TNA User Report

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Project title	Microbial Life in Frost Flowers: determining seeding sources and spatial structure
Name of the accessed chamber	RvG-ASIC (formerly ASIBIA)
Number of users in the project	2
Project objectives (max 100 words)	In order to better understand and predict the distribution of bacteria in frost flowers, we need to carry out laboratory experiments where we can control multiple parameters, such as growth, temperature and seeding sources and be able to replicate our studies. Our objectives are to experimentally determine a) whether frost flower microbial communities are seeded by the underlying sea/ice/brine system and b) the spatial heterogeneity of these frost flower microbial communities. The results from this study will not only enable us to provide novel quantitative data on poorly characterized ecosystems with global significance (help model climate change), but also a mechanistic understanding of colonization processes through various stages of sea-ice.
Description of work (max 100 words):	Using unfiltered sea water, sea-ice and frost flowers will be grown at -30°C for 1 week. After frost flower formation, we collected atmosphere, frost flower, sea-ice, brine and sea water samples for microbial and chemical analysis using sterilized sampling equipment. The sampling surface was divided into sections that were sampled vertically (i.e. frost flowers, ice, brine, sea-water). Samples were transported back to our research facility at the University of Lyon and sub-sampled for chemical analyses.

Principal Investigator's and group's information	
First name	Catherine
Family name	Larose
Nationality	French
Activity domain[1]	Earth Sciences & Environment
Home institution	CNRS
Institution legal status[2]	UNI
Email	Catherine.larose@ec-lyon.fr
Gender	female
User status[3]	RES
New user	yes

User 1 Information[4]	
First name	Rose
Family name	Layton
Nationality	UK
Activity domain	Earth Sciences & Environment
Home institution	ENOVEO
Institution legal status	SME
Email	r.layton@enoveo.com
Gender	female
User status	PGR
New user	yes

[1] Physics; Chemistry; Earth Sciences & Environment; Engineering & Technology; Mathematics; Information & Communication Technologies; Material Sciences; Energy; Social sciences; Humanities.
[2] UNI= University and Other Higher Education Organisation;
RES= Public Research Organisation (including international research organisations and private research organisations controlled by public authority);
SME= Small and Medium Enterprise;
PRV= Other Industrial and/or Profit Private Organisation;
OTH= Other type of organization.
[3] UND= Undergraduate; PGR= Post graduate; PDOC= Post-doctoral researcher; RES= Researcher ENG= Engineer; ACA= Academic; TEC= Technician.
[4] Reproduce the table for each user who accessed the infrastructure

Trans-National Access (TNA) Scientific Report

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Instructions

Please limit the report to max 5 pages, you can include tables and figures. Please make sure to address any comments made by the reviewers at the moment of the project evaluation (if applicable, in this case you were informed beforehand). Please do not alter the layout of the document and keep it in Word version. The report will be made available on the eurochamp.org website. Should any information be confidential or not be made public, please inform us accordingly (in this case it will only be accessible by the European Commission, the EUROCHAMP-2020 project partners, and the reviewers). Please include:

- Introduction and motivation
- Scientific objectives
- Reason for choosing the simulation chamber/ calibration facility
- Method and experimental set-up
- Data description
- Preliminary results and conclusions
- Outcome and future studies
- References

Name of the PI: Catherine Larose
Chamber name and location: RvG-ASIC (formerly ASIBIA), University of East Anglia
Campaign name and period: Microbial Life in Frost Flowers: determining seeding sources and spatial structure
Text:

