Detection and Quantification of Cells Exhibiting 'Selfish' Uptake in samples taken during R/V Endeavor cruise EN638 in the Western North Atlantic in May 2019

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Data Type: Cruise Results, experimental
Version: 1
Version Date: 2025-11-03

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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Lloyd, Chad	University of North Carolina at Chapel Hill (UNC-Chapel Hill)	Student
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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 carbon (POC), 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. Here we present, in collaboration with colleagues from the Max Planck Institute for Marine Microbiology, the detection and quantification of microbial cells exhibiting selfish uptake behavior of fluorescently-labeled HMW polysaccharides. This dataset includes sample collection metadata, environmental variables, experimental variables, the number of cells detected exhibiting "selfish" uptake, and total cellular abundance.

Table of Contents

Coverage

Location: Western North Atlantic, stations 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**:34.6369 **W**:-72.0021
Temporal Extent: 2019-05-17 - 2019-05-25

Methods & Sampling

Water was collected via Niskin bottles mounted on a rosette, equipped with a CTD, or from mesocosm (large volume) 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 at each depth from bottom water, water from the depth at which oxygen showed a minimum, and deep chlorophyll maximum (DCM) water, according to the CTD. 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 at approximately 3 d, 5 or 7 d, 10 d, 15 d, and 30 d for multiple assays including: bacterial production using 3H-Leucine, dissolved organic carbon (DOC), nutrients, bacterial cell counts, peptidase and glucosidase activity measurements in addition to the potential of the seawater microbial community to hydrolyze six high-molecular-weight polysaccharides (arabinogalactan, chondroitin sulfate, fucoidan, laminarin, pullulan, and xylan). Bacterial cell counts presented here are from unamended incubations

For each depth and mesocosm sample, 20-30 ml of 1% formaldehyde (FA) fixed sample were filtered through a 0.2 µm pore size poly-carbonate filter, applying a maximum vacuum of 200 mbar. Nucleic acids of filtered cells were counterstained with 4',6-diamidino-2-phenylindole (DAPI) and mounted using a Citifluor/VectaShield (4:1) solution. A fully automated epifluorescence microscope (Zeiss AxioImager.Z2 microscope stand, Carl Zeiss Jena, Germany) equipped with a cooled charged-coupled-device (CCD) camera (AxioCam MRm + Colibri LED light source, Carl Zeiss), a light-emitting diode for DAPI (UV-emitting LED, 365 nm) and a HE-62 multi filter module with a triple emission filter (425/50 nm, 527/54 nm, LP 615 nm, including a triple beam splitter of 395/495/610, Carl Zeiss). As described by Bennke *et al.,* 2016, a minimum of 45 fields of view (FOV) per sample were acquired using a 63x magnification oil immersion plan apochromatic objective with a numerical aperture of 1.4 (Carl Zeiss). Cell counting was performed with the image analysis software ACMETOOL (Zeder, M. 2005-2021, Software for Biology, http://www.technobiology.ch and Max Planck Institute for marine microbiology, Bremen). Validation of the automated counts was done by manual cell counting.

Data Processing Description

Image analysis was performed with ACMETOOL (Zeder, M. 2005-2021, Software for Biology, http://www.technobiology.ch and Max Planck Institute for marine microbiology, Bremen, version 3) and Zen software package (Carl Zeiss).

BCO-DMO Processing Description

- Opened "20250811_EN638_cellcount_selfish_BCODMO.csv" in Excel and removed all the replicate measurements, leaving only the sample information
- Removed the duplicate rows in this file to produce a unique list of all sample instances
- Saved file as "unique_sample_only.csv" and imported into the BCO-DMO system

- Imported "20250811_EN638_cellcount_selfish_BCODMO.csv" into the BCO-DMO system
- Duplicated "20250811_EN638_cellcount_selfish_BCODMO.csv" three times and filtered by "variable" creating three duplicates, each containing only one variable
- Joined these filtered datasets to the "unique_sample_only.csv" dataset
- Exported file as "988179_v1_en638_cellcount_selfish.csv"

[table of contents | back to top]

Related Publications

Bennke, C. M., Krüger, K., Kappelmann, L., Huang, S., Gobet, A., Schüler, M., Barbe, V., Fuchs, B. M., Michel, G., Teeling, H., & Amann, R. I. (2016). Polysaccharide utilisation loci of Bacteroidetes from two contrasting open ocean sites in the North Atlantic. Environmental Microbiology, 18(12), 4456–4470. Portico. https://doi.org/10.1111/1462-2920.13429
Methods

Bergauer, K., Fernandez-Guerra, A., Garcia, J. A. L., Sprenger, R. R., Stepanauskas, R., Pachiadaki, M. G., Jensen, O. N., & Herndl, G. J. (2017). Organic matter processing by microbial communities throughout the Atlantic water column as revealed by metaproteomics. Proceedings of the National Academy of Sciences, 115(3). https://doi.org/10.1073/pnas.1708779115
Methods

