Phosphohydrolysis rates from samples collected in the coastal western North Atlantic on R/V Endeavor cruise EN588 during September 2016

Website: https://www.bco-dmo.org/dataset/767022

Data Type: Cruise Results

Version: 1

Version Date: 2019-05-08

Project

» Collaborative Research: Exploring the role of exogenous polyphosphate in the precipitation of calcium phosphate minerals in the marine environment (PolyP and P-minerals)

Contributors	Affiliation	Role
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Abstract

Phosphohydrolysis rates from samples collected in the coastal western North Atlantic on R/V Endeavor cruise EN588 during September 2016.

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Coverage

Spatial Extent: **N**:41.54397 **E**:-70.66917 **S**:39.41208 **W**:-73.24917

Temporal Extent: 2016-09 - 2016-09

Dataset Description

Seawater samples were collected at five field sites and amended with inorganic polyphosphate to determine maximum potential hydrolysis rates compared to a common fluorogenic probe, 4-methylumbelliferyl phosphate (MUF-P).

Acquisition Description

Field sampling: Surface seawater (5–35 m) was collected in September 2016 during two sampling campaigns in the coastal western North Atlantic (Diaz et al., 2018; Supplementary Table S1). Three sites were sampled aboard the R/V Endeavor using a Niskin rosette sampler and incubated immediately in order to determine rates of P hydrolysis. Two sites accessible by small boat in Woods Hole Harbor and Buzzard's Bay, MA, were sampled utilizing a peristaltic pump. These samples were transported on ice packs and analyzed for P hydrolysis rates within 5–6 hours of collection. Additional samples were preserved and analyzed for chlorophyll, bacteria and phytoplankton abundance, and soluble reactive P (SRP), as detailed below.

Chlorophyll: In the dark, 250 mL of seawater was filtered onto 25 mm GF/F filters. Samples were stored in the dark at -20C until analyzed according to protocols adapted from Strickland and Parsons (1972). Briefly, samples were extracted in 90% acetone in the dark (4C, 9 hr) and measured using a 10AU fluorometer (Turner). Sample signals were calibrated using a chlorophyll-a standard (Sigma) and were corrected for phaeopigments by accounting for the fluorescence of extracts before and after acidification to 0.003 M HCl.

Abundance of bacteria and phytoplankton: Seawater samples were preserved for flow cytometry with 0.5% glutaraldehyde (final concentration), flash frozen in liquid nitrogen and stored at -80°C until analysis. Bacteria and group-specific phytoplankton counts were conducted on a 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}) for 3 min. To enumerate bacteria, samples were diluted (1:100) with filtered seawater (0.01 µm). Samples and filtered seawater blanks were stained with SYBR Green I (Invitrogen) according to the manufacturer's instructions and incubated in a 96-well plate in the dark at room temperature for 1 hr. Bacterial cells were counted based on diagnostic forward scatter vs. green fluorescence signals. Major phytoplankton groups were distinguished based on plots of forward scatter vs. orange (phycoerythrin-containing Synechococcus sp.), and forward scatter vs. red (eukaryotes). Size classes of eukaryotic phytoplankton were further distinguished based on forward scatter (pico-, nano- and large eukaryotes).

Soluble reactive P: Seawater samples were collected from Niskin rosette bottles or the peristaltic pump into acid cleaned, high density polyethylene bottles. Samples used for determining in situ SRP concentrations were frozen and stored upright at -20°C until analysis. Field samples and diatom filtrates were both analyzed for SRP using a standard colorimetric method (Hansen and Koroleff, 1999). To determine in situ SRP concentrations in field samples, SRP analysis was conducted using a 4 cm glass spectrophotometry cell on triplicate subsamples, and the detection limit, defined as three times the standard deviation of replicate blank measurements, was 115 nmol L^{-1} SRP. For incubations to determine P hydrolysis rates (see below), replicate samples were analyzed in clear 96-well plates on a multimode plate reader (Molecular Devices) with a detection limit of 800 nmol L^{-1} P.

P-hydrolysis of model DOP substrates: Field samples were incubated with the fluorogenic probe 4-methylumbeliferone phosphate (MUF-P) and two inorganic polyphosphate compounds with an average chain length of 3 or 45 P atoms.

Samples were amended with each substrate at a final concentration of 20 M P. This concentration was assumed to be rate-saturating based on preliminary experiments. Hydrolysis of polyphosphates was determined from the production of phosphate using the colorimetric protocol outlined above. Hydrolysis of the fluorogenic probe MUF-P was monitored using a standard fluorescence technique. Briefly, hydrolysis of MUF-P to 4-methylumbellierone (MUF) was measured (excitation: 359 nm, emission: 449 nm) and

calibrated with a multi-point standard curve of MUF (10–500 nmol L^{-1}). In both methods, samples were corrected for substrate autohydrolysis by accounting for negative controls, which were filtered (0.2 m) and boiled (99C, 15 min) prior to P amendment in order to eliminate enzyme activity. See Diaz et al. 2018 Frontiers in Marine Science 5: 380 for full methods.

