Measurements of peptidase and glucosidase activities in large volume mesocosm incubations on RV/Endeavor EN584, July 2016 (Patterns of activities project)

Website: https://www.bco-dmo.org/dataset/717532
Data Type: experimental
Version: 1
Version Date: 2022-12-07

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
Soenen, Karen	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Measurements of peptidase and glucosidase activities in large volume mesocosm incubations on RV/Endeavor EN584, July 2016 (Patterns of activities project)

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Coverage

Temporal Extent: 2016-07-01

Dataset Description

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].

See Niskin Bottle and Cast List EN584 to link specific casts and bottles to each experiment: BCO-DMO dataset 717427 (see related datasets).

Methods & Sampling

For mesocosm (large volume) incubation experiments (referred to as “LV” incubations), a 30L Niskin bottle rosette was used to collect the water. Separate casts were used to collect surface water, bottom water, and water from the depth at which oxygen showed a minimum, according to the CTD. From each depth, 20L seawater from single Niskin bottles was dispensed using cleaned silicon tubing into a single carboy. Prior to filling, carboys were rinsed 3x with water from the same Niskin bottle used to fill the carboy. Four carboys were filled at each depth. Triplicate 20L carboys were amended with ca. 500 mg (exact mass was recorded for each addition) of HMW Thalassiosira; unamended single carboys were used for controls. All mesocosms were incubated in the dark at near in-situ temperatures. Mesocosms were sub-sampled at the start of incubation (0 days), and then after 2 d, 7d, and 16d for the following assays: bacterial production using 3H-Leucine, dissolved organic carbon (DOC), nutrients, bacterial cell counts, peptidase and glucosidase activity measurements.

Two substrates, a-glucose and b-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].

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
- added cruise_id column
- replaced 'na' with 'nd' (no data)

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Data Files

File
en584_lv_platereader.csv (Comma Separated Values (.csv), 576.72 KB) MD5:02ec21d2c1571cc05420330b6ea98eda Primary data file for dataset ID 717532

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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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Related Datasets

References

Arnosti, C. (2017) **Notes on Niskin bottle sampling and use: depth of sample, operator, observations, type of experiments run on the sample, from RV/Endeavor EN556 and EN584, 2015 and 2016 (Patterns of activities project).** Biological and Chemical Oceanography Data Management Office (BCO-DMO). Version Date 2017-10-20 http://lod.bco-dmo.org/id/dataset/717427 [view at BCO-DMO]
Relationship Description: See Niskin Bottle and Cast List EN584 to link specific casts and bottles to each experiment.

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Parameters

Parameter	Description	Units
timepoint	Sampling point post incubation	unitless
time_elapsed_hr	Elapsed time, incubation time	hours (hr)
cruise_id	cruise identifier	unitless
station	station number	unitless
cast	cast number	unitless
depth_id	depth identifier, sequence of depths sampled	unitless
depth_m	Actual depth of water collection	meters (m)
meso_no	Mesocosm number	unitless
treatment	treatment, amended or unamended	unitless
substrate	Substrates for measurement of enzymatic activities: a-glu = substrate to measure alpha glucosidase: 4-methylumbelliferyl-a-D-glucopyranoside; b-glu = substrate to measure beta glucosidase: 4-methylumbelliferyl-ß-D-glucopyranoside; 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
subtimepoint	Mesocosm subsampling	unitless
timepoint_average	Average of three plate replicates at an indiviual timepoint for a given Mesocosm subsampling	nmol/L/hr
timepoint_std_dev	Standard deviation of average enzymatic activity at an indiviual timepoint for a given Mesocosm subsampling	nmol/L/hr
subtimepoint_average	Average enzymatic activity across timepoints for a given Mesocosm subsampling	nmol/L/hr
subtimepoint_std_dev	Standard deviation of average enzymatic activity across timepoints for a given Mesocosm subsampling	nmol/L/hr

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Instruments

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
Dataset-specific Description	Used to measure fluorescence of incubated 96-well plate. Settings: 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.

Dataset-specific Instrument Name	
Generic Instrument Name	Shipboard Incubator
Generic Instrument Description	A device mounted on a ship that holds water samples under conditions of controlled temperature or controlled temperature and illumination.

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Deployments

EN584

Website	https://www.bco-dmo.org/deployment/717087
Platform	R/V Endeavor
Start Date	2016-06-29
End Date	2016-07-13
Description	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/EN584)

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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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