Introduction and motivation

As the climate changes, with a transition from a dominance of multiyear ice to new and young sea ice forms in the Arctic (Kwok, 2007; Maslanik et al., 2011; Barber et al., 2012), it is expected that the areal extent and periodicity of frost flowers on sea ice will increase because of later fall freezeup and thinner and more mobile pack ice in the winter (Isleifson et al., 2013). Frost flowers are relatively short-lived clusters of ice crystals that form dendritic structures at the interface between a warm ice surface and a cold atmosphere (Perovich and Richter-Menge, 1994). They can either be formed by atmospheric deposition of water vapor onto the ice surface or through sublimation or evaporation of warm ice into the atmospheric boundary layer (Domine et al., 2005) and a key element in their formation is the presence of brine-wetted surface ice (Roscoe et al., 2011) or a source of brine that can then be wicked up into the already formed frost flowers (Martin et al., 1995; Style and Worster, 2009). The biogeochemical, characteristics of these surfaces are poorly understood, as are their role in climate, ecology, and biogeochemistry-related processes across the ocean-sea ice-atmosphere interface. Bowman and Deming (2010) demonstrated that frost flowers also constitute unique habitats for microorganisms and more recent work has suggested that microorganisms may undergo a selective colonization process based on the enrichment of a specific microbial taxa in frost flowers relative to the underlying ice and sea water, leading to a distinctive microbial community (Bowman et al., 2013).

These communities have the genetic potential to participate in key biogeochemical cycles involving mercury, dimethylsulfide, and halocarbons (Bowman et al., 2014) and might therefore contribute to critical biogeochemical processes in polar environments. An enrichment of certain taxa such as Rhizobium may be the result of transport processes from the ice to the surface and into frost flowers via the brine, but no related sequences were found in the winter first-year ice study of Collins and colleagues (2010) or the summer multiyear ice study of Bowman and colleagues (2011). Another colonization mechanism might be through atmospheric deposition, given the high specific surface area and chemical reactivity of frost flowers. However, studying the ecology of frost flowers in the field is challenging – they can be difficult to access and sample volumes are generally limited. In order to better understand and predict the distribution of bacteria in frost flowers, we need to carry out laboratory experiments where we can control multiple parameters, such as growth, temperature and seeding sources and be able to replicate our studies.

Scientific objectives

Our objectives were to experimentally determine a) whether frost flower microbial communities are seeded by the underlying sea/ice/brine system and b) the spatial heterogeneity of these frost flower microbial communities. The results from this study will not only enable us to provide novel quantitative data on poorly characterized ecosystems with global significance (help model climate change), but also a mechanistic understanding of colonization processes through various stages of sea-ice.

Reason for choosing the simulation chamber/ calibration facility

In order to carry out this study, we used the Roland von Glasow air-sea-ice chamber at the Centre for Ocean and Atmospheric Sciences at the University of East Anglia, Norwich in collaboration with James France and Jan Kaiser. With this chamber, we were able to replicate first year sea-ice formation and frost flower growth in a controlled setting where sea water, ice and atmospheric conditions are monitored in real time. To our knowledge, this is the only large scale chamber of its kind, and therefore it provides a unique opportunity for us to study biological and chemical air-sea-ice-frost flower interactions.

Method and experimental set-up

Using unfiltered sea water from the North Sea, sea-ice and frost flowers were grown at -30 °C for 1 week. Salinity and light measurements were carried out throughout. After frost flower formation, atmospheric samples were collected for microbial community analysis using a KNF LABOPORT N 86 KT.18 electric mini vacuum pump with a flow rate of 5.5 l/min. A total volume of 3.63 m^3 was collected. Subsequently, frost flower, sea-ice, brine and sea water samples for microbial and chemical analysis were collected using sterilized sampling equipment. Briefly, the sampling surface was divided into 18 sections, surfaces were scraped to collect frost flowers that were then stored in sterile sampling bags. Following frost flower collection, sea-ice was collected using a sterilized kovax drill. A total of 16 cores were drilled, 4 were frozen intact, while the others were sub-sampled for brine using the slow-melt technique. Additionally, samples of sea water were collected both before and after the experiment. Samples were transported back to our research facility at the University of Lyon and sub-sampled for chemical analyses (salinity, pH and extracellular polymeric substances (EPS)). Both pH and salinity were measured in the melted samples using a conductivity and pH probe. Between 50 and 100 ml of sample were filtered onto 0.4 µm Millipore filters and the filtrate was conserved for both particulate and dissolved EPS quantification using the phenol-sulfuric acid (PSA) method described in Kremps et al. 2011. Briefly, EPS gives an orange-yellow color when treated with phenol and sulfuric acid that can then be quantified spectrophotometrically.

