Energy-dispersive X-ray spectroscopy (EDS) spectra and scanning electron microscope (SEM) images from phytoplankton Si utilization experiments during 8-day laboratory cultures in 2016 and 2017.

Website: https://www.bco-dmo.org/dataset/858840
Data Type: experimental
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Project
» NSFGEO-NERC: An unexpected requirement for silicon in coccolithophore calcification: physiological, ecological and evolutionary implications (Coccolithophore Silicon Requirements)

Contributors	Affiliation	Role
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Abstract
Energy-dispersive X-ray spectroscopy (EDS) spectra and scanning electron microscope (SEM) images from phytoplankton Si utilization experiments during 8-day laboratory cultures in 2016 and 2017. Coccolithophore Si utilization was assessed via Si drawdown from growth media.

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Coverage

Temporal Extent: 2016-12-20 - 2017-09-13

Dataset Description

Raw SEM images and EDS spectra files are accessible from the "Data Files" section on this page. EDS sample metadata and atomic percentages and atomic weight percentages are available as a tabular dataset from this dataset landing page which is available in a variety of formats (see file access at the top of this page).

Acquisition Description

The methodology below describes all data collected from this experiment. This dataset landing page serves the "Si Depletion Experiment: SEM and EDS" data. Other datasets can be found in the "Related Datasets" section of this page.

Experiment Overview: Phytoplankton Si utilization during an 8 d culture experiment was assessed. A variety of silicifying and non-silicifying species were grown in fully amended LH and F/2 media supplemented with 5 M Si. All species were grown in quadruplicates except for *Thalassiosira weissflogii* and the no cells control. Aliquots of cultures for cell counts, dissolved Si (DSi), and biogenic Si incorporated into the cells (BSi) were taken daily from T0 – T4, then on T6 and T8 and subsequently processed as described below. In addition, EDS analysis was used to determine if Si was present in biomineral structures.

Cell Counting: Cells were counted on each collection day using a hemocytometer or Sedgwick-Rafter chamber. A minimum of 300 cells were counted per sample. Growth curves were plotted and specific growth rates were calculated for each species throughout the 8 d sampling period.

DSi Method: Autoclaved and filtered Gulf Stream seawater was amended with LH or F/2 nutrients and [Si] measured prior to adding sufficient $NaSiO_3$ to reach a starting [Si] of 5 M. Cells were harvested from cultures at early exponential phase and gently washed in Si-free media using a 0.4 m Nalgene polycarbonate filter unit. Replicate 200 mL cultures were seeded with washed cells for a starting density of 1-5 x 10^4 mL^{-1}. Each sampling day, 15 mL culture aliquots were 0.2 m filtered (Merck Millipore Ltd.) and filtrate was stored at 4C prior to DSi analysis. The filters were frozen for later BSi analysis (see below). For AutoAnalyzer nutrient analysis, the molybdate method was used (modified from Brzezinski and Nelson (1995) and Brzezinski *et al.* (1997)). The system was washed with sodium dodecyl sulfate (SDS) to lubricate the Si lines. Oxalic acid concentrations were increased to saturated levels (143 g/L) to overcome any phosphate interference. Molybdate was made fresh for each run. The tubing diameter for the oxalic line was also increased to 0.035 in, with a flow rate of 0.41 at 40%.

BSi Method: The filters (See DSi explanation above) with cells were frozen at -20C prior to BSi analysis. For BSi determination, silicifying species were collected on 0.2 m polycarbonate filters, and processed using the alkaline digestion method as described by Brazinski and Nelson (1995), with modifications from Paasche (1973) and Krausse (1983). For coccolithophores, filters were first treated with 1mL 0.5 M HCl to fully dissolve coccoliths (Moheimani & Borowitzka, 2006), then treated with 4 mL 0.2 M NaOH to neutralize, before the alkaline digestion. For the alkaline digestion, each filter was placed in a 15 mL polymethylpentene tube (Diagenode, Inc.) with 4 mL of 0.2 M NaOH and brought to 100^{o}C in a water bath for 20 min, cooled, and neutralized with 1 mL of 0.5 M HCl. The digest was centrifuged at 10,000 rpm for 9 minutes and aliquots of supernatant were removed and diluted for autoanalyzer analysis as appropriate. Analytical blanks with filter only (no cells) were included for each run. Autoanalyzer conditions are the same as described under the DSi Collection section.

EDS Method: For EDS analysis 1-3 mL of culture were filtered onto 13 mm 0.4 m isopore filters [Merck Millipore Ltd.] and rinsed with Nanopure water buffered to pH 8.0 with 1 mM HEPES to remove salts. Filters were air-dried and mounted onto a SEM stub with carbon adhesive tabs before coