Large substrate hydrolysis of bulk water samples taken aboard the R/V Endeavor EN638, May 2019 in the Northern Atlantic.

Website: https://www.bco-dmo.org/dataset/821801
Data Type: Other Field Results
Version: 1
Version Date: 2020-08-26

Project
» A mechanistic microbial underpinning for the size-reactivity continuum of dissolved organic carbon degradation (Microbial DOC Degradation)

Contributors	Affiliation	Role
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Abstract
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Table of Contents

- Coverage
- Dataset Description
  - Methods & Sampling
  - Data Processing Description
- Data Files
- Related Publications
- Parameters
- Instruments
- Deployments
- Project Information
- Funding

Coverage

Spatial Extent: **N**:42.83954 **E**:-53.3949 **S**:34.50102 **W**:-75.67819
Temporal Extent: 2019-05-14 - 2019-05-25

Dataset Description

The potential of the seawater microbial community to hydrolyze six high-molecular-weight polysaccharides (arabinogalactan, chondroitin sulfate, fucoidan, laminarin, pullulan, and xylan) was investigated in surface and bottom water. Seawater has been collected during the EN638 cruise on the R/V Endeavor in May 2019.

Methods & Sampling

Water was collected via Niskin bottles mounted on a rosette, equipped with a CTD.

Seawater was transferred to 20 L carboys that were rinsed three times with water from the sampling depth and then filled with seawater from a single Niskin bottle, using silicone tubing that had been acid washed then rinsed with distilled water prior to use. From each carboy, water was dispensed into smaller glass containers that were cleaned and pre-rinsed three times with water from the carboy prior to dispensing. This water was used to measure cell counts, bacterial productivity, and the activities of polysaccharide hydrolases, peptidases, and glucosidases. A separate glass Duran bottle was filled with seawater from the carboy and sterilized in an

autoclave for 20-30 minutes to serve as a killed control for microbial activity measurements.

The potential of the seawater microbial community to hydrolyze six high-molecular-weight polysaccharides (arabinogalactan, chondroitin sulfate, fucoidan, laminarin, pullulan, and xylan) was investigated in surface and bottom water. For each substrate, three 50 mL falcon tubes were filled with seawater and one 50 mL falcon tube was filled with autoclaved seawater to serve as a killed control. Substrate was added at 3.5 µM monomer-equivalent concentrations, except for fucoidan, which was added at 5 µM concentrations (a higher concentration was necessary for sufficient fluorescence signal). Two 50 mL falcon tubes – one with seawater and one with autoclaved seawater – with no added substrate served as blank controls. Incubations were stored in the dark at as close to in situ temperature as possible.

Subsamples of the incubations were collected at time zero, and at a sequence of subsequent time points. At each time point, 2 mL of seawater was collected from the 50 mL falcon tube using a sterile syringe, filtered through a 0.2 µm pore size syringe filter, and stored frozen until processing.

Data Processing Description

The hydrolysis of high molecular weight substrate to lower molecular weight hydrolysis products was measured using gel permeation chromatography with fluorescence detection, after the method of Arnosti [1996, 2003]. In short, the subsample was injected onto a series of columns consisting of a 21 cm column of G50 and a 19 cm column of G75 Sephadex gel. The fluorescence of the column effluent was measured at excitation and emission wavelengths of 490 and 530 nm, respectively.

Hydrolysis rates were calculated from the change in molecular weight distribution of the substrate over time, as described in detail in Arnosti [2003]. Scripts to calculate hydrolysis rates are available in the associated Github repository [Hoarfrost, 2017].

BCO-DMO processing notes:

- Added ISO_DateTime_UTC column
- Adjusted date column to ISO format (yyyy-mm-dd)
- Adjusted column headers to comply with database requirements

[table of contents | back to top]

Data Files

File
fla_rates_bulk.csv (Comma Separated Values (.csv), 161.44 KB) MD5:747279e5d0cc76d701b934a9c80f907b Primary data file for dataset ID 821801

[table of contents | back to top]

Related Publications

Arnosti, C. (1996). A new method for measuring polysaccharide hydrolysis rates in marine environments. Organic Geochemistry, 25(1-2), 105–115. doi:10.1016/s0146-6380(96)00112-x https://doi.org/10.1016/S0146-6380(96)00112-X
Methods