Samples were filter concentrated onto 0.2 µm Millipore filters for microbial community analysis using sterilized filtration equipment and the filtrate was conserved for free DNA and mobile genetic element analysis. DNA will be extracted from filtered samples and the filtrate, PCR amplified to target the 16S rRNA gene and the ITN region (i.e. taxonomic markers) and sequenced using Illumina MiSeq in order to determine community structure. Microorganisms will also be quantified using qPCR to target bacteria and eukaryotes.

Statistical analysis will be used to compare community structure and composition a) across different frost flower samples to determine microbial heterogeneity and b) among the sampled habitats to determine the seeding source of frost flower microorganisms. Different multivariate techniques will be used.

Data description

A total of 44 samples were collected - 2 atmosphere, 4 bulk ice cores, 3 drained upper ice cores, 3 drained bottom ice cores, 12 brine samples, 18 frost flower samples and 2 seawater. This large data set consisting of basic chemistry (salinity, pH, EPS content), cell count estimates (16S, 18S) and microbial community structure (16S and ITN sequencing data will be made publicly available following publication.

Preliminary results and conclusions

Based on our initial analysis, we were able to observe differences among the sample types in terms of their chemistry. We observed a strong gradient in salinity with frost flowers showing the highest salinity and pH, followed by brine, sea water, and ice (figure 1).

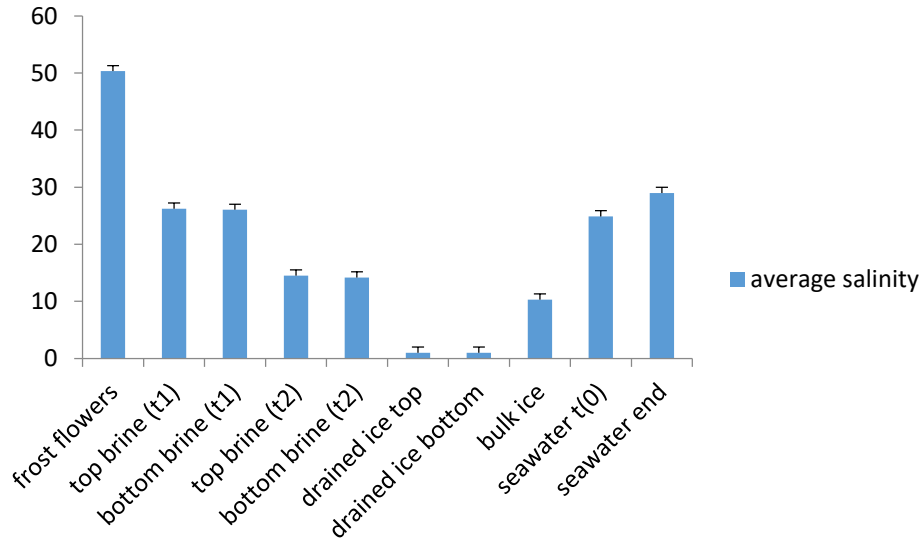

Figure 1: Average salinity for the different sample types

Salinity in frost flowers was nearly double that observed in seawater and brine, which would suggest a form of enrichment. Drained ice cores showed low salinity values, close to those observed in glacial ice. The initial brine fractions (collected by slow-melting over 4 hours) had the same salinity as the seawater, while the second fraction was much lower.

We also observed similar trends in average pH values.

