Hydrolysis rates from bulk samples, plate reader results from RV/Endeavor EN556, 2015 (Patterns of activities project)

Website: https://www.bco-dmo.org/dataset/719487
Data Type: experimental
Version: 1
Version Date: 2017-11-16

Project
» Latitudinal and depth-related contrasts in enzymatic capabilities of pelagic microbial communities: Predictable patterns in the ocean? (Patterns of activities)

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

Abstract
This dataset includes polysaccharide hydrolysis rates to measure microbial enzyme activities and bacterial productivity, from bulk samples, plate reader results from RV/Endeavor EN556, 2015.

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Coverage

Spatial Extent: **N**:40.0725 **E**:-68.4078 **S**:37.6675 **W**:-71.0086
Temporal Extent: 2015-04-27 - 2015-05-02

Dataset Description

This dataset includes polysaccharide hydrolysis rates to measure microbial enzyme activities and bacterial productivity.

See Niskin Bottle and Cast List EN556 to link specific casts and bottles to each experiment: https://www.bco-dmo.org/dataset/717427.

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 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.

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. Hydrolysis rates of the substrates were measured as an increase in fluorescence as the fluorophore was hydrolyzed from the substrate over time [as in Hoppe, 1993; *Obayashi and Suzuki*, 2005]. 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 L seawater for experimental incubations; triplicate wells were filled with 200 L 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 spectrafluor plus; 360 nm excitation, 460 emission), with time points taken every 4-6 hours. 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 and produce the figures shown here are available in the associated Github repository [*Hoarfrost*, 2017].

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 six subsequent time points (t1-t6): 2 days, 5 days, 10 days, 17 days, 30 days, and 42 days. 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

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- removed 'cast00' and 'stn0' from data records for the cast and station columns

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Data Files

File	
EN556_bulk_platereader.csv	(Comma Separated Values (.csv), 8.33 KB) MD5:36c31835f90fec56d3c00504fee21a97
Primary data file for dataset ID 719487	

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Related Publications

Hoarfrost, A., Gawarkiewicz, G., & Arnosti, C. (2017, May 15). Ahoarfrost/Shelf1234: Shelf1234 Initial Release. Zenodo. https://doi.org/10.5281/zenodo.580059
Methods

Hoppe, HG. (1993). Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement

of bacteria, p. 423-431. In P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole (ed.), Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, FL 978-0873715645
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
cruise_id	cruise identifier	unitless
cast	cast number	unitless
station	station number	unitless
depth_no	depth description: sequence of depths sampled with 1 is surface and higher numbers at greater depths	unitless
depth_m	actual depth at which water collected	meters
substrate	substrates for measurement of enzymatic activities: a-glu = alpha glucosidase: 4-methylumbelliferyl-a-D-glucopyranoside; b-glu = beta glucosidase: 4-methylumbelliferyl-beta-D-glucopyranoside; L = leucine aminopeptidase (L-leucine-7-amido-4 MCA); AAF = chymotrypsin activity: ala-ala-phe-MCA; AAPF = chymotrypsin activity: N-succinyl-ala-ala-pro-phe-MCA; QAR = trypsin activity: Boc-gln-ala-arg-MCA; FSR = trypsin activity: N-t-boc-phe-ser-arg-MCA	unitless
rep1_rate	replicate 1 of enzymatic hydrolysis rate	nanomol monosaccharide/liter/hour
rep2_rate	replicate 2 of enzymatic hydrolysis rate	nanomol monosaccharide/liter/hour
rep3_rate	replicate 3 of enzymatic hydrolysis rate	nanomol monosaccharide/liter/hour
average	average of the 3 hydrolysis rates	nanomol monosaccharide/liter/hour
std_dev	standard deviation of the 3 hydrolysis rates	nanomol monosaccharide/liter/hour

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	30 liter Niskin bottles
Generic Instrument Name	Niskin bottle
Dataset-specific Description	Used to collect water for large volume mesocosm experiments
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset-specific Instrument Name	TECAN spectrafluor plus
Generic Instrument Name	plate reader
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

EN556

Website	https://www.bco-dmo.org/deployment/717216
Platform	R/V Endeavor
Start Date	2015-04-27
End Date	2015-05-02
Description	Project: Latitudinal and Depth-Related Contrasts in Enzymatic Capabilities of Pelagic Microbial Communities. Cruise track obtained from rvdata.us control-point navigation (http://www.rvdata.us/catalog/EN556)

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Project Information

Latitudinal and depth-related contrasts in enzymatic capabilities of pelagic microbial communities: Predictable patterns in the ocean? (Patterns of activities)

Coverage: Atlantic Ocean, Arctic Ocean, Pacific Ocean, Greenland

NSF Award Abstract:
Heterotrophic microbial communities are key players in the marine carbon cycle, transforming and respiring organic carbon, regenerating nutrients, and acting as the final filter in sediments through which organic matter passes before long-term burial. Microbially-driven carbon cycling in the ocean profoundly affects the global carbon cycle, but key factors determining rates and locations of organic matter remineralization are unclear. In this study, researchers from the University of North Carolina at Chapel Hill will investigate the ability of pelagic microbial communities to initiate the remineralization of polysaccharides and proteins, which together constitute a major pool of organic matter in the ocean. Results from this study will be predictive on a large scale regarding the nature of the microbial response to organic matter input, and will provide a mechanistic framework for interpreting organic matter reactivity in the ocean.

Broader Impacts: This study will provide scientific training for undergraduate and graduate students from underrepresented groups. The project will also involve German colleagues, thus strengthening international scientific collaboration.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1332881

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