Giljan, G., Arnosti, C., Kirstein, I. V., Amann, R., & Fuchs, B. M. (2022). Strong seasonal differences of bacterial polysaccharide utilization in the North Sea over an annual cycle. Environmental Microbiology, 24(5), 2333–2347. Portico. https://doi.org/10.1111/1462-2920.15997
Methods

Giljan, G., Brown, S., Lloyd, C. C., Ghobrial, S., Amann, R., & Arnosti, C. (2023). Selfish bacteria are active throughout the water column of the ocean. ISME Communications, 3(1). https://doi.org/10.1038/s43705-023-00219-7
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
deployment	Cruise ID	unitless
station	Station number for cruise (18, 19, or 20)	unitless
latitude	Latitude, south is negative	decimal degrees
longitude	Longitude, west is negative	decimal degrees
ISO_DateTime_UTC	Date and time of sample collection in ISO format, US Eastern Time (UTC-05:00)	unitless
cast_number	Cast number (refers to cast of CTD/Niskin bottles on cruise)	unitless
depth_name	Water column feature or oceanic zone sampled (Surface, DCM, 300m, or bottom/near bottom)	unitless

depth_actual	Actual depth at which water was collected	meters (m)
sample_type	Sample from bulk water or Large Volume incubation	unitless
unamended_amended	Whether high molecular weight organic matter was added or not; U for unamended	unitless
substrate	Polysaccharide used for incubation: ara = arabinogalactan, chn = chondroitin sulfate, fuc = fucoidan, lam = laminarin, pul = pullulan, xyl = xylan, or control (no substrate added)	unitless
timepoint_days	Days post amendment when subsample was taken for substrate addition and enzymatic activity measurement	days
timepoint_hours	Hours post amendment when subsample was taken for substrate addition and enzymatic activity measurement	hours
replicate_1_selfish_percent	Replicate sample 1 of selfish percent. Blank value indicates sample not available for counting or autofluorescence	percent
replicate_2_selfish_percent	Replicate sample 2 of selfish percent. Blank value indicates sample not available for counting or autofluorescence	percent
replicate_3_selfish_percent	Replicate sample 3 of selfish percent. Blank value indicates sample not available for counting or autofluorescence	percent
average_selfish_percent	Average selfish percent of the three replicates. Blank value indicates sample not available for counting or autofluorescence	percent
standard_deviation_selfish_percent	Standard deviation of the average selfish percent of the three replicates. Blank value indicates sample not available for counting or autofluorescence	percent
replicate_1_substratecells	Replicate sample 1 of number of substrate cells per ml. Blank value indicates sample not available for counting or autofluorescence	Cells/ml
replicate_2_substratecells	Replicate sample 2 of number of substrate cells per ml. Blank value indicates sample not available for counting or autofluorescence	Cells/ml
replicate_3_substratecells	Replicate sample 3 of number of substrate cells per ml. Blank value indicates sample not available for counting or autofluorescence	Cells/ml

average_substratecells	Average number of substrate cells per ml of the three replicates. Blank value indicates sample not available for counting or autofluorescence	Cells/ml
standard_deviation_substratecells	Standard deviation of the average number of substrate cells per ml of the three replicates. Blank value indicates sample not available for counting or autofluorescence	Cells/ml
replicate_1_cells	Replicate sample 1 of number of cells per ml. Blank value indicates sample not available for counting or autofluorescence	Cells/ml
replicate_2_cells	Replicate sample 2 of number of cells per ml. Blank value indicates sample not available for counting or autofluorescence	Cells/ml
replicate_3_cells	Replicate sample 3 of number cells per ml. Blank value indicates sample not available for counting or autofluorescence	Cells/ml
average_cells	Average number of cells per ml of the three replicates. Blank value indicates sample not available for counting or autofluorescence	Cells/ml
standard_deviation_cells	Standard deviation of the average number of cells per ml of the three replicates. Blank value indicates sample not available for counting or autofluorescence	Cells/ml

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Zeiss LSM780 with Airyscan (Carl Zeiss)
Generic Instrument Name	Confocal Laser Scanning Microscope
Dataset-specific Description	Zeiss LSM780 with Airyscan (Carl Zeiss) using a 405 nm, a 488 nm, and a 561 nm laser with detection windows of 420–480 nm, 500–550 nm, and LP 605 nm, respectively.
Generic Instrument Description	A laser scanning confocal microscope is a type of confocal microscope that obtains high-resolution optical images with depth selectivity, in which a laser beam passes through a light source aperture and then is focused by an objective lens into a small (ideally diffraction-limited) focal volume within or on the surface of a specimen. The confocal microscope uses fluorescence optics. 'Confocal' means that the image is obtained from the focal plane only, any noise resulting from sample thickness being removed optically. 'Laser scanning' means the images are acquired point by point under localized laser excitation rather than full sample illumination, as in conventional widefield microscopy.

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, or from mesocosm (large volume) incubations.
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	Zeiss AxioImager.Z2 microscope stand, Carl Zeiss
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Zeiss AxioImager.Z2 microscope stand, Carl Zeiss - Fully automated epifluorescence microscope
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

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, or from mesocosm (large volume) incubations.
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

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

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1736772

[table of contents | back to top]