2018-2019 Ocean Metaproteome Intercalibration and Intercomparison Standards

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Introduction

With increasing interest in the direct measurement of proteins and their biogeochemical functions within the oceans, the metaproteomic datatype is beginning to establish itself as a valuable research and monitoring tool. However, given rapid changes in technology and methods, as well as the overall youth of the metaproteomic field, there is a need to develop confidence in reproducibility and accuracy of these methods. This document describes the ongoing effort to conduct the first ocean metaproteomic intercomparison in 2018-2019.

Methods

Intercalibration Sample Collection and Metadata

Ocean metaproteome intercalibration filter samples were collected at the Bermuda Atlantic Time Series (31° 40'N 64° 10'W) on expedition BATS 348 in June 16th 2018 between 01:00 and 05:00am. Two horizontal McLane pumps were clamped together and attached at the same depth (80m) with two filter heads on each pump and a flow meter downstream of each filter head. Each 142mm filter head contained a 0.2 μm Supor filter with an upstream 3.0 μm Supor filter. The pumps were set to run for 240 min at 3 L/min starting at 01:00 local time. Volume filtered were measured by three gauges on each pump, one downstream of each pump head, and one on the total outflow (Table 1). Individual pump head gauges summed to the total gauge for pump 1 (within 1L; 447L and 446.2L), but deviated by 89L on pump 2 (478L and 388.9L). Given that the total gauge is further downstream, we plan to use the pump head gauges [Data from McLane data log?].

The pump heads were removed from the McLane pumps immediately upon retrieval, decanted of excess seawater by vacuum, and placed in coolers with frozen blue ice packs, and brought into a fabricated clean room environment aboard the ship. 0.2 μm filters were then cut in eight equivalent pieces and frozen at -80°C in 2 mL cryovials, creating 16 samples that were co-collected temporally and in very close proximity to each other. The sample naming scheme associated with the different pumps and pumps heads is described in Table 1. Note that pump 1A and 1B samples accidentally had two 3.0 μm filters above the 0.2 μm filter, and 1B had a small puncture in it, although neither of these seemed to affect biomass on it (presumably the puncture occurred after sampling was completed).

Proposed Intercalibration Process

We propose to send two samples to 16 laboratories interested in participating in the intercomparison/intercalibration project. These would be from different pump heads, so total protein abundances may shift with volume filtered and can be corrected for, and the biological diversity within each sample should be close to identical given the sampling approach. We also could provide an annotated metagenomics database (provided by Chris Dupont of the JC Venter Institute most likely) as well as a heterologous overexpressed synthetic protein encoding a suite of isotopically labeled tryptic

peptide standards (~30) that are representative of this geographic region. Samples will be shipped out in October-December of 2018, prioritizing US laboratories conducting oceanographic research, but also including several additional microbiome laboratories to diversify participation, until samples are exhausted. A workshop in Woods Hole will be planned for fall of 2019 to compare results. An advisory committee consisting of Ben Neely (NIST), Michael Janech (College of Charleston), and Dasha Leary (Naval Research Laboratory) have agreed to provide feedback regarding procedures throughout this process.

Acknowledgements

Funds for distributing intercomparison samples and a future workshop to discuss results is being supported by Ocean Carbon and Biogeochemistry (OCB). Funds for the research expedition where samples were collected were supported by NSF and the Gordon and Betty Moore Foundation.

Table 1. Volumes filtered through intercomparison sample heads.

Pump / Pump head / sample name	Volume filtered (L)	Volume per slice (L)
Pump 2L / BATS 1 / pump 1A	221.6*	27.7
Pump 2R / BATS 2 / pump 1B	167.3*	20.9
Pump 1L / BATS 3 / pump 2A	235.1+	29.4
Pump 1R / BATS 4 / pump 2B	211.1+	26.3

* Pump 1 total gauge = 447L, sum of two pump gauges = 446.2L

+ Pump 2 total gauge = 478L, sum of two gauges = 388.9L, discrepancy of 89L, gauges on pump head are assumed more accurate, as leaks in system could create the additional flow for the total pump gauge.

