Cell abundance, growth rate, cellular P quotas, and alkaline phosphatase activity from a laboratory experiment examining the response of three species of marine phytoplankton grown under different phosphorus (P) conditions

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Project
» Phosphonate Utilization by Eukaryotic Phytoplankton: Who, How, and Where? (Euk Phn Utilization)

Contributors	Affiliation	Role
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Abstract
A laboratory experiment was carried out to characterize the growth and physiological response of three species of eukaryotic phytoplankton grown with inorganic phosphate and phosphonate as the sole sources of phosphorus (P). Data reported are cell abundance, growth rate, cellular P quotas, and alkaline phosphatase activity.

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Dataset Description

Three species of marine phytoplankton – *Micromonas commoda*, *Emiliania huxleyi*, and *Phaeodactylum tricornutum* – were grown under four phosphorus (P) conditions. These include phosphate (Pi) replete and deplete conditions and the phosphonate conditions where cultures received either methylphosphonate (MPN) or 2-aminoethylphosphonate (2-AEPN) as the sole source of phosphorus at replete levels. Samples for cell abundance were collected throughout the experiment to monitor growth. In addition, particulate P, to calculate cellular P quota, as well as alkaline phosphatase activity was measured at a single time point when cells were in exponential growth.

Acquisition Description

Axenic cultures of the pico-prasinophyte *Micromonas commoda* (CCMP 2709), the coccolithophore *Emiliania huxleyi* (CCMP 2090), and the diatom *Phaeodactylum tricornutum* (CCMP 2561) were obtained

from the National Center for Marine Algae and Microbiota (Bigelow Laboratory for Ocean Sciences, East Boothbay, Maine). The cultures remained axenic throughout the experiments as determined by SYTO-staining and flow cytometric counting on a BD FACSJazz cell sorter; all cultures were free of bacteria during these experiments. Phytoplankton were grown in artificial sea water amended with L1 media with (*P. tricornutum*) or without (*M. commoda and E. huxleyi*) silica. The P source was added separately to achieve the desired growth conditions; Pi-replete media contained 36 µM PO_4^{3-}, the Pi-deficient condition received 0.1 µM PO_4^{3-}, and the phosphonate treatments received either 36 µM MPN or 2-AEPN. The Pi-deficient treatment (0.1 µM) represents a control for the low level of contaminating Pi measured in the phosphonate media; thus, an increase in growth in the MPN and 2-AEPN conditions above that measured in the Pi-deficient condition is due to phosphonate utilization. The potential for abiotic breakdown of phosphonate to Pi was investigated in media-only tubes exposed to the experimental temperature and light conditions for 10 days. Pi levels did not change throughout the experimental period (MPN average Pi = 0.11 µM ± 0.02; 2-AEPN average Pi = 0.10 µM ± 0.02), strongly supporting the notion of active enzymatic breakdown of phosphonates for growth. Natural P concentrations are much lower than those used in this study; the replete nutrient concentrations were used to support high cell yields necessary for analytical measurements. Cultures were acclimated to the four growth conditions described above as they had been maintained in each P treatment for a minimum of two transfers (20 days). Cultures were grown at 20°C in a 14h light/10h dark cycle at ~100 µE m^{-2} s^{-1} with a starting concentration of ~$1x10^4$ cells mL^{-1} in 25 mL culture volumes. Phytoplankton growth was monitored by fluorescence measurements using a Turner TD-700 fluorometer and cell counts analyzed by flow cytometry. Specific growth rates (µ) were calculated from the linear regression of the natural log of cell counts during the exponential growth phase of cultures. Quadruplicate cultures were setup for each treatment; three replicates were harvested in the late exponential phase of growth for physiological measurements, while the fourth was used to monitor cell abundances later in the growth cycle.

Physiological measurements:
Cell samples (5 mL culture volume) for particulate P were collected onto precombusted 25 mm Whatman glass fiber filters, rinsed with 0.17 M Na_2SO_4, and stored frozen at -20°C until analysis. Determinations were made as previously described (Lomas et al. 2010). Briefly, filters were rinsed with 0.017 M $MgSO_4$, dried at 90°C, and combusted at 500°C for 2 h. Upon cooling, 0.2 M HCl was added and hydrolyzed at 80°C for 30 min. After cooling, mixed reagent was added, the samples were centrifuged, and absorbance was read at 885 nm using a Genesys 10 spectrophotometer.

Alkaline phosphatase content (APA) measurements were made by quantifying the hydrolysis of 6,8-difluoro-4-methylumbelliferyl phosphate using a Molecular Devices FilterMax F5 microplate reader. Abiotic substrate hydrolysis was accounted for in killed controls that were boiled and cooled prior to substrate addition, as well as in media-only controls. The fluorescent reference standard, 6,8-difluoro-4-methylcoumarin was used to calculate the rate of hydrolysis, which was then normalized to cell abundance to determine APA per cell.

Processing Description

Cell abundance data were processed from flow cytometry files by gating logic using Sortware software. Nutrient concentrations, both dissolved and particulate, were calculated based upon the instrument absorbance response to known concentration standards. Alkaline phosphatase activity was calculated based on fluorescence response of a known standard.

Any samples that were not collected are represented by 'nd' in the dataset.

BCO-DMO Processing:
- renamed fields;
- replaced 'NaN' with 'nd' (no data).

