# Polysaccharide hydrolase activities from water samples collected at various sites under varying hydrostatic pressures in the Western North Atlantic aboard R/V Atlantic Explorer cruise AE2413 in May 2024

Website: <a href="https://www.bco-dmo.org/dataset/968956">https://www.bco-dmo.org/dataset/968956</a>
<a href="Data Type">Data Type</a>: Cruise Results, Other Field Results</a>

Version: 1

Version Date: 2025-07-23

## **Project**

» <u>Collaborative Research: Pressure effects on microbially-catalyzed organic matter degradation in the deep</u> ocean (Pressure effects on microbes)

Contributors	Affiliation	Role
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#### **Abstract**

Heterotrophic bacteria and archaea (here: microbes) are critical drivers of the ocean's biogeochemical cycles, active throughout the depth of the ocean. Their capabilities and limitations help determine the rates and locations at which carbon and nutrients are regenerated, as well as the extent to which organic matter is preserved (Hedges 1992). In the deep ocean, at bathy- and abyssopelagic depths (ca. 1000-6000m), these communities are dependent upon the sinking flux of particulate organic matter (POM) from the surface ocean (Bergauer et al. 2018). This dependence means that heterotrophic microbial communities must produce the extracellular enzymes required to solubilize and hydrolyze high molecular weight (HMW) POM to sizes substrates suitable for cellular uptake. A recent global-scale investigation of deep-sea microbes in fact found that the genetic potential for exported (extracellular) enzymes among bacteria in deep waters was far greater than for communities in surface or mesopelagic waters (Zhao et al. 2020). We have new evidence that a substantial fraction of bacteria in bottom water from the North Atlantic Ocean use a specialized set of extracellular enzymes to rapidly take up HMW polysaccharides (Gilian et al. 2021), a substrate processing mechanism that would not be detected with the low molecular weight substrates used in most prior studies of microbial activity in the deep ocean (Nagata et al. 2010). Through our collaboration with the Danish Center for Hadal Research, we were able to use pressurization systems and in situ specialized equipment to investigate the effects of pressures characteristic of bathy- and abyssopelagic depths on microbial communities and their extracellular enzymes in the open North Atlantic Ocean. Here we present the measurement of polysaccharide hydrolase activities above that measured for killed controls from various sites under varying hydrostatic pressures in the Western North Atlantic aboard R/V Atlantic Explorer, cruise AE2413 in May 2024.

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## Coverage

**Location**: Western North Atlantic stations 35° 00' N, 73° 03' W; 35° 00' N, 68° 15' W; 42° 10' N, 60° 02' W.

Total water column depths were 4260m, 5280m, and 4310m. Various depths sampled.

**Spatial Extent**: **N**:42.17828 **E**:-60.0479 **S**:34.99333 **W**:-73.03333

**Temporal Extent**: 2024-05-10 - 2024-05-19

## Methods & Sampling

Water was collected via Niskin bottles mounted on a rosette, equipped with a CTD in the Western North Atlantic aboard R/V Atlantic Explorer, cruise AE2413 in May 2024. Sampling was done at the following locations:

stn24: 34.99333, -73.03333
stn25: 34.99685, -68.24842
stn26: 42.17828, -60.0479

At each station, 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 cell counts, and the activities of polysaccharide hydrolases under varying hydrostatic pressures (0.1, 20, and 40 MPa). A separate glass Duran bottle was filled with seawater from the carboy and sterilized in an autoclave for over 30 minutes to serve as a killed control for various measurements.

For each substrate and time point, three 3 mL Exetainer vials were filled with seawater and one 3 mL Exetainer vial was filled with autoclaved seawater to serve as a killed control. Substrate was added at 3.5  $\mu$ M monomer-equivalent concentrations, except for fucoidan, which was added at 5  $\mu$ M concentrations (a higher concentration was necessary for sufficient fluorescence signal). Two 3 mL Exetainer vials – one with seawater and one with autoclaved seawater – with no added substrate served as blank controls. Vials for each substrate were pressurized to either 0.1, 20, or 40 MPa in individual pressure vessels for each time point and stored in the dark at 4°C for 0, 5, 12, or 22 days.