Arnosti, C. (2003). Fluorescent derivatization of polysaccharides and carbohydrate-containing biopolymers for measurement of enzyme activities in complex media. Journal of Chromatography B, 793(1), 181–191. doi:10.1016/s1570-0232(03)00375-1 https://doi.org/10.1016/S1570-0232(03)00375-1
Methods

Hoarfrost, A., & Arnosti, C. (2017). Heterotrophic Extracellular Enzymatic Activities in the Atlantic Ocean Follow Patterns Across Spatial and Depth Regimes. Frontiers in Marine Science, 4. doi:10.3389/fmars.2017.00200
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
deployment	Cruise ID	unitless
station	Station number for cruise	unitless
longitude	Longitude, south is negative	decimal degrees
latitude	Latitude, west is negative	decimal degrees
date	Date of sample collection in ISO format (yyyy-mm-dd), US Eastern Time (UTC-05:00)	unitless
time	Time of sample collection in ISO format (hh:mm:ss), US Eastern Time (UTC-05:00)	unitless
cast_number	Cast number (refers to cast of CTD/Niskin bottles on cruise)	unitless
depth_sequence	Sequence of depths sampled (1 is surface; higher numbers at greater depths)	unitless
depth_actual	Actual depth at which water was collected	meters
sample_type	Sample from bulk water (Bulk) or Large Volume (LV) incubation	unitless
unammended_ammended	Refers to whether high molecular weight thalassiosira weissflogii extract was added or not; A, B, C refers to incubation depth, and the following number corresponds to incubation replicate.	unitless
substrate	Substrate of sample collection: ara = substrate to measure alpha glucosidase: 4-methylumbelliferyl-α-D-, chn = substrate to measure beta glucosidase: 4-methylumbelliferyl-β-D-, fuc = substrate to measure leucine aminopeptidase (L-leucine-7-amido-4 MCA), lam = substrate to measure chymotrypsin activity: ala-ala-phe-MCA, pul = substrate to measure chymotrypsin activity: N-succinyl-ala-ala-pro-phe-MCA, xyl = substrate to measure trypsin activity: Boc-gln-ala-arg-MCA	unitless
timepoint_number	Timepoint_Number of sample collection	unitless
timepoint_days	Timepoint_Days of sample collection	unitless
rate_x	Hydrolysis rate of killed control incubation at subsampled timepoint	nM*hr-1
rate_1	Hydrolysis rate of incubation replicate #1 at subsampled timepoint	nM*hr-1
rate_2	Hydrolysis rate of incubation replicate #2 at subsampled timepoint	nM*hr-1
rate_3	Hydrolysis rate of incubation replicate #3 at subsampled timepoint	nM*hr-1
rate_mean	Mean hydrolysis rate of incubation replicates at subsampled timepoint	nM*hr-1
rate_sd	Standard deviation of mean hydrolysis rates at subsampled timepoint	nM*hr-1
rate_x_kc	Kill control corrected hydrolysis rate of killed control incubation	nM*hr-1
rate_1_kc	Kill control corrected hydrolysis rate of incubation replicate #1	nM*hr-1
rate_2_kc	Kill control corrected hydrolysis rate of incubation replicate #2	nM*hr-1

rate_3_kc	Kill control corrected hydrolysis rate of incubation replicate #3	nM*hr-1
rate_mean_kc	Mean hydrolysis rate of kill control corrected incubation replicates	nM*hr-1
rate_sd_kc	Standard deviation of mean hydrolysis rate of kill control corrected incubation replicates	nM*hr-1
ISO_DateTime_UTC	Datetime of sample collection in ISO format in UTC timezone (yyyy-mm-dd:hh:mm:ssZ)	yyyy-MM-dd'T'HH:mm:ss'Z'

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	HPLC system with Hitachi fluorescence detec
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	HPLC system with Hitachi fluorescence detectors (L-7485, L-2485, Chromaster - 5440)
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

[table of contents | back to top]