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Related Publications

Lomas, M. W., Burke, A. L., Lomas, D. A., Bell, D. W., Shen, C., Dyhrman, S. T., & Ammerman, J. W. (2010). Sargasso Sea phosphorus biogeochemistry: an important role for dissolved organic phosphorus (DOP). Biogeosciences, 7(2), 695–710. doi:10.5194/bg-7-695-2010
Related Research

Whitney, L. P., & Lomas, M. W. (2016). Growth on ATP Elicits a P-Stress Response in the Picoeukaryote Micromonas pusilla. PLOS ONE, 11(5), e0155158. doi:10.1371/journal.pone.0155158
Related Research

Whitney, L. P., & Lomas, M. W. (2018). Phosphonate utilization by eukaryotic phytoplankton. Limnology and Oceanography Letters, 4(1), 18–24. doi:10.1002/lol2.10100
Results

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Related Datasets

Different Version

Whitney, L. A. P., & Lomas, M. W. (2018). Data from: Phosphonate utilization by eukaryotic phytoplankton (Version 1) [Data set]. Dryad. https://doi.org/10.5061/DRYAD.B765BR4
https://doi.org/10.5061/dryad.b765br4

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Parameters

Parameter	Description	Units
Strain_No	National Center for Marine Algae and Microbiota numerical designation (CCMPxxxx)	unitless
Taxonomic_ID	Genus species identification of strain	unitless
Phosphorus_source	Compound containing phosphorus included in the growth media	unitless
Phosphorus_condition	Status of phosphorus addition at the beginning of the experiment	unitless
Time	Days since the start of the experiment	days
Average_Cell_abundance	Direct phytoplankton cell counts	cells per milliliter (cells/mL)
SD_of_Cell_abundance	Standard deviation of replicate cell counts	cells per milliliter (cells/mL)
Average_Growth_rate	Calculated from the linear regression of the natural log of cell counts during the exponential growth phase	growth per day
SD_of_growth_rate	Standard deviation of the linear regression of the natural log of cell counts during the exponential growth phase	growth per day
Average_QP	Cellular P quota	femtomoles per cell (fmol/cell)
SD_of_QP	Standard Deviation of cellular P quota	femtomoles per cell (fmol/cell)
Average_APA	Alkaline phosphatase activity	femtomoles P per cell per hour (fmol P cell-1 h-1)
SD_of_APA	Standard Deviation of alkaline phosphatase activity	femtomoles P per cell per hour (fmol P cell-1 h-1)

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Instruments

Dataset-specific Instrument Name	BD FACSJazz cell sorter
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	Turner TD-700 fluorometer
Generic Instrument Name	Turner Designs 700 Laboratory Fluorometer
Generic Instrument Description	The TD-700 Laboratory Fluorometer is a benchtop fluorometer designed to detect fluorescence over the UV to red range. The instrument can measure concentrations of a variety of compounds, including chlorophyll-a and fluorescent dyes, and is thus suitable for a range of applications, including chlorophyll, water quality monitoring and fluorescent tracer studies. Data can be output as concentrations or raw fluorescence measurements.

Dataset-specific Instrument Name	Genesys 10 spectrophotometer
Generic Instrument Name	Spectrophotometer
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

Dataset-specific Instrument Name	Molecular Devices FilterMax F5 microplate reader
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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Project Information

Phosphonate Utilization by Eukaryotic Phytoplankton: Who, How, and Where? (Euk Phn Utilization)

Coverage: Laboratory culture studies

NSF Award Abstract: Phosphorus (P) is an essential nutrient for all living cells. It is a central component of genetic material and cellular membranes and is integral to energy production and regulating enzyme activity. In the marine environment, P occurs as inorganic (Pi) and dissolved organic (DOP) forms; the availability and concentration of these different forms of P is an important control on marine phytoplankton growth. Marine phytoplankton are single-celled photosynthetic organisms and can be both prokaryotic bacteria and eukaryotic plants. While Pi is the preferred form of P for marine phytoplankton, in large regions of the oceans it is at such low levels that it restricts phytoplankton growth. In these regions, DOP is the most important P source. The composition of the DOP pool can generally be divided into two major groups: P esters and phosphonates. All marine phytoplankton are capable of using P esters to support growth; in contrast, phosphonates have only been shown to be an important source of P in the nutrition of bacteria to date. This project will determine the ability of marine eukaryotic phytoplankton to use phosphonates as a source of P for growth. Genomic analyses will determine the metabolic response of eukaryotic phytoplankton species to growth on phosphonates as well as the relevance of phosphonate use by natural populations. It is critical to understand the metabolic capabilities of phytoplankton which control marine nutrient cycling. In addition, the project is of great value in understanding the potential impacts of a changing ocean on phytoplankton growth. The project supports reseach opportunities for undergraduates from a local community college as well as hands-on enrichment programs for an afterschool program that serves a diverse student population. Comprising up to 10% of the marine DOP pool, phosphonates have been shown to be a dynamic P pool both being assimilated and produced by marine photosynthetic bacteria. The ability of eukaryotic phytoplankton to supplement their growth with phosphonates remains vastly unexplored. Several eukaryotic phytoplankton species have been shown to use glyphosate, a chemically synthesized herbicidal phosphonate, as a P source; it remains unknown if open ocean eukaryotic phytoplankton can utilize phosphonates found naturally in the marine environment. Preliminary experiments suggest at least some eukaryotic phytoplankton are able to directly utilize extracellular phosphonates. This project characterizes the pervasiveness of phosphonate utilization within eukaryotic phytoplankton lineages and identifies the cellular underpinnings that support the acquisition of and growth on naturally occuring phosphonates. The project uses whole-cell transcriptomics and functional gene complementation assays, in addition to phylogenetic analyses, to understand the bioavailability of phosphonates and relevance of phosphonate utilization by natural eukaryotic phytoplankton populations. It is critical to understand the metabolic capabilities of phytoplankton which control marine biogeochemical cycles. This is especially important given the prediction that future oceans may become more stratified which could increase the importance of DOP, including phosphonates, in supporting phytoplankton growth.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756271

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