Small 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/820973
Data Type: Cruise Results
Version: 1
Version Date: 2020-08-14

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

Contributors	Affiliation	Role
Arnosti, Carol	University of North Carolina at Chapel Hill (UNC-Chapel Hill)	Principal Investigator, Contact
Soenen, Karen	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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

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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

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

Methods & Sampling

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 the activities of 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.

Two substrates, -glucose and -glucose linked to a 4-methylumbelliferyl (MUF) fluorophore, were used to

measure glucosidase activities. Five substrates linked to a 7-amido-4-methyl coumarin (MCA) fluorophore, one amino acid – leucine – and four oligopeptides – the chymotrypsin substrates alanine-alanine-phenylalanine (AAF) and alanine-alanine-proline-phenylalanine (AAPF), and the trypsin substrates glutamine-alanine-arginine (QAR) and phenylalanine-serine-arginine (FSR) – were used to measure exo- and endo-acting peptidase activities, respectively. Incubations with the seven low molecular weight substrates were set up in a 96-well plate. For each substrate, triplicate wells were filled with a total volume of 200 uL seawater for experimental incubations; triplicate wells were filled with 200 uL autoclaved seawater for killed control incubations. Substrate was added at saturating concentrations. A saturation curve was determined with surface water from each station to determine saturating concentrations of substrate. The saturating concentration was identified as the lowest tested concentration of substrate at which additional substrate did not yield higher rates of hydrolysis. Fluorescence was measured over 24-48 hours incubation time with a plate reader (TECAN infiniteF200; 360 nm excitation, 460 emission), with timepoints taken every 4-6 hours.

Data Processing Description

Hydrolysis of the substrates was measured as an increase in fluorescence as the fluorophore was hydrolyzed from the substrate over time [as in Hoppe, 1983; Obayashi and Suzuki, 2005].

Hydrolysis rates were calculated from the rate of increase of fluorescence in the incubation over time relative to a set of standards of known concentration of fluorophore. Scripts to calculate hydrolysis rates are available in the associated Github repository (Hoarfrost, 2017).

BCO-DMO processing notes:

- Adjusted column names to comply with database requirements
- Added ISO_DateTine_UTC column
- Converted data to ISO format (yyyy-mm-dd)

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Data Files

File
bulk_plate_rate.csv (Comma Separated Values (.csv), 22.28 KB) MD5:bdf2b6f7540f9c33672a5535fc86775c Primary data file for dataset ID 820973

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Related Publications

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

Hoppe, H.-G. (1983). Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Marine Ecology Progress Series, 11, 299–308. doi:10.3354/meps011299
Methods

Obayashi, Y., & Suzuki, S. (2005). Proteolytic enzymes in coastal surface seawater: Significant activity of endopeptidases and exopeptidases. Limnology and Oceanography, 50(2), 722–726. doi:10.4319/lo.2005.50.2.0722
Methods

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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 (m)
sample_type	Sample from bulk water or Large Volume incubation	unitless
unammended_ammended	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
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'
replicate_1	plate replicate 1 hydrolysis rate	nmol L-1 h-1
replicate_2	plate replicate 2 hydrolysis rate	nmol L-1 h-1
replicate_3	plate replicate 3 hydrolysis rate	nmol L-1 h-1
average	average of hydrolysis rates	nmol L-1 h-1

standard_deviation	standard deviation of hydrolysis rates	nmol L-1 h-1
substrate	Substrates for measurement of enzymatic activities: • a-glu: substrate to measure alpha glucosidase: 4-methylumbelliferyl-α-D- • b-glu: substrate to measure beta glucosidase: 4-methylumbelliferyl-β-D- • L: substrate to measure leucine aminopeptidase (L-leucine-7-amido-4 MCA) • AAF: substrate to measure chymotrypsin activity: ala-ala-phe-MCA • AAPF: substrate to measure chymotrypsin activity: N-succinyl-ala-ala-pro-phe-MCA • QAR: substrate to measure trypsin activity: Boc-gln-ala-arg-MCA • FSR: substrate to measure trypsin activity: N-t-boc-phe-ser-arg-MCA	unitless

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Instruments

Dataset-specific Instrument Name	TECAN infiniteF200
Generic Instrument Name	plate reader
Dataset-specific Description	TECAN infiniteF200; 360 nm excitation, 460 emission
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

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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

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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.

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Funding

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

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