Deployments

EN638

Website	https://www.bco-dmo.org/deployment/820578
Platform	R/V Endeavor
Start Date	2019-05-15
End Date	2019-05-30
Description	Underway datasets (and their DOIs) provided by R2R are the following. Click the cruise DOI for more general information ADCP: 10.7284/134159 Anemometer: 10.7284/134174 Anemometer: 10.7284/134176 CTD: 10.7284/134160 GNSS: 10.7284/134158 GNSS: 10.7284/134167 GNSS: 10.7284/134168 GNSS: 10.7284/134170 Gyrocompass: 10.7284/134161 Gyrocompass: 10.7284/134162 Met Station: 10.7284/134166 Radiometer: 10.7284/134163 Radiometer: 10.7284/134164 Singlebeam Sonar: 10.7284/134172 Speed Log: 10.7284/134169 Time Server: 10.7284/134171 TSG: 10.7284/134165 TSG: 10.7284/134173 Winch: 10.7284/134175

[table of contents | back to top]

Project Information

A mechanistic microbial underpinning for the size-reactivity continuum of dissolved organic carbon degradation (Microbial DOC Degradation)

Coverage: Northern Atlantic, Southern Indian Ocean, Svalbard

NSF Award Abstract:
Marine dissolved organic matter (DOM) is one of the largest actively-cycling reservoirs of organic carbon on the planet, and thus a major component of the global carbon cycle. The high molecular weight (HMW) fraction of DOM is younger in age and more readily consumed by microbes than lower molecular weight (LMW) fractions of DOM, but the reasons for this difference in reactivity between HMW DOM and LMW DOM are unknown. Two factors may account for the greater reactivity of HMW DOM: (i) targeted uptake of HMW DOM by specific bacteria, a process the PI and her collaborators at the Max Planck Institute for Marine Microbiology (MPI) recently identified in surface ocean waters; and (ii) a greater tendency of HMW DOM to aggregate and form gels and particles, which can be colonized by bacteria that are well-equipped to breakdown organic matter. Scientists and students from the University of North Carolina (UNC) - Chapel Hill will collaborate with researchers at the MPI for Marine Microbiology (Bremen, Germany) to investigate this breakdown of HMW DOM by marine microbial communities. These investigations will include a field expedition in the North Atlantic, during which HMW DOM degradation rates and patterns will be compared in different water masses and under differing conditions of organic matter availability. DOM aggregation potential, and degradation rates of these aggregates, will also be assessed. Specialized microscopy will be used in order to pinpoint HMW DOM uptake mechanisms and rates. The work will be complemented by ongoing studies of specific bacteria that breakdown HMW DOM, their genes, and their proteins. Graduate as well as undergraduate students will participate as integral members of the research team in all aspects of the laboratory and field work; aspects of the project will also be integrated into classes the scientist teaches at UNC.

The existence of a size-reactivity continuum of DOM - observations and measurements showing that HMW DOM tends to be younger and more reactive than lower MW DOM - has been demonstrated in laboratory and field investigations in different parts of the ocean. A mechanistic explanation for the greater reactivity of HMW DOM has been lacking, however. This project will investigate the mechanisms and measure rates of HMW DOM degradation, focusing on identifying the actors and determining the factors that contribute to rapid cycling of HMW DOM. Collaborative work at UNC and MPI-Bremen recently identified a new mechanism of HMW substrate uptake common among pelagic marine bacteria: these bacteria rapidly bind, partially hydrolyze, and transport directly across the outer membrane large fragments of HMW substrates that can then be degraded within the periplasmic space, avoiding production of LMW DOM in the external environment. This mode of substrate processing has been termed selfish, since targeted HMW substrate uptake sequesters resources away from other members of microbial communities. Measurements and models thus must account for three modes of substrate utilization in the ocean: selfish, sharing (external hydrolysis, leading to low molecular weight products), and scavenging (uptake of low molecular weight hydrolysis products without production of extracellular enzymes). Using field studies as well as mesocosm experiments, the research team will investigate the circumstances and locations at which different modes of substrate uptake predominate. A second focal point of the project is to determine the aggregation potential and microbial degradation of aggregated HMW DOM. Preliminary studies have demonstrated that particle-associated microbial communities utilize a broader range of enzymatic capabilities than their free-living counterparts. These capabilities equip particle-associated communities to effectively target a broad range of complex substrates. The project will thus focus on two key aspects of HMW DOM - the abilities of specialized bacteria to selectively sequester HMW substrates, as well as the greater potential of HMW substrates to aggregate ? and will quantify these factors at different locations and depths in the ocean. The project will thereby provide a mechanistic underpinning for observations of the DOC size-reactivity continuum, an essential part of developing an overall mechanistic understanding of organic matter degradation in the ocean.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1736772

[table of contents | back to top]