Processing Description

BCO-DMO Processing: modified parameter names (replaced spaces and hyphens with underscores, removed units)

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Related Publications

Diaz, J. M., Holland, A., Sanders, J. G., Bulski, K., Mollett, D., Chou, C.-W., … Duhamel, S. (2018). Dissolved Organic Phosphorus Utilization by Phytoplankton Reveals Preferential Degradation of Polyphosphates Over Phosphomonoesters. Frontiers in Marine Science, 5. doi:10.3389/fmars.2018.00380

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Parameters

Parameter	Description	Units
Station	Station name	unitless
Lat	Latitude North	decimal degrees
Long	Longitude East (negative values = West)	decimal degrees
Depth	Depth	meters (m)
Temperature	Temperature	degrees Celsius
Salinity	Salinity	PSU?
Inorganic_poly_P_hydrolysis	Inorganic poly-P hydrolysis	nanomoles P per liter per hour (nmol P/L/hr)
MUF_P_hydrolysis	MUF-P hydrolysis	nmol P/L/hr
Soluble_reactive_P	Soluble reactive P	nanomoles per liter (nmol/L)
Chlorophyll	Chlorophyll	micrograms per liter (ug/L)
Bacterial_abundance	Bacterial abundance	10^5 cells per milliiter (10^5 cells/mL)
Total_phytoplankton	Total phytoplankton	10^4 cells/mL
Synechococcus_spp	Synechococcus spp.	10^4 cells/mL
Picoeukaryotic_phytoplankton	Picoeukaryotic phytoplankton	10^3 cells/mL
Nanoeukaryotic_phytoplankton	Nanoeukaryotic phytoplankton	10^3 cells/mL
Large_eukaryotic_phytoplankton	Large eukaryotic phytoplankton	10^2 cells/mL
Bacterial_abundance_to_Total_phytoplankton	Ratio of Bacterial abundance:Total phytoplankton	unitless

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Niskin bottle
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	10AU fluorometer (Turner)
Generic Instrument Name	Turner Designs Fluorometer -10-AU
Generic Instrument Description	The Turner Designs 10-AU Field Fluorometer is used to measure Chlorophyll fluorescence. The 10AU Fluorometer can be set up for continuous-flow monitoring or discrete sample analyses. A variety of compounds can be measured using application-specific optical filters available from the manufacturer. (read more from Turner Designs, turnerdesigns.com, Sunnyvale, CA, USA)

Dataset-specific Instrument Name	Guava EasyCyte HT flow cytometer (Millipore)
Generic Instrument Name	Flow Cytometer
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)

Dataset-specific Instrument Name	peristaltic pump
Generic Instrument Name	Pump
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

Dataset-specific Instrument Name	multimode plate reader (Molecular Devices)
Generic Instrument Name	plate reader
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

EN588

Website	https://www.bco-dmo.org/deployment/766973
Platform	R/V Endeavor
Start Date	2016-08-30
End Date	2016-09-08

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Project Information

Collaborative Research: Exploring the role of exogenous polyphosphate in the precipitation of calcium phosphate minerals in the marine environment (PolyP and P-minerals)

NSF Award Abstract: Phosphorous is an important nutrient sustaining all forms of life. In particular, in the ocean, phosphorous is a key limiting nutrient, controlling levels of primary productivity across large swaths of the ocean. Removal of phosphorous occurs largely via formation of stable apatite minerals in ocean sediments. However, average ocean conditions generally inhibit the formation of apatite, thus the abundance of apatite minerals in marine sediments is a mystery. This research aims to determine the mechanisms of apatite formation in the ocean to answer this century-old question. Evaluating these mechanisms will greatly advance current understanding of phosphorous cycling in the ocean. A more detailed understanding of phosphorous cycling can be applied across the disciplines of ocean science, and because of the importance of phosphorous as a nutrient and an element with a variety of interactions with other elements, it will be applicable to a variety of other research questions. The researchers are dedicated to promoting diversity in ocean science and plan to include undergraduate students from underrepresented groups in the study. They will also mentor a postdoc and communicate their science to the public and K-12 teachers via a blog entitled ? Britannica Blog?, the Atlanta Science Festival, a rock show, and educational material, the latter two to be developed as part of this work. Marine phosphorous burial via authigenic stable apatite formation in sediments is a major pathway for phosphorous removal in the ocean. However, in most marine environments, under natural conditions, this process is kinetically inhibited. It has been a mystery for more than a century as to how it is therefore possible for apatite to be oversaturated in large areas of marine sediments. A possible mechanism that could explain 95% of the apatite burial flux is that apatite minerals are precipitated as fine-grained particles from exogenous polyphosphate intermediates. Exogenous polyphosphates have been understudied, despite this possible importance as a mechanism for phosphorous removal. As a consequence this research could revolutionize current understanding of phosphorous cycling in the ocean for the major aim is to make a thorough and detailed study of the mechanisms behind marine apatite formation, focusing on the role of exogenous polyphosphate particles. Phosphorous is an element with widespread importance in ocean sciences, and more clearly understanding its burial will have applications across the disciplines.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1559124
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