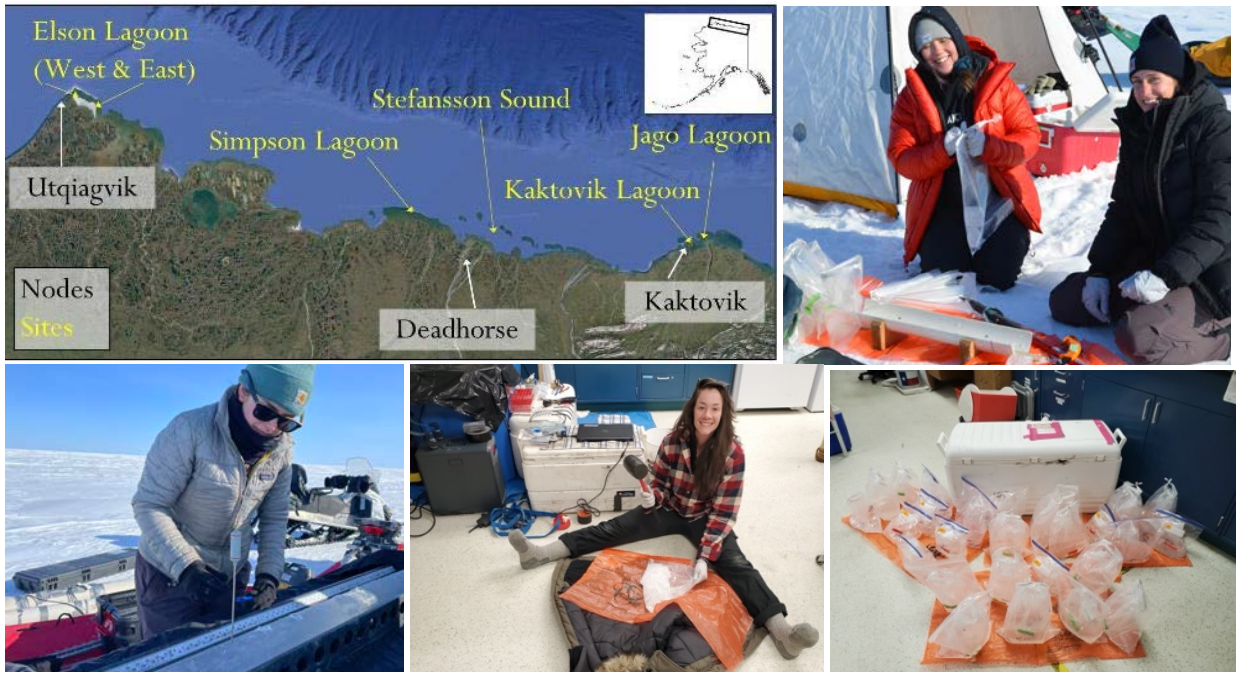

Figure 1. Map of the Beaufort Sea coast of Alaska showing lagoons routinely sampled by the BLE-LTER, and photos of ice core processing in April, 2022 from Stefansson Sound (middle left, Sydney Wilkinson and Anne Krone, photo by Ken Dunton), and Elson Lagoon (middle right, Natasha Griffin, photo by Alina Spera; bottom left, smashing ice in bags to speed thawing, photo by Natasha Griffin; bottom right, ice thawing in bags, photo by Natasha Griffin).

Objectives

We sought to understand the roles of sea-ice microbial communities on lagoon ecology in a warming Arctic by measuring microbial community parameters in sea ice and water throughout the annual ice-water cycle, combining microbiological and biogeochemical techniques with metagenomics and metatranscriptomics, and by leveraging support of the BLE-LTER. Our proposal for this pilot study described three objectives:

Objective 1. Develop and validate ice-melt metatranscriptomic techniques for quantifying metabolic responses of microbes to in-ice emplacement and from-ice release (performed in the UW-APL ice laboratory).

