Peptidase and glucosidase activities from mesocosm and bulk water incubations from waters taken aboard the R/V Endeavor in the Western North Atlantic during the research cruise EN683 in May and June, 2022.

Website: https://www.bco-dmo.org/dataset/956085
Data Type: Other Field Results
Version: 1
Version Date: 2025-03-17

Project
» Substrate structural complexity and abundance control distinct mechanisms of microbially-driven carbon cycling in the ocean (Substrate complexity and microbes)

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

Abstract
This dataset includes the measurements of leucine aminopeptidase, glucosidase, and endo-acting (mid-chain cleaving) peptidase activities from bulk and mesocosm incubations from waters taken aboard the R/V Endeavor in the Western North Atlantic during the research cruise EN683 (2022-05-24 to 2022-06-12). Waters for bulk incubation were taken at three sites and various depths, mesocosm incubations were taken at two sites and two depths. This research tested the hypothesis that the mechanism of polysaccharide processing is related to the cost to a cell of producing the enzymes required for its hydrolysis, and the probability that a cell will receive sufficient return on investment for producing the enzymes. Our conceptual model suggests that external (extracellular) hydrolysis is favored when organic matter is abundant, or when enzyme production costs can be shared (e.g., on particles, in biofilms); selfish uptake (hydrolysis without production of low molecular weight products in the environment) would be a better strategy when high molecular weight (HMW) organic matter is scarce, and particularly when the HMW organic matter is very complex. Seawater was sampled from four depths at our initial station, and the deep chlorophyll maximum and bottom water from two subsequent stations differing in typical extent of primary productivity. We incubated mesocosms of seawater from the deep chlorophyll maximum and from bottom water from two stations differing in typical extent of primary productivity. Organic matter availability was amended by adding high molecular weight organic matter (dissolved and particulate) from diatoms, or by adding the polysaccharide fucoidan, or by adding the polysaccharide arabinogalactan. Amendments were performed in triplicate mesocosms, while one mesocosm was left unamended for a total of ten mesocosms from each depth and station tested These data help test this model because they provide information about the activities of exo-acting glucosidases and an exo-acting peptidase, plus endo-acting peptidases, all of which are used to hydrolyze high molecular weight organic matter. Activities of these enzymes differed by station as well as by depth

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Table of Contents

- Project Information
- Funding

Coverage

Location: Western North Atlantic
Spatial Extent: **N**:42.870093 **E**:-53.873585 **S**:33.7441666 **W**:-75.296666
Temporal Extent: 2022-05-22 - 2022-06-09

Methods & Sampling

Mesocosm incubations:

For mesocosm (large volume) incubation experiments (referred to as “LV” incubations), 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. Four carboys were filled from bottom water and deep chlorophyll maximum (DCM) water each, according to the CTD. Triplicate 20L carboys (a total of 9 carboys) were amended with high molecular weight material isolated from the diatom Thalassiosira, or the polysaccharide fucodian, or the polysaccharide arabinogalactan; unamended single carboys were used for controls. 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 to serve as a killed control for microbial activity measurements. All mesocosms were incubated in the dark at near in-situ temperatures. Mesocosms were sub-sampled for peptidase and glucosidase activity measurements at the start of incubation (0 days), and then after at approximately 5d, 10d, 15d, and 25d. To measure gluosidase and peptidase activities at each timepoint, water was collected from the mesocosms as described above.

For these measurements, seven glucosidase and peptidase substrates were set up in a 96-well plate. The substrates used included alpha-glucose and beta-glucose linked to a 4-methylumbelliferyl (MUF) fluorophore to measure exo-acting glucosidase activities. Five substrates linked to a 7-amido-4-methyl coumarin (MCA) fluorophore, including 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. 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 identify 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. Bottom water measurements were made in a cold van/cold room at 4 C; water from the deep chlorophyll maximum was incubated at room temperature.

Bulk water incubations:

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, alpha-glucose and beta-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 identify 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 0-72 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. Calculations followed the procedure outlined in the tutorial available in the associated Github repository (https://github.com/ArnostiLab/ArnostiLab-RScript-Demo-PlateRdr/tree/master/scripts (DOI: 10.5281/zenodo.14783119).

BCO-DMO Processing Description

* Merged bulk water and mesocosm incubations data into 1 dataset (added Sample_type parameter to differentiate between the 2 datasets)
* Added ISO_DateTime UTC variable to data
* Merged bulk water and mesocosm incubations metadata into 1 landing page
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[table of contents | back to top]

Data Files

File
956085_v1_peptidase.csv (Comma Separated Values (.csv), 274.50 KB) MD5:67a1cca8ac8dce5a3e798a0ca97c89cb Primary data file for dataset ID 956085, version 1

[table of contents | back to top]

Related Publications

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

ahoarfrost. (2025). ArnostiLab/ArnostiLab-RScript-Demo-PlateRdr: ArnostiLab-RScript-Demo-PlateRdr (Version v1.0.0) [Computer software]. Zenodo. https://doi.org/10.5281/ZENODO.14783119 https://doi.org/10.5281/zenodo.14783119
Software

[table of contents | back to top]

Related Datasets

IsRelatedTo

Ghobrial, S., Arnosti, C. (2025) **Bacterial Productivity measurement of bulk seawater and mesocosm experiments taken aboard the R/V Endeavor in the Western North Atlantic during the research cruise EN683 in May and June, 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-10-02 http://lod.bco-dmo.org/id/dataset/985783 [view at BCO-DMO]
Relationship Description: Includes data from mesocosm experiments performed using seawater samples collected on EN683.