At each time point, three pressure vessels were depressurized (one at 0.1, 20, and 40 MPa), vials were removed and using a sterile syringe, incubations were filtered through a 0.2  $\mu$ m pore size syringe filter, and stored frozen until analysis.

Hydrolysis rates were calculated from the change in molecular weight distribution of the substrate over time, as described in detail in Arnosti (2003).

## **Data Processing Description**

Hydrolysis rates were calculated from the change in molecular weight distribution of the substrate over time, as described in detail in Arnosti (2003). Scripts to calculate hydrolysis rates are available in the associated Github repository (Hoarfrost, 2017).

#### **BCO-DMO Processing Description**

- Imported "20250505 BCODMO AE2413 FlaPS-bulk.csv" into BCO-DMO system
- -Convert date to ISO YYYY-MM-DD date format
- -Create ISO datetime, using "date" and "time"
- -Renamed fields to remove spaces and special characters in keeping with BCO-DMO system and style guidelines
- -Exported file as "968956 v1 ae2413 flaps bulk.csv"

## **Problem Description**

Station 26 DCM was not measured due to sampling error.

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## **Data Files**

#### File

**968956\_v1\_ae2413\_flaps\_bulk.csv**(Comma Separated Values (.csv), 106.90 KB)

MD5:bc5c285fcab013192cc1ad3c98d32eaa

Primary data file for dataset ID 968956, version 1

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## **Related Publications**

Arnosti, C. (1996). A new method for measuring polysaccharide hydrolysis rates in marine environments. Organic Geochemistry, 25(1-2), 105–115. doi:10.1016/s0146-6380(96)00112-x  $\frac{\text{https://doi.org/10.1016/S0146-6380(96)00112-X}}{\text{https://doi.org/10.1016/S0146-6380(96)00112-X}}$ 

Methods

Arnosti, C. (2003). Microbial Extracellular Enzymes and their Role in Dissolved Organic Matter Cycling. Aquatic Ecosystems, 315–342. https://doi.org/10.1016/b978-012256371-3/50014-7 <a href="https://doi.org/10.1016/B978-012256371-3/50014-7">https://doi.org/10.1016/B978-012256371-3/50014-7</a>

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

Hedges, J. I. (1992). Global biogeochemical cycles: progress and problems. Marine Chemistry, 39(1–3), 67–93. https://doi.org/10.1016/0304-4203(92)90096-s <a href="https://doi.org/10.1016/0304-4203(92)90096-5">https://doi.org/10.1016/0304-4203(92)90096-S</a> *Methods* 

Hoarfrost, A., Gawarkiewicz, G., & Arnosti, C. (2017, May 15). Ahoarfrost/Shelf1234: Shelf1234 Initial Release. Zenodo. https://doi.org/10.5281/zenodo.580059 Software

Lloyd, C. C., Ghobrial, S., & Arnosti, C. (2025). Polysaccharide Hydrolase Activities in Danish Coastal Seawater and Sediments under Varying Hydrostatic Pressures [Data set]. Zenodo. https://doi.org/10.5281/ZENODO.14673558 <a href="https://doi.org/10.5281/zenodo.14673558">https://doi.org/10.5281/zenodo.14673558</a> <a href="https://doi.org/10.5281/zenodo.14673558">https://doi.org/10.5281/zenodo.14673558</a> <a href="https://doi.org/10.5281/zenodo.14673558">https://doi.org/10.5281/zenodo.14673558</a>