We addressed this objective with a set of laboratory experiments designed to determine the degree to which prokaryotic gene expression in sea ice, measured with metatranscriptomics, is altered by the process of ice melt and RNA preservation. To do this we created artificial sea-ice cores by inoculating artificial seawater (ASW) with a pure culture of the model marine psychrophile *Colwellia psychrerythraea 34H* at environmentally relevant concentrations, freezing the ASW, melting, and then preserving the sample using several techniques, both common to sea-ice research and novel.

Specifically, techniques tested included 1) "direct" melts followed by RNALater fixation, where sea-ice cores are allowed to melt with no additional material or liquid, typically thought to produce the greatest osmotic strain on organisms entrained in the sea-ice brine matrix; 2) isohaline melts followed by RNALater fixation, where ice cores are melted in an equal volume of isohaline solution (in this case, ASW), theoretically reducing the osmotic stress on organisms; 3) RNALater liquid melts – a novel method to reduce the osmotic stresses prior to cell fixation by melting directly into RNALater solution; 4) RNALater salt melts, a novel approach to reduce osmotic stresses as in #3, while also reducing required volumes for ease of fieldwork, where cores are melted by introduction of RNALater salts instead of liquid RNAlater solution. 5) Our controls for these experiments were bacteria in unfrozen ASW incubated at -1.0 C ("brine"), and parallel incubations of bacteria in small-volume, high-cell-concentration samples undergoing the same thawing and preservation techniques described above, which could be frozen and then rapidly thawed and preserved ("fastmelt"). We ran two experiments with a range of different treatments, extracted RNA and analyzed the metatranscriptomes of the various samples.

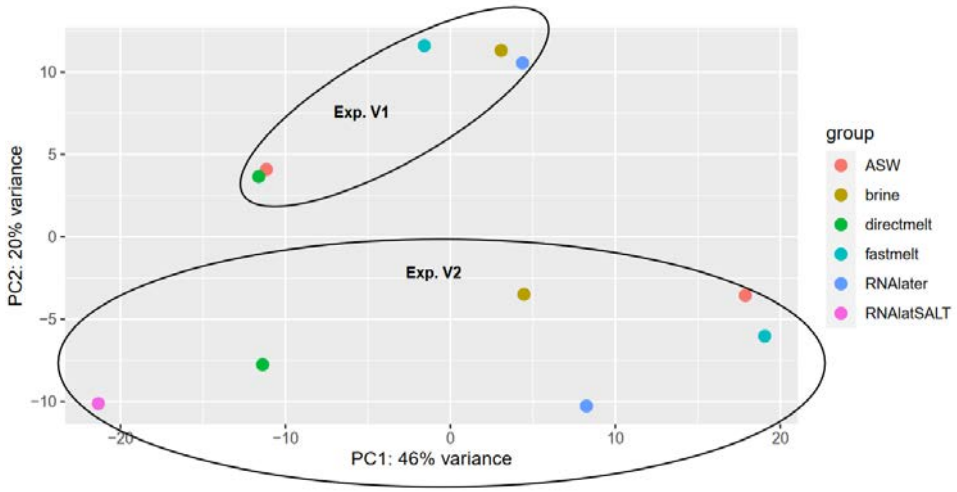

Figure 2. Gene expression patterns for a pure culture of bacteria frozen in artificial sea water and thawed and preserved with several methods during two experiments (V1 and V2), visualized with principal components analysis of relative transcript abundance for expressed genes annotated to the KEGG database (Kyoto Encyclopedia of Genes and Genomes). Colors indicate ice melt treatment.

Our results showed that gene expression patterns for samples thawed overnight in RNAlater were consistently similar to expression in "fastmelt" controls (Figure 2). Other thawing methods produced gene expression patterns that were not consistently similar to controls, including thawing with no added materials ("directmelt"), thawing with undissolved RNAlater salts ("RNAlatSALT"), and standard overnight thawing in artificial seawater (ASW). These results suggest that thawing sea ice in RNAlater is the best method for preserving in situ gene expression patterns.

Objective 2. Characterize changes in microbial signatures (whole-cell numbers [total and active cells], metagenomic and metatranscriptomic diversity) in ice and water samples in winter and spring to establish baseline microbial life-metrics throughout the water-ice-water system evolution (UW-APL, OSU, UAF).

