Peptidase and glucosidase activities from bulk water incubations from waters taken aboard the R/V Thomas G. Thompson in the Southern Indian Ocean during the research cruise TN362 in November and December, 2018

Website: https://www.bco-dmo.org/dataset/996093
Data Type: Cruise Results
Version: 1
Version Date: 2026-04-06

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

Contributors	Affiliation	Role
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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 existence of a size-reactivity continuum of DOM - observations and measurements showing that HMW (high-molecular-weight) DOM tends to be younger and more reactive than lower MW (molecular-weight) 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. Here we investigated the potential of seawater microbial communities from different water masses to hydrolyze six high-molecular-weight polysaccharides (arabinogalactan, chondroitin sulfate, fucoidan, laminarin, pullulan, and xylan). This dataset includes the measurement of peptidase and glucosidase activities from samples taken from bulk water incubations from various stations and depths aboard the R/V Thomas G. Thompson in the Southern Indian Ocean during the research cruise TN362 in November and December, 2018.

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Coverage

Location: Southern Indian Ocean; Various stations from approximately 30 to 42 South and 78 to 110 East; Sampling depths varied from 3 to 5090 meters.
Spatial Extent: **N**:-31.299 **E**:100.482 **S**:-41.646 **W**:96.55
Temporal Extent: 2018-11-09 - 2018-12-12

Methods & Sampling

Water was collected via Niskin bottles mounted on a rosette, equipped with a CTD.

From the Niskin bottle, water was dispensed into smaller glass containers that were cleaned and pre-rinsed three times with water from the Niskin bottle 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 Niskin bottle 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 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 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) and 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 (Hoarfrost et al., 2017).

BCO-DMO Processing Description

- Loaded CSV file "20251229_Plate_RatesBulk_Timepoint_TN362_BCO-DMO.csv" with header row 1; treated "", "nd", and "NA" as missing values; resource named by filename
- Converted date field from "%Y.%m.%d" format to ISO "%Y-%m-%d" date type
- Exported file as "996093_v1_plate_rates_bulk_water_tn362.csv"

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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
,
Software

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 3, 6, 13, 18, or 31	unitless
latitude	Latitude of sampling site, south is negative	decimal degrees
longitude	Longitude of sampling site, west is negative	decimal degrees
date	Date of sample collection	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	m
sample_type	Sample from bulk water (bulk) or Large Volume incubation (LV)	unitless
Incubation_temp	Temperature of incubation. RT = Room Temperature (~20 C)	degrees Celsius
unamended_amended	Whether high molecular weight organic matter was added or not; U for unamended.	unitless
substrate	Substrates for measurement of enzymatic activities: • a.glu= substrate to measure alpha glucosidase: 4-methylumbelliferyl-_-D- • b.glu=substrate to measure beta glucosidase: 4-methylumbelliferyl-_-D- • Leu=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
t1_rate	Average of three plate replicates taken at ~ 6 hours	nmol L-1 hr-1
t1_sd	Standard deviation of hydrolysis rates for t1	nmol L-1 hr-1

t2_rate	Average of three plate replicates taken at ~ 12 hours	nmol L-1 hr-1
t2_sd	Standard deviation of hydrolysis rates for t2	nmol L-1 hr-1
t3_rate	Average of three plate replicates taken at ~ 18 hours	nmol L-1 hr-1
t3_sd	Standard deviation of hydrolysis rates for t3	nmol L-1 hr-1
t4_rate	Average of three plate replicates taken at ~ 24 hours	nmol L-1 hr-1
t4_sd	Standard deviation of hydrolysis rates for t4	nmol L-1 hr-1
t5_rate	Average of three plate replicates taken at ~ 36 hours	nmol L-1 hr-1
t5_sd	Standard deviation of hydrolysis rates for t5	nmol L-1 hr-1
t6_rate	Average of three plate replicates taken at ~ 48 hours	nmol L-1 hr-1
t6_sd	Standard deviation of hydrolysis rates for t6	nmol L-1 hr-1
t7_rate	Average of three plate replicates taken at ~ 72 hours	nmol L-1 hr-1
t7_sd	Standard deviation of hydrolysis rates for t7	nmol L-1 hr-1
max_mean	Maximum rate calculated from all timepoints	nmol L-1 hr-1
max_sd	Standard deviation of maximum rate calculated from timepoints	nmol L-1 hr-1
max_timepoint_id	Time point at which maximum rate was calculated	unitless
avg_potential_rate	Average rate from all timepoints	nmol L-1 hr-1
potential_sd	Standard deviation of average rate from all timepoints	nmol L-1 hr-1

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Instruments

Dataset-specific Instrument Name	CTD
Generic Instrument Name	CTD Sea-Bird SBE 911plus
Dataset-specific Description	Water was collected via Niskin bottles mounted on a rosette, equipped with a CTD.
Generic Instrument Description	The Sea-Bird SBE 911 plus is a type of CTD instrument package for continuous measurement of conductivity, temperature and pressure. The SBE 911 plus includes the SBE 9plus Underwater Unit and the SBE 11plus Deck Unit (for real-time readout using conductive wire) for deployment from a vessel. The combination of the SBE 9 plus and SBE 11 plus is called a SBE 911 plus. The SBE 9 plus uses Sea-Bird's standard modular temperature and conductivity sensors (SBE 3 plus and SBE 4). The SBE 9 plus CTD can be configured with up to eight auxiliary sensors to measure other parameters including dissolved oxygen, pH, turbidity, fluorescence, light (PAR), light transmission, etc.). more information from Sea-Bird Electronics

Dataset-specific Instrument Name	Niskin bottles
Generic Instrument Name	Niskin bottle
Dataset-specific Description	Water was collected via Niskin bottles mounted on a rosette, equipped with a CTD.
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 infiniteF200
Generic Instrument Name	plate reader
Dataset-specific Description	Methods Description: 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. Instrument 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

TN362

Website	https://www.bco-dmo.org/deployment/996056
Platform	R/V Thomas G. Thompson
Start Date	2018-11-07
End Date	2018-12-19
Description	Project: Indian Ocean coring

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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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