Nagata, T., Tamburini, C., Arístegui, J., Baltar, F., Bochdansky, A. B., Fonda-Umani, S., Fukuda, H., Gogou, A., Hansell, D. A., Hansman, R. L., Herndl, G. J., Panagiotopoulos, C., Reinthaler, T., Sohrin, R., Verdugo, P., Yamada, N., Yamashita, Y., Yokokawa, T., & Bartlett, D. H. (2010). Emerging concepts on microbial processes in the bathypelagic ocean – ecology, biogeochemistry, and genomics. Deep Sea Research Part II: Topical Studies in Oceanography, 57(16), 1519–1536. https://doi.org/10.1016/j.dsr2.2010.02.019 *Methods* 

Zhao, Z., Baltar, F., & Herndl, G. J. (2020). Linking extracellular enzymes to phylogeny indicates a predominantly particle-associated lifestyle of deep-sea prokaryotes. Science Advances, 6(16). https://doi.org/10.1126/sciadv.aaz4354

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## **Related Datasets**

## IsRelatedTo

Lloyd, C., Ghobrial, S., Arnosti, C. (2025) **Polysaccharide Hydrolase activities in Danish coastal seawater and sediments under varying hydrostatic pressures on samples collected in September 2023.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-06-10 doi:10.26008/1912/bco-dmo.963382.1 [view at BCO-DMO]

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## **Parameters**

Parameter	Description	Units
deployment	Cruise ID	unitless
station	Station number 24, 25, or 26	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
time_local_est	Time of sample collection (local time), US Eastern Time (UTC-05:00)	unitless
ISO_DateTime_UTC	Datetime of sample collection in ISO 8601 format, UTC	unitless
cast_number	Cast number (refers to cast of CTD/Niskin bottles on cruise)	unitless
depth	Water column feature or oceanic zone sampled (DCM, OMZ, Bathy, or Deep (bottom or near bottom). Station 26 DCM was not measured due to sampling error	unitless
depth_actual	Actual depth at which water was collected	m
in_situ_temp	Temperature of the samples in-situ	°C
sample_type	The type of sample, whether it was incubated using water from the bulk water column, sediments, or amended	unitless

incubation_pressure	Amount of pressure applied during incubation	МРа
incubation_Temp	Temperature of incubation	°C
unamended_amended	Whether the sample was amended (A) or unamended (U)	unitless
substrate	Polysaccharide used for incubation: ara = arabinogalactan, chn = chondroitin sulfate, fuc = fucoidan, lam = laminarin, man = mannan, pul = pullulan, xyl = xylan	unitless
timepoint_no	The timepoint number sampled for each incubation	unitless
timepoint_days	The amount of time that has elapsed at each timepoint	days
ratex_nM_hr	The hydrolysis rate for the kill-control	nmol L- 1 hr-1
rate1_nM_hr	The hydrolysis rate for the first replicate	nmol L- 1 hr-1
rate2_nM_hr	The hydrolysis rate for the second replicate	nmol L- 1 hr-1
rate3_nM_hr	The hydrolysis rate for the third replicate	nmol L- 1 hr-1
mean_rate_nM_hr	The average hydrolysis rate for all replicates	nmol L- 1 hr-1
sd_rate_nM_hr	The standard deviation of the hydrolysis rates for all replicates	nmol L- 1 hr-1
kcratex_nM_hr	The kill-corrected hydrolysis rate for the kill-control	nmol L- 1 hr-1
kcrate1_nM_hr	The kill-corrected hydrolysis rate for the first	nmol L- 1 hr-1
kcrate2_nM_hr	The kill-corrected hydrolysis rate for the second	nmol L- 1 hr-1
kcrate3_nM_hr	The kill-corrected hydrolysis rate for the third replicate	nmol L- 1 hr-1
mean_kcrate_nM_hr	The average kill-corrected hydrolysis rate for all replicates	nmol L- 1 hr-1
sd_kcrate_nM_hr	The standard deviation of the kill-corrected hydrolysis rates for all replicates	nmol L- 1 hr-1