Our survey of phylogenetic and metatranscriptomic diversity in sea ice revealed variation with depth in ice and differences from underlying seawater. With the help of collaborator PI Andy Mahony and collaborators from the BLE-LTER project we collected sea ice cores in Elson Lagoon and Stefansson Sound and RNA and DNA was obtained from specific ice sections (top 50 cm, bottom 50 cm, bottom 10 cm). Taxonomic surveys of bacteria and archaea (16S rRNA gene amplicon sequencing) showed that sea ice communities were clearly distinct from planktonic communities during ice cover, break-up, and open water seasons, and varied with depth in ice (Figure 3).

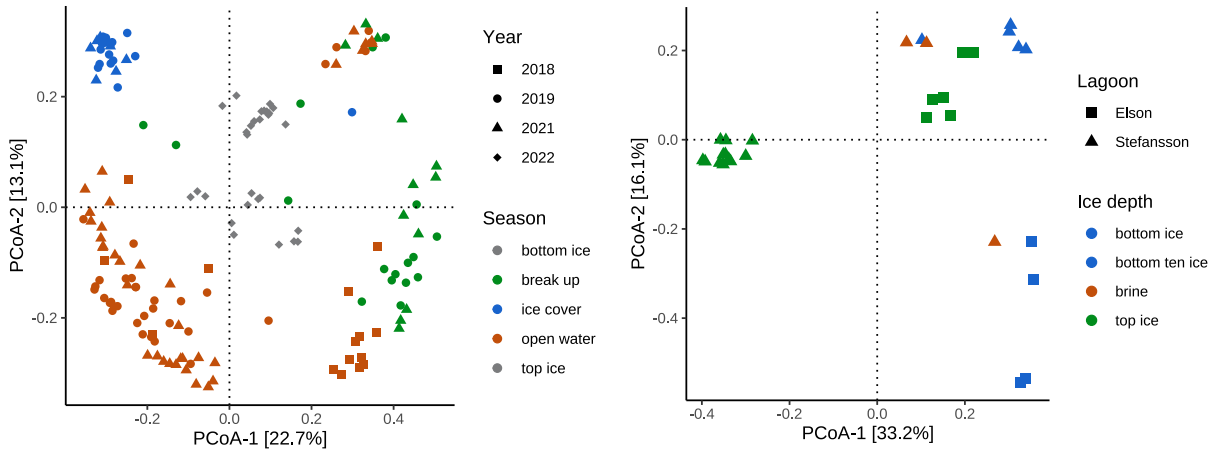

Figure 3. Microbial betadiversity (Principal coordinates analysis of 16S rRNA gene amplicon sequences) for (A) water column and sea ice samples, and (B) sea ice samples only.

We conducted metatranscriptomic DNA sequencing on two cores from Elson Lagoon, and two cores from Stefansson Sound, and thus far have completed metatranscriptomics analysis on the Elson Lagoon cores. For each pair of cores, samples from one core were thawed in an equal volume of sterile artificial seawater (ASW), and samples from the other core were thawed in 1.75 volumes of sterile RNAlater preservative.

Differential expression analysis using DeSeq software in R found no differentially expressed genes between the two melt treatments but identified 687 genes that were differentially

expressed by communities at different depths within the ice cores (Figure 4). These results suggests that contrary to our lab results standard ASW thawing may be adequate to capture the gene expression patterns of sea ice microbial communities.

Variable	Δ in expression	Genes
Depth in ice	Up regulated (LFC > 0)	91
	Down regulated (LFC < 0)	596
	Total	**687**
Melt treatment	Up regulated (LFC > 0)	0
	Down regulated (LFC < 0)	0
	Total	**0**