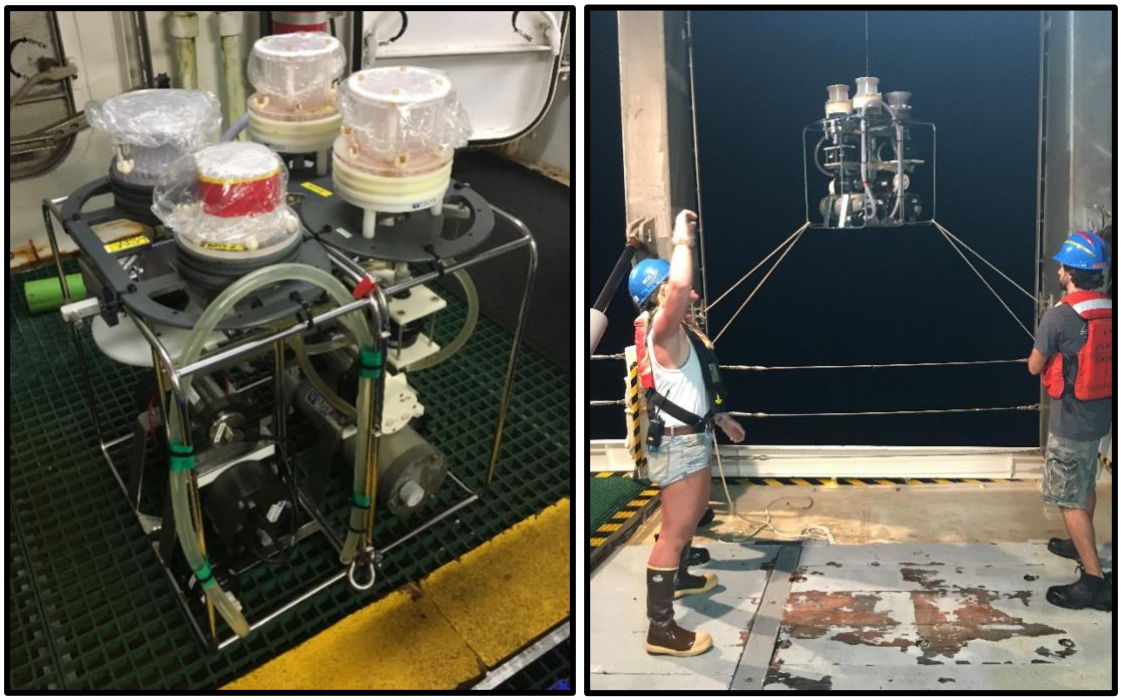

Figure 1. Two McLane pump samplers with two Mini-MULVS sampler heads clamped together and deployed on BATS expedition 348 on June 16th 2018 aboard the R/V Atlantic Explorer.

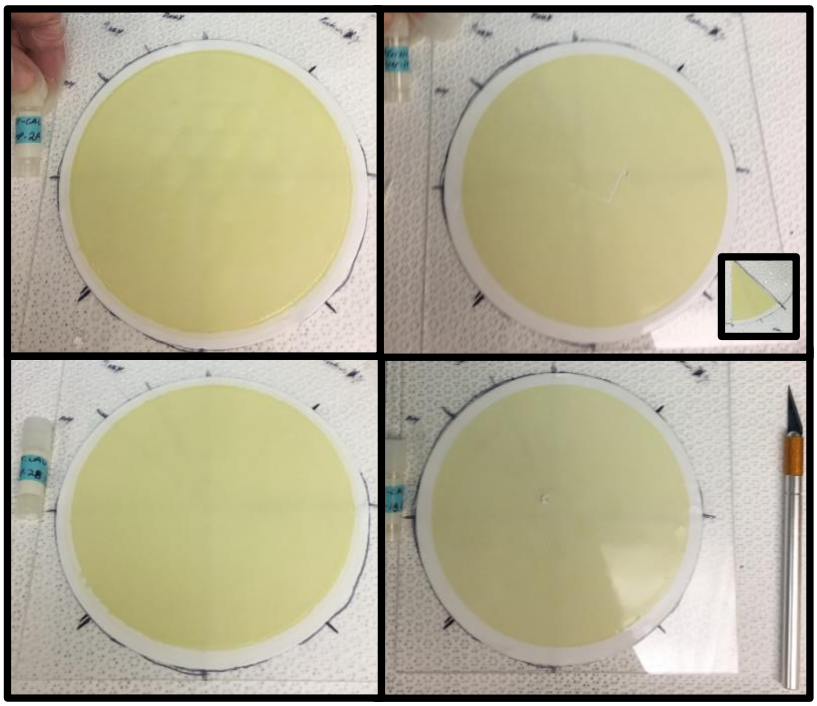

Figure 2. The four 142 mm filter 0.2 μm filters used for this intercomparison study collected by McLane pump (X-Acto knife for scale). Each filter was sliced into 8 fractions (inset) and frozen at -80C in a cryovial. Samples were labeled by pump and pump head (Table 1; pump-2A upper left; pump-1A upper right; pump-2B lower left; pump-1B lower right).

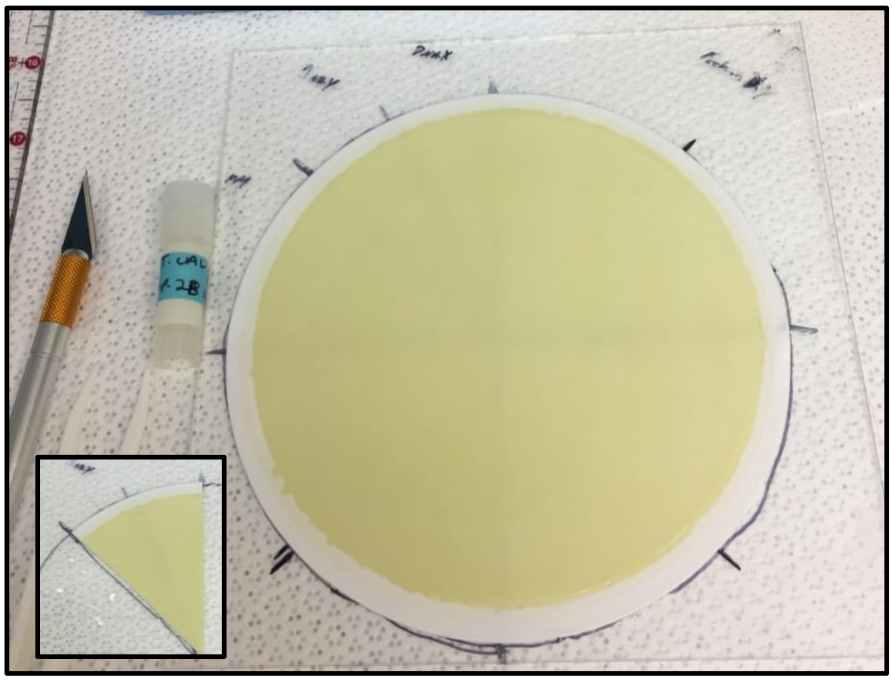

Figure S1. Additional figure of intercomparison samples