Microbial enzymatic activities from seawater and from particle-associated seawater communities from Greenland, August 2015 (Patterns of activities project)

Website: https://www.bco-dmo.org/dataset/717660
Data Type: experimental
Version: 1
Version Date: 2017-10-30

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

Contributors	Affiliation	Role
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Abstract
Bacterial activity as measured by hydrolysis rates from unfiltered seawater and particle-associated communities collected near shore in northeastern Greenland in August 2015.

Table of Contents

Coverage

Spatial Extent: **Lat**:74.46 **Lon**:-20.574
Temporal Extent: 2015-08-02

Dataset Description

Bacterial activity as measured by hydrolysis rates from unfiltered seawater and particle-associated communities collected near shore in northeastern Greenland in August 2015.

Methods & Sampling

Using a small boat, samples were collected in 20L carboys in Tylerfjord-Young Sound. Three rivers that feed into Tyrolerfjord-Young Sound (Tyroler River, Lerbugten River and Zackenberg River) were sampled; surface and subsurface water samples were also collected at transition sites where the rivers feed into the fjord (Tyro_01, Zac_30, Ler_30, altogether referred to as 'river transition sites'). Enzyme activities were measured in unfiltered water. In addition, water was size-fractionated using gravity filtration through a GF/A filter to capture ≥1.6 µm particles.

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]. All substrates were used to measure enzyme activities in unfiltered water, as well as particle-associated (≥1.6 µm) enzymatic activities.

In unfiltered water, enzyme activities were measured by adding 4 mL of water to triplicate cuvettes. One incubation containing autoclaved water served as the killed control. This procedure was applied to each of the 7 substrates and one live blank and autoclave blank (no substrate addition). Each cuvette containing either live or autoclaved water was amended with one substrate to a concentration of 100 µM. Fluroescence was measured using a Promega Quantifluor solid-state single-cuvette fluorimeter; excitation and emission maxima were 365 nm and 410–450 nm, respectively,

To measure particle-associated enzyme assays, 1/12th piece of a GF/A filter through which water had been gravity filtered was put into a cuvette containing 4 mL of cooled, autoclaved water from the same station/depth as the live samples. In addition, killed controls were set up using sterile GF/A filters cut into 1/12th pieces. Bulk water and particle-associated enzyme assays were incubated for up to 24 and 16 hours, respectively; timepoints were taken at specific intervals. Incubations were kept in the dark either at 0°C, 5°C, or 8°C, depending on in situ water temperature at the time of sampling.

Activities of polysaccharide hydrolases were measured using fluorescently labeled polysaccharides (Arnosti 2003). Activities of enzymes that hydrolyze pullulan, laminarin, xylan, fucoidan, arabinogalactan, and chondroitin sulfate were measured in unfiltered water, and using GF/A filters through which water had been gravity-filtered. For these measurements, substrate was added (of 3.5 µM monomer equivalent) to 15 mL of water; autoclaved ambient water served as the killed control. Particle-associated activities were measured by submerging 1/12th of a GF/A filter in 15 ml autoclaved seawater. Samples were incubated in the dark at near in situ temperature (0°C, 5°C, or 8°C), and sub-sampled at specific time intervals—t0 (0h, upon substrate addition), t1 (120 h), t2 (240 h), t3 (360 h) and t4 (600 h). Sub-samples from each timepoint were filtered using 0.2 µM pore size SFCA (surfactant-free cellulose acetate) syringe filters, and the filtrate was collected in tubes and frozen at -20°C until processing in the lab. Sub-samples were processed using gel permeation chromatography (Arnosti, 2003).

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 trip_id, bulk_or_not, FLA_MCAMUF columns
- reduced the decimal precision of time_elapsed_hr and the rates columns to 3.
- sorted by sample_type, fluorophore, station, cast, depth_id

[table of contents | back to top]

Data Files

File	
Greenland_hydrolysis_all.csv	(Comma Separated Values (.csv), 96.64 KB) MD5:537026237ab33549bb14a3729e2cf27d
Primary data file for dataset ID 717660	

[table of contents | back to top]

Related Publications

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

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

[table of contents | back to top]

Parameters

Parameter	Description	Units
trip_id	sampling trip identifier; YS means Young Sound	unitless
sample_type	indication of whether sample was filtered (GF) or not (bulk)	unitless
fluorophore	fluorescent molecules used to measure hydrolysis rates: fluorescently-labeled polysaccharides (FLA) or small substrate proxies tagged with methylcoumarine (MCA) and methylumbelliferone (MUF) fluorophores.	unitless
cast	cast identifier	unitless
station	station identifier	unitless
depth_id	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: ara = arabinogalactan; chn = chondroitin sulfate; fuc = fucoidan; lam = laminarin; pul = pullulan; xyl = xylan	unitless
timepoint	sampling time point (0; 1; 2; etc.) post-incubation	unitless
time_elapsed_hr	hours elapsed to reach a specific timepoint	hours
rep1_rate	replicate 1 hydrolysis rate	nanomol monomer/liter/hour
rep2_rate	replicate 2 hydrolysis rate	nanomol monomer/liter/hour
rep3_rate	replicate 3 hydrolysis rate	nanomol monomer/liter/hour
rate_average	average of the 3 hydrolysis rates	nanomol monomer/liter/hour
rate_std_dev	standard deviation of the 3 hydrolysis rates	nanomol monomer/liter/hour
filter_um	filter size	nanomol monomer/liter/hour

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Promega Quantifluor solid-state single-cuvette fluorimeter
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	20 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.

[table of contents | back to top]

Deployments

zodiac_Arnosti

Website	https://www.bco-dmo.org/deployment/717659
Platform	small boat Zackenber Res. Sta.
Start Date	2015-08-02
End Date	2015-08-17
Description	Sample collection and experiments for project "Latitudinal and depth-related contrasts in enzymatic capabilities of pelagic microbial communities: Predictable patterns in the ocean?"

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

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

[table of contents | back to top]