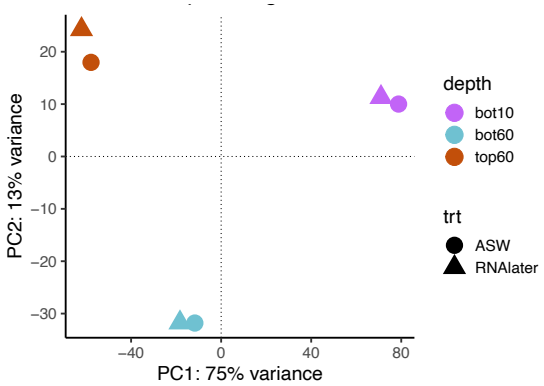

Figure 4. Left: Number of genes identified as differentially expressed by depth and by melt treatment (Padj. <0.1, LFC = log2 fold change). Right: Principal components analysis of relative transcript abundance for the top 5000 most highly expressed genes annotated to the KEGG database (Kyoto Encyclopedia of Genes and Genomes). Colors indicate depth in ice (top 60 cm, bottom 60 cm, and bottom 10 cm), and symbols indicate melt treatment.

Diverse communities of proteobacteria, cyanobacteria, and fusobacteria were responsible for most of the gene expression in sea ice, with higher relative expression by Cyanobacteria and Fusobacteria in surface ice (Figure 5). Prokaryotic cell abundance was similar in the top 60 cm ($1.5x10^4$ cells ml^{-1}) and bottom 60 cm ($1.4x10^4$ cells ml^{-1}) but was greater in the bottom 10 cm of ice ($4.4x10^4$ cells ml^{-1}), suggesting that the bottom 10cm of ice supports more total gene expression than other ice fractions.

Expression by eukaryotic organisms was more variable (Figure 5), and taxonomic assignments were less certain because of the limited availability of eukaryotic genomes to annotate metatranscriptomes. However, we found big differences between surface and bottom ice, with surface ice featuring relatively greater expression by pennate and centric diatoms and green algae, and bottom ice featuring relatively greater expression of fungi, insects, and annelid worms. We also identified expression of genes annotated to a diverse collection of protists, roundworms, cnidaria, limpets, leech, and lancelets.

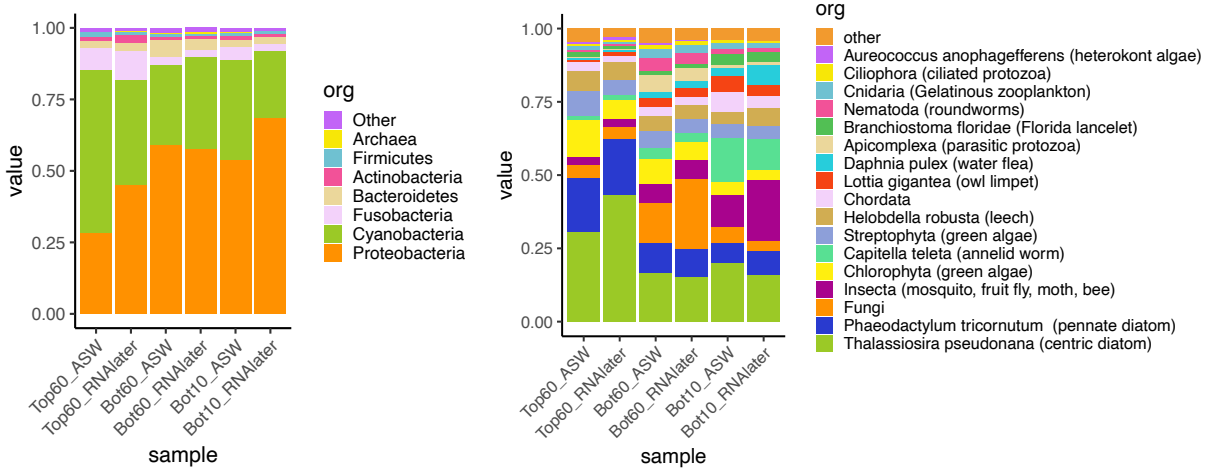

Figure 5. Proportion of mRNA transcripts in metatranscriptomes annotated to prokaryotic (left) and eukaryotic (right) taxonomic groups for sea ice communities from the top 60 cm (Top60), bottom 60 cm (Bot60), and bottom 10 cm (Bot10) of sea ice cores from Elson Lagoon thawed either in an equal volume of artificial seawater (ASW), or 1.75 volumes or RNAlater (RNAlater).