[table of contents | back to top]

Parameters

Parameter	Description	Units
Incubation	Mesocosm or Bulk Water Incubation	unitless
deployment	Cruise ID	unitless
Station	Station number for cruise	unitless
longitude	Longitude, west is negative	decimal degrees
latitude	Latitude, south 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
ISO_DateTime_UTC	DateTime of sample collection in ISO format in GMT/UTC Time	unitless
cast_number	Cast number (refers to cast of CTD/Niskin bottles on cruise)	unitless
depth_actual	Actual depth at which water was collected	meters (m)
sample_type	Sample from bulk water or Large Volume incubation	unitless
Incubation_Temp	Temperature of incubation, RT (~20-25°C).	Degrees Celsius (°C)

unamended_amended	Whether high molecular weight organic mater was added or not; U for unamended, F, A, T refer to type of organic mater added (Fucodian, Arabinogalactan, Thalassiosira extract), the following number corresponds to amended incubation replicate.	unitless
Sub_sample_day	Days post amendment when subsample was taken prior to substate addition and enzymatic activity measurement.	days
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
rate_6hr	Measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-0
sd_6hr	Standard deviation of measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1
rate_12hr	Measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1
sd_12hr	Standard deviation of measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1
rate_18hr	Measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1
sd_18hr	Standard deviation of measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1
rate_24hr	Measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1
sd_24hr	Standard deviation of measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1
rate_36hr	Measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1

sd_36hr	Standard deviation of measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1
rate_48hr	Measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1
sd_48hr	Standard deviation of measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1
rate_72hr	Measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1
sd_72hr	Standard deviation of measured hydrolysis rate at indicated time interval (6hr, 12hr, 18hr, ...) post substrate addition. Blank = not measured	nmol L-1 h-1

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	TECAN infiniteF200; 360 nm excitation, 460 emission
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.

[table of contents | back to top]

Deployments

EN683

Website	https://www.bco-dmo.org/deployment/956118
Platform	R/V Endeavor
Start Date	2022-05-24
End Date	2022-06-12

[table of contents | back to top]

Project Information

Substrate structural complexity and abundance control distinct mechanisms of microbially-driven carbon cycling in the ocean (Substrate complexity and microbes)

Coverage: Western North Atlantic

Substrate Structural Complexity and Abundance Control Distinct Mechanisms of Microbially-Driven Carbon Cycling in the Ocean

Almost half of the organic carbon produced in the ocean is processed by bacteria. Bacteria use extracellular (outside the cell) enzymes to break down large organic molecules to small sizes that can be transported into their cells. It has recently been discovered that bacteria use extracellular enzymes in two ways: 'selfish uptake' and 'external hydrolysis'. External hydrolysis releases low molecular weight products to the environment where they can be used by other organisms. 'Selfish uptake' releases little or no products. This research will determine the extent and location of 'selfish uptake' in ocean waters. This process affects the distribution of organic carbon in the ocean, the flow of small organic molecules to feed a wider range of bacteria, and the composition and dynamics of the bacterial community. Recent results show that 'selfish' bacteria are active in deep ocean waters, where they take up complex polysaccharides (sugars) that are not hydrolyzed externally. These results inspired a new model that links 'selfish uptake' and external hydrolysis to the amount and complexity of the organic matter that is used by bacteria. This project will test the model by describing the polysaccharide fraction of marine organic matter, and studying the relationships between organic matter abundance, structural complexity, and extracellular enzyme use. Graduate and undergraduate students will participate in the project as members of the research team in the field and in the laboratory.

This research will test the hypothesis that the mechanism of polysaccharide processing is related to the cost to a cell of producing the enzymes required for its hydrolysis, and the probability that a cell will receive sufficient return on investment for producing the enzymes. The conceptual model that will be tested suggests that external hydrolysis is favored when organic matter is abundant, or when enzyme production costs can be shared (e.g., on particles, in biofilms); selfish uptake would be a better strategy when high molecular weight (HMW) organic matter is scarce, and particularly when the HMW organic matter is very complex. This study will test this model by characterizing the structure of polysaccharide-containing components of dissolved organic matter (DOM) and particulate organic matter (POM) collected from the ocean, by determining the extent of selfish uptake and rates of external hydrolysis of different polysaccharides by natural microbial communities from the surface and the deep ocean, and by incubation experiments that control for the abundance of polysaccharides of different structural complexity. This project will be carried out in collaboration with colleagues at the Max Planck Institute for Marine Microbiology, whose expertise in carbohydrate chemistry and structural analyses, and in advanced microscopy and analysis of complex microbial communities, are central to the project.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2022952

[table of contents | back to top]