# 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	High-Performance Liquid Chromatograph
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset- specific Description	HPLC system with Hitachi fluorescence detectors (L-7485, L-2485, Chromaster - 5440)
Instrument	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset- specific Instrument Name	Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset- specific Description	Water was collected via Niskin bottles mounted on a rosette, equipped with a CTD.
	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	Pressure vessels
Generic Instrument Name	Pressure Vessel
Dataset- specific Description	Vials for each substrate were pressurized to either 0.1, 20, or 40 MPa in individual pressure vessels for each time point and stored in the dark at $4^{\circ}$ C for 0, 5, 12, or 22 days.
Generic Instrument Description	A pressure vessel is a container designed to hold gases or liquids at a pressure substantially different from the ambient pressure. Construction methods and materials may be chosen to suit the pressure application, and will depend on the size of the vessel, the contents, working pressure, mass constraints, and the number of items required. Examples include glassware, autoclaves, compressed gas cylinders, compressors (including refrigeration), vacuum chambers and custom designed laboratory vessels.

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## **Deployments**

#### **AE2413**

Website	https://www.bco-dmo.org/deployment/963451	
Platform	R/V Atlantic Explorer	
Start Date	2024-05-08	
End Date	2024-05-28	

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## **Project Information**

Collaborative Research: Pressure effects on microbially-catalyzed organic matter degradation in the deep ocean (Pressure effects on microbes)

**Coverage**: Western North Atlantic, hadal depths of the Pacific

## NSF Award Abstract:

Microbes are important players in the carbon cycle in the ocean. These organisms consume organic carbon and produce carbon dioxide in marine systems. Because the average depth of the ocean is 4000 m, microbes must work at high pressures typical of the deep ocean (>1000 m). Although high pressure is known to affect marine microbes, their carbon cycling activities have mostly been measured at surface ocean pressures. As a result, it remains unknown how closely these measurements reflect the activities of deep-sea microbes at high pressures. As a result of collaborations with scientists in Denmark and Germany, this project will be able to use special equipment to investigate the effects of high pressures on marine microbes and their carbon cycling activities. This work is necessary to quantify rates of carbon cycling and identify the microbes involved, especially in deep waters. The project will provide training for diverse undergraduate and graduate students, and a postdoc who will conduct novel research in the U.S., Denmark, and Germany, both at sea and in the lab. The scientists will also teach middle school students about the role of microbes in the carbon cycle and pressure effects on life in the ocean. The project will provide internships for high school students, focusing on first-generation students who would like to go to college. This work may aid in future efforts to identify enzymes that function well under high pressure.

Heterotrophic microbes (e.g., bacteria and archaea) are found throughout the ocean. Their biogeochemical

functions help determine the rates and locations at which carbon and nutrients are regenerated, as well as the extent to which organic matter is preserved. Although research has shown that pressure profoundly affects the activities of marine microbes, most investigations of microbial communities of the deep sea are conducted at atmospheric pressure, due to the limited availability of specialized equipment. In collaboration with the Danish Center for Hadal Research at the University of Southern Denmark, this study will identify the effects of pressure on microbial communities and their extracellular enzymes of pressures characteristic of bathy- and abyssopelagic depths. At sea and in the lab, the scientific team will compare the effects of depressurization on the activities of enzymes produced by microbial communities of the deep ocean, as well as the effects of high pressure on surface-water derived enzymes and communities. Fieldwork will take place in Danish coastal waters, well as in the open North Atlantic and Pacific Oceans. Using pressurization systems and in situ incubations, this study will measure hydrolysis rates of peptides and polysaccharides, two of the major classes of marine organic matter. Project activities will also focus on developing the means to measure enzyme activities in situ in the deep ocean. In collaboration with colleagues from the Max Planck Institute for Marine Microbiology in Germany, this proect will additionally investigate whether pressure affects the selfish uptake of polysaccharides. These studies will provide new insight into understudied but key factors that help determine the fate of organic matter in the deep ocean.

This project is funded by the Biological and Chemical Oceanography Programs.

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.

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# **Funding**

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2241720

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