Carbohydrate microarray (epitope) analyses of POM-derived carbohydrates in the Western North Atlantic Ocean in May 2019

Website: https://www.bco-dmo.org/dataset/985786
Data Type: Cruise Results
Version: 1
Version Date: 2025-10-03

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 carbon (DOC) is one of the largest actively-cycling carbon reservoirs on earth—comparable in magnitude to atmospheric CO2 (Hansell 2013)—and thus is an essential component of the global carbon cycle. DOC has a multitude of sources, including phytoplankton productivity, grazing, excretion, solubilization from particulate organic matter (POM), viral lysis, and riverine input; the major DOC sink in the ocean is consumption by heterotrophic microbial communities (Carlson & Hansell 2015). Measurement of bulk DOC characteristics such as 14C age and molecular size have demonstrated that the high molecular weight (HMW) DOC fraction is generally younger and more biologically reactive than the low molecular weight (LMW) fraction (e.g. Guo et al. 1996; Walker et al. 2016). We know that a substantial fraction of HMW DOC consists of carbohydrates, including neutral sugars, and that its concentration is lower in the deep ocean than in the upper mesopelagic/surface ocean (Benner & Amon 2015). Characterization of the HMW DOC fraction has primarily used chemical measurements that provide information about monomeric constituents (Benner et al. 1992; Kaiser & Benner 2009), but yield no information on the order in which such constituents are linked together, or about the 3D structure of the intact HMW DOC. Beyond these observations and measurements, however, the specific factors controlling the rate, location, and extent to which DOC is transformed and remineralized by heterotrophic microbial communities in the ocean are still not well understood. A key focus of this project's field work is investigating the potential of marine heterotrophic microbial communities from different water masses and under differing conditions of organic matter availability to hydrolyze six well-characterized high-molecular-weight (HMW) polysaccharides (arabinogalactan, chondroitin sulfate, fucoidan, laminarin, pullulan, and xylan). To better contextualize this hydrolysis, bulk water characterization of the deep chlorophyll maximum (DCM), oxygen minimum zone (OMZ), and bottom waters used in our mesocosm incubation experiments was performed. This dataset includes the measurement of carbohydrate microarray (epitope) analyses of POM-derived carbohydrates from bulk waters collected in the western North Atlantic Ocean aboard R/V Endeavor (EN638) during May 2019. Note that the carbohydrate microarray data are only semiquantitative; while comparisons can be made for the abundance of a given epitope between stations and depths, the signal intensity cannot be used to compare signals of different epitopes, since the binding affinity for individual epitopes differs between antibodies. Note also that analyses were performed for all sampled depths at all stations. There was, however, no positive signal at depths that are shown as "0" in this dataset.

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Coverage

Location: Western North Atlantic, stations 17, 18, 19, and 20. Water samples were taken at the depth of the deep chlorophyll maximum, the oxygen minimum zone, and at the bottom.
Spatial Extent: **N**:42.83954 **E**:-53.3949 **S**:34.50102 **W**:-75.67819
Temporal Extent: 2019-05-14 - 2019-05-25

Methods & Sampling

Collection

Water was collected via Niskin bottles mounted on a rosette, equipped with a CTD in the western North Atlantic Ocean aboard R/V Endeavor (EN638) during May 2019. Seawater was transferred to carboys that were rinsed three times with water from the sampling depth and then filled with seawater from a single Niskin bottle.

Particulate organic matter (POM) was harvested by filtering between 5-15 liters of seawater through a 47-mm pre-combusted (400°C for 6 hours) glass fiber filter (GF/F; nominal pore size 0.7 µm; for volumes filtered at each depth and station, see the dataset). Filers were stored at -80C until further analysis. The same filter was used for both particulate organic carbon (POC) measurements and monosaccharide composition analysis.

Analysis

Polysaccharide extraction for microarray analyses of POM

POM samples were prepared for polysaccharide analysis according to Vidal-Melgosa et al. (2021). Polysaccharides were sequentially extracted from four filter piece punches (11.2 mm diameter) from GF/F filters. The samples were first extracted with autoclaved MilliQ water, followed by 50 mM EDTA, and finally 4 M NaOH with 0.1% $NaBH_4$. The supernatant containing extracted polysaccharides was collected from each of the sequential steps and stored at 4 °C.

Carbohydrate microarray analysis to determine structural complexity of POM

The polysaccharides extracted as described above were analyzed following Vidal-Melgosa et al. (2021). In brief, the polysaccharide extracts were first diluted in printing buffer (55.2% glycerol, 44% water, 0.8% Triton X-100), and then printed on 0.45 µm pore size nitrocellulose membrane (Whatman) using a microarray robot (Sprint, Arrayjet, Roslin, UK) at 20 °C and 50% humidity. The membranes were probed with one of 9 monoclonal antibodies, washed multiple times, and probed with secondary antibodies (anti-rat, anti-mouse, or anti-His tag) conjugated to alkaline phosphatase for 2 hours. The arrays were developed using 5-bromo-4-chloro-3-indolyphosphate and nitro blue tetrazolium in alkaline phosphatase buffer (100 mM NaCl, 5 mM $MgCl_2$, 100 mM Tris-HCl, pH 9.5). The microarrays were scanned and signal intensity was acquired using the software Array-Pro Analyzer 6.3 (Media Cybernetics). Signals were normalized among samples; higher signals correspond to a higher abundance of a given polysaccharide epitope. Note that the carbohydrate microarray data are only semiquantitative; while comparisons can be made for the abundance of a given epitope between stations and depths, the signal intensity cannot be used to compare signals of different epitopes.

