Phytoplankton and heterotrophic bacterial cell abundance over the incubation period in bioassay experiments with seawater from R/V Savannah cruise SAV-19-02 in the NW Atlantic Ocean in Spring of 2019

Website: https://www.bco-dmo.org/dataset/864199
Data Type: Cruise Results, experimental
Version: 1
Version Date: 2021-11-02

Project
» Collaborative Research: Assessing the role of compound-specific phosphorus hydrolase transformations in the marine phosphorus cycle (P-hydrolase)

Contributors	Affiliation	Role
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Abstract
Phytoplankton and heterotrophic bacterial cell abundance over the incubation period in bioassay experiments with seawater collected during R/V Savannah cruise SAV-19-02 from March to April of 2019 in the Northwestern Atlantic from the surface to 50 m depth.

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Coverage

Spatial Extent: **N**:31.7635 **E**:-79.8421 **S**:31.0175 **W**:-80.7965
Temporal Extent: 2019-03-30 - 2019-04-10

Methods & Sampling

Sampling and analytical procedures:

Bioassay experiments were conducted at station 1 and stations 3. At each station, inorganic and organic phosphate amendments were performed on seawater with and without nitrogen enrichment (NH_4Cl, $NaNO_3$). Bioassay Experiments consisted in incubating, over an incubation period of 48h, surface seawater (5m) with inorganic or organic phosphate compounds (20 µM; final concentration of P) including, polyphosphate (polyp), inorganic phosphate (Pi), nucleotides (ATP or AMP) and methylphosphonate (Mepn). In each incubation experiment, a control treatment (surface seawater) was included.

Samples for cell abundance (2 mL) were subsampled from experimental incubation bottle, fixed with 0.5% glutaraldehyde (final concentration), flash frozen in liquid nitrogen and then transferred into a -80 °C freezer until analysis. Frozen samples were thawed at room temperature and were analyzed using the Guava EasyCyte HT flow cytometer (Millipore). Instrument specific beads were used to calibrate the cytometer.

Samples were analyzed at a low flow rate (0.24 µL s^{-1}) during 3 min. For heterotrophic bacteria cell counts, samples were incubated with SYBR Green II solution 1:10 (Molecular Probes) for 15 min in the dark, in order to stain the nucleic acids. Bacterial cells were detected and enumerated based on diagnostic plots of forward scatter vs. green fluorescence. Group-specific phytoplankton were distinguished based on plots of forward scatter vs. orange fluorescence (phycoerythrin containing *Synechococcus* sp.), and SSC *vs.* red fluorescence (eukaryotes).

Instrument: Cell abundance of both phytoplankton and heterotrophic bacterial cell abundance were determined using the Guava EasyCyte HT flow cytometer (Millipore).

Location: Northwestern Atlantic surface waters. Depth: surface-50 m.

Data Processing Description

Data were organized using MATLAB and output as .mat files. Gaps in data were filled with NaN in the .mat files.

BCO-DMO Data Manager Processing Notes:

* Data from source files BioassayExperiments_Datasets.xslx. Sheets: CellAbundance_St1, CellAbundance_St1_NN, CellAbundance_St3 were imported into the BCO-DMO data system and combined into one data table.
* Data columns were added to contain information that was in the Excel sheet names. Column "station" added from information in the sheet name. The original excel sheet name was added as column data_subset_name.
* Original table had two rows of column header information. The two rows of header information were combined into one column name row for the table as is required to import into the BCO-DMO data system. Then data contained in the column names were extracted out of the header and into data columns (cell_type, Incubation_hours) by performing an "unpivot" of the table.
* Data table then sorted by {data_subset_name}{cell_type}{Id_treatments}{Incubation_hours}
* Parameters (column names) renamed to comply with BCO-DMO naming conventions. See https://www.bco-dmo.org/page/bco-dmo-data-processing-conventions
* Missing data values (Blank/Null) values in this dataset are displayed according to the format of data you access. For example, in csv files it will be blank values. In Matlab .mat files it will be NaN values. When viewing data online at BCO-DMO, the missing value will be shown as "nd" meaning "no data."