Our preliminary examination of differentially expressed functional genes revealed that bottom ice prokaryotes had greater expression of genes for photosystems, sporulation, iron oxidation, and growth (e.g., ribosomal proteins; Figure 5). This suggests that the more porous ice at the interface with underlying water hosts a more active and more rapidly growing photoautotrophic microbial community compared to top ice. Top ice prokaryotes showed greater expression of genes for osmotic regulation (e.g., OmpR), salt stress, and cyanate decomposition, suggesting that this community invests more energy into mechanisms for survival in the much higher salinity in top ice brines. By focusing in on expression of genes involved in energy metabolism for prokaryotes and eukaryotes, we found that genes involved in photosynthesis and carbon fixation were more highly expressed in bottom ice, while genes for the heterotrophic process of oxidative phosphorylation were more highly expressed in top ice (Figure 6). Genes involved in methane, nitrogen, and sulfur metabolism were expressed in all samples albeit at a lower level.

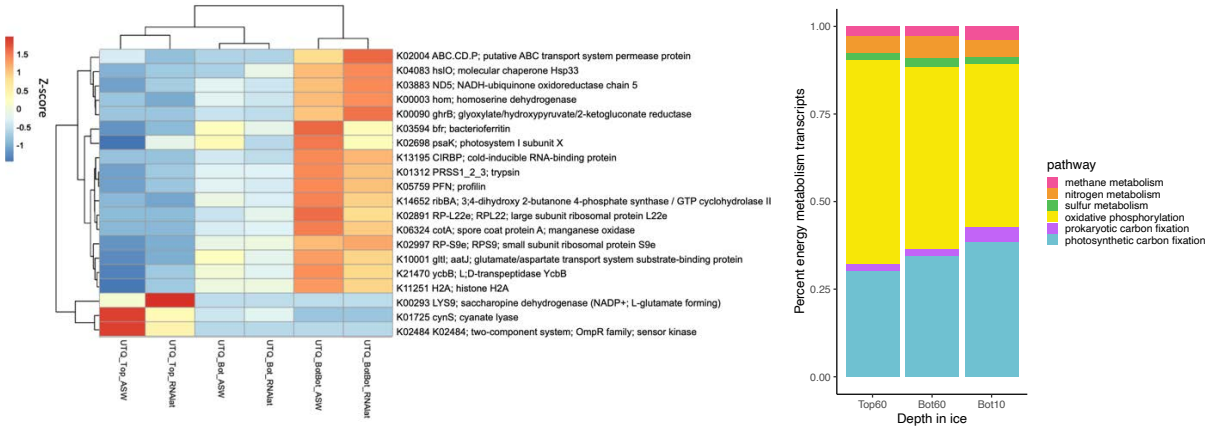

Figure 6. Left: Heatmap of Z-score normalized gene expression levels for the top 20 most differentially expressed prokaryotic genes. Right: Proportion of mRNA transcripts in metatranscriptomes annotated to different energy metabolism pathways. Samples grouped by depth in ice. Photosynthetic carbon fixation and oxidative phosphorylation are predominant metabolisms. Relative abundance of photosynthetic carbon fixation transcripts increased with depth in ice.

Objective 3. Correlate baseline life-metrics and environmental variables measured through the BLE-LTER to functional gene abundance and expression (metagenomes and metatranscriptomes) of microbial populations in ice and water and quantify the impact or exchange between the two systems (OSU).

Progress on this objective is ongoing and will be reported in a master's thesis and peer-reviewed publication first-authored by graduate student Anne Krone supported through this grant.

People and Collaborations

This development grant supported two PIs (Karen Junge, Byron Crump), research scientist Erin Firth, and master's student Anne Krone who presented the findings at two conferences (Coastal and Estuarine Research Federation, Portland, OR, Nov. 12-16, 2023; Alaska Marine Science Symposium, Anchorage, AK, Jan. 29-31, 2024) It contributed to the development of new research directions and potential future collaborations with Bonnie Light (University of Washington) and Amber Hardison (Virginia Institute for Marine Biology) who joined our NSF proposal. It also stimulated several new PI-driven research projects on sea ice within the Beaufort Lagoons LTER project, which provided logistical support for our field sampling.

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