Alkaline phosphatase activity (APA) Michaelis-Menten kinetic parameters (Vmax and Km) in both whole and filtered seawater collected during R/V Savannah cruise SAV-19-02 in the NW Atlantic Ocean in the Spring of 2019

Website: https://www.bco-dmo.org/dataset/864269
Data Type: Cruise Results
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
Alkaline phosphatase activity (APA) Michaelis-Menten kinetic parameters (Vmax and Km) in both whole and filtered (<0.2 µm) 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:

Seawater samples were collected in triplicate from Niskin bottles (12 L), at three stations (St. 1, St. 2 and St. 3) along a transect from coastal Georgia to offshore waters. Latitude (lat) and longitude (lon) of sampling sites are provided in .mat files. Stations 1 and 2 were on the continental shelf (15–30 m depth), while Station 3 was on the shelf break (220 m depth) adjacent to the Gulf Stream. At each station, samples were collected at 2-3 depths between the surface (4–5 m) and 50 m for alkaline phosphatase activity measurements (APA).

APA was determined fluorometrically using 4-methylumbelliferyl phosphate (MUF-P), as representative of phosphomonoesterase activity. APA was measured in triplicate samples from both bulk (total APA) and filtered (<0.2 µm, dissolved APA) seawater for each sampling depth. Hydrolysis rates were determined by separately

incubating 200 µL of seawater with 8 different concentrations (0, 0.1, 0.2, 0.5, 1, 5, 10, 20 µM; final concentrations) of MUF-P in 96-well black microtiter plates. To ensure linearity of the hydrolysis rate, the increase in fluorescence was measured (excitation/emission wavelength: 359/449 nm) at multiple time points over an incubation period of 24 h. Maximum hydrolysis rate (Vmax) and Michaelis-Menten constant (Km) were determined, using a nonlinear regression based on the rectangular hyperbolic function following: V=Vmax x S/ Km+ S, where S and V are the concentrations of the substrate and the hydrolysis rates, respectively.

Instruments: Sampling was performed using Niskin bottles (12 L) mounted on a rosette. Reading of fluorescence was performed on a plate reader (SpectraMax® M2, Molecular Devices).

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.

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Niskin bottle
Dataset-specific Description	Sampling was performed using Niskin bottles (12 L) mounted on a rosette.
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	SpectraMax® M2, Molecular Devices
Generic Instrument Name	plate reader
Dataset-specific Description	Sampling was performed using Niskin bottles (12 L) mounted on a rosette. Reading of fluorescence was performed on a plate reader (SpectraMax® M2, Molecular Devices).
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

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
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