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Guava EasyCyte HT flow cytometer (Millipore)
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Cell abundance of both phytoplankton and heterotrophic bacterial cell abundance were determined using the Guava EasyCyte HT flow cytometer (Millipore).
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

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Deployments

SAV-19-02

Website	https://www.bco-dmo.org/deployment/864191
Platform	R/V Savannah
Start Date	2019-03-30
End Date	2019-04-11
Description	Cruise synonym: Zephyr (Zooming in on Enzymatic PhosphoHYdrolysis Reactions)

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Project Information

Collaborative Research: Assessing the role of compound-specific phosphorus hydrolase transformations in the marine phosphorus cycle (P-hydrolase)

NSF Award Abstract:
Phosphorus (P) is an essential building block for life. Because P is in short supply over vast areas of the ocean, P availability may control biological productivity, such as photosynthesis and carbon fixation, which has implications for uptake of the greenhouse gas carbon dioxide and thus climate regulation. Marine microorganisms must satisfy their nutritional requirement for P by obtaining it from seawater, where P is present in a variety of chemical forms, from simple phosphate ions (Pi) to complex dissolved organic phosphorus (DOP) molecules. The concentration of DOP vastly exceeds Pi over most ocean areas, therefore DOP is a critically important source of P for marine microbial nutrition and productivity. However, much remains unknown about the contribution of specific DOP compounds to the P nutrition, productivity, and structure of marine microbial communities. In this project, the investigators will conduct field experiments in the Atlantic Ocean and perform a series of controlled laboratory studies with pure enzymes and microbial cultures to determine how and to what extent different DOP compounds are degraded to Pi in the marine environment. Furthermore, the contribution of these compound-specific DOP molecules to microbial P nutrition, carbon fixation, and community structure will be determined, thus advancing the current state of knowledge regarding the factors that control the activity and distribution of microbial species in the ocean, and the ocean?s role in the climate system. This project will support two female junior investigators, a postdoctoral researcher, and graduate and undergraduate students. The undergraduate students will be recruited from the

Marine Sciences program at Savannah State University, an Historically Black Colleges and Universities. In addition, results will be incorporated into new hands-on K-12 educational tools to teach students about microbial P biogeochemistry, including a digital game and formal lesson plans with hands-on demos. These tools will be validated with K-12 educators and will be widely accessible to the public through various well-known online platforms. These activities will thus reach a broad audience including a significant fraction of underrepresented groups.

P is a vital nutrient for life. Marine microorganisms utilize P-hydrolases, such as alkaline phosphatase (AP), to release and acquire phosphate (Pi) from a wide diversity of dissolved organic P (DOP) compounds, including P-esters (P-O-C bonds), phosphonates (P-C), and polyphosphates (P-O-P). Compound-specific DOP transformations have the potential to exert critical and wide-ranging impacts on marine microbial ecology (e.g. variable DOP bioavailability among species), biogeochemistry (e.g. P geologic sequestration via formation of calcium Pi), and global climate (e.g. aerobic production of the greenhouse gas methane by dephosphorylation of methylphosphonate). However, the mechanisms and comparative magnitude of specific DOP transformations, in addition to their relative contributions to microbial community-level P demand, productivity, and structure, are not completely understood. This study will fill these knowledge gaps by tracking the fate of specific DOP pools in the marine environment. Specifically, this project will test four hypotheses in the laboratory using recombinant enzymes and axenic cultures representative of marine eukaryotic and prokaryotic plankton from high and low nutrient environments, and in the field using observational and experimental approaches along natural Pi gradients in the Atlantic Ocean. In particular, the investigators will reveal potential differences in the hydrolysis and utilization of specific DOP compounds at the community- (bulk enzymatic assays), taxon- (cell sorting of radiolabeled cells in natural samples), species- (axenic cultures) and molecular-levels (pure enzyme kinetic studies and cell-associated proteomes and exoproteomes). Results from our proposed work will provide a robust understanding of the enzymatic basis involved in the transformation of specific forms of DOP and create new knowledge on the relative contribution of these specific P sources to Pi production, marine microbial nutrition, community structure, primary productivity, and thus global carbon cycling and climate. In particular, our refined measurements of the concentration of bioavailable DOP and our unique estimates of DOP remineralization fluxes will provide critical new information to improve models of marine primary production and P cycling.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1736967
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