Data Processing Description

Data processed using Microsoft Excel.

BCO-DMO Processing Description

- Imported "20250724_Carbohydrate Microarray (epitope) Analyses_Data_BCODMO.csv" into the BCO-DMO system
- Converted "date" and "time" to one date time parameter, "ISO_DateTime_EST"
- Created a new datetime parameter in UTC "ISO_DateTime_UTC"
- Removed "date" and "time" as redundant
- Replaced "Stn" string with "stn" string in the "station" field to be consistent with other datasets in this project
- Exported file as "985786_v1_carb_microarray_epitope.csv"

Problem Description

Note that the carbohydrate microarray data are only semiquantitative; while comparisons can be made for the abundance of a given epitope between stations and depths, the signal intensity cannot be used to compare signals of different epitopes, since the binding affinity for individual epitopes differs between antibodies. Note also that analyses were performed for all sampled depths at all stations. There was, however, no positive signal at depths that are shown as "0" in this dataset.

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Related Publications

Vidal-Melgosa, S., Sichert, A., Francis, T. B., Bartosik, D., Niggemann, J., Wichels, A., Willats, W. G. T., Fuchs, B. M., Teeling, H., Becher, D., Schweder, T., Amann, R., & Hehemann, J.-H. (2021). Diatom fucan polysaccharide precipitates carbon during algal blooms. Nature Communications, 12(1). https://doi.org/10.1038/s41467-021-21009-6
Methods

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Related Datasets

IsRelatedTo

Lloyd, C., Vidal, S., Arnosti, C., Ghobrial, S. (2025) **Particulate organic carbon concentrations and monosaccharide composition of POM-derived carbohydrates from samples taken during R/V Endeavor cruise EN638 in the Western North Atlantic in May 2019.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-10-03 http://lod.bco-dmo.org/id/dataset/985784 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
deployment	Cruise ID on R/V Endeavor	unitless
station	Cruise station number (17, 18, 19, 20)	unitless
latitude	Latitude, south is negative	decimal degrees
longitude	Longitude, west is negative	decimal degrees

ISO_DateTime_Local	Datetime of sample collection in ISO format, US Eastern Time (ET)	unitless
ISO_DateTime_UTC	Datetime of sample collection in ISO format, UTC	unitless
cast_number	Cast number at station (refers to cast of CTD/Niskin bottles on cruise at each station)	unitless
depth_sequence	Sequence of depths sampled (d1 is surface; higher numbers at greater depths; d2 is DCM = deep chlorophyll maximum as indicated by chlorophyll-a detection via CTD, etc.)	unitless
depth_actual	Actual depth at which water was collected	meters
POM_L_filtered	The amount of seawater filtered (liters) for carbohydrate microarray (epitope) analyses of POM-derived carbohydrates	Liters
epitope	The specific polysaccharide epitope detected for each sample. Note these are relative to the specific antibodies; therefore, you can compare signals between stations/depths between an individual epitope, but cannot compare signals between epitopes, as the binding affinity for a specific polysaccharide structure varies between epitopes	unitless
H2O	The semi-quantiative signal output for each antibody when H2O was used (initially) for extraction of the polysaccharides. Note that this was part of a sequential extraction, so polysaccharides that were fully extracted with H2O may not show up in subsequent extractions	unitless
EDTA	The semi-quantiative signal output for each antibody when EDTA was used for extraction of the polysaccharides following H2O. Note that this was a sequential extraction, so polysaccharides that were fully extracted may not show up in subsequent extractions	unitless
NaOH	The semi-quantiative signal output for each antibody when NAOH was used for extraction of the polysaccharides following H2O and EDTA	unitless

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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 in the western North Atlantic Ocean aboard R/V Endeavor (EN638) during May 2019.
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	Array-Pro Analyzer 6.3 (Media Cybernetics)
Generic Instrument Name	microarray scanner
Dataset-specific Description	The microarrays were scanned and signal intensity was acquired using the software Array-Pro Analyzer 6.3 (Media Cybernetics).
Generic Instrument Description	Microarray scanners are instruments used to detect and quantify the intensity of fluorescence of spots on a microarray slide, by selectively exciting fluorophores with a laser and measuring the fluorescence. A microarray scanner typically consists of lasers, a special microscope, and a camera. The DNA material in the microarray is labeled with fluorescents which become excited by the lasers in the scanner. The microscope and camera work together to create a digital image of the array.

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 in the western North Atlantic Ocean aboard R/V Endeavor (EN638) during May 2019.
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.

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Deployments

EN638

Website	https://www.bco-dmo.org/deployment/820578
Platform	R/V Endeavor
Start Date	2019-05-15
End Date	2019-05-30
Description	Underway datasets (and their DOIs) provided by R2R are the following. Click the cruise DOI for more general information ADCP: 10.7284/134159 Anemometer: 10.7284/134174 Anemometer: 10.7284/134176 CTD: 10.7284/134160 GNSS: 10.7284/134158 GNSS: 10.7284/134167 GNSS: 10.7284/134168 GNSS: 10.7284/134170 Gyrocompass: 10.7284/134161 Gyrocompass: 10.7284/134162 Met Station: 10.7284/134166 Radiometer: 10.7284/134163 Radiometer: 10.7284/134164 Singlebeam Sonar: 10.7284/134172 Speed Log: 10.7284/134169 Time Server: 10.7284/134171 TSG: 10.7284/134165 TSG: 10.7284/134173 Winch: 10.7284/134175

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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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