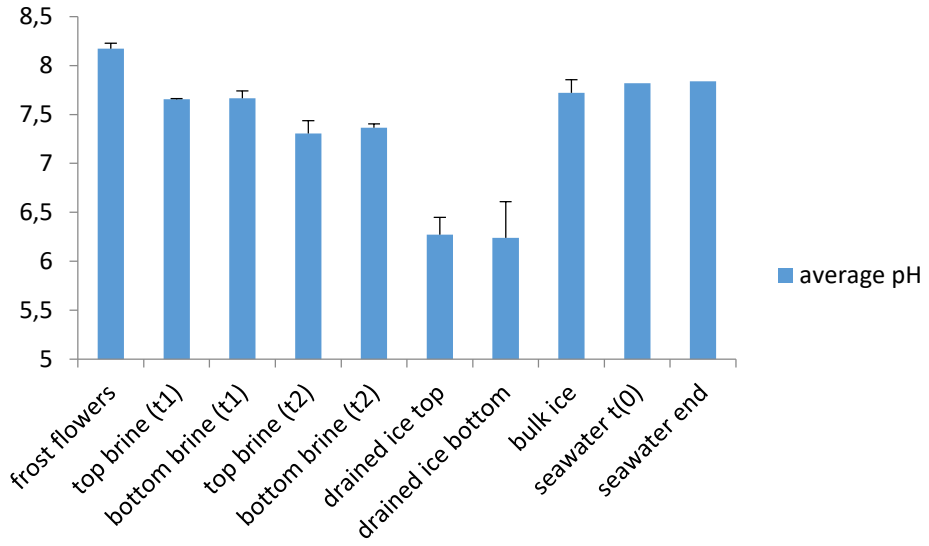

Figure 2: Average pH values in different samples collected

We also observed initial differences in the biology of the sample types. DNA was isolated from all the samples and the DNA concentration measured and corrected for initial sample volume. DNA concentration in the frost flowers was nearly 7 times higher than any other sample, suggesting an enrichment of cells (Figure 3a). Similarly, brine DNA concentrations were also dramatically higher relative to both the drained ice and sea water concentrations (Figure 3b).

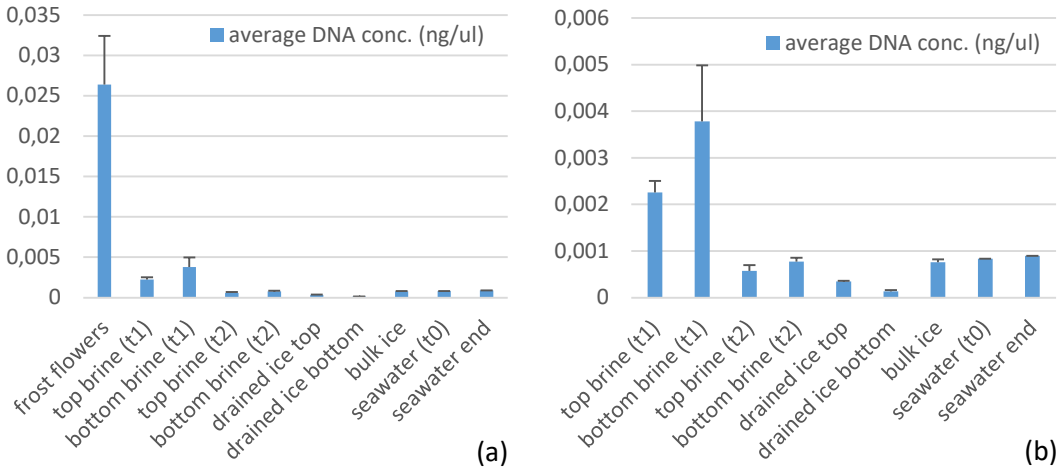

Figure 3: Average DNA concentrations in (a) all samples collected and (b) all samples collected excluding frost flowers.

While we have yet to complete our analysis, this suggests that the different environments sampled represent unique habitats with specific constraints that the microbial community must adapt to.

Outcome and future studies

The results from this study will be published in a peer-reviewed journal and we will include the access provider and staff involved either as co-authors or in the acknowledgements. We anticipate that the first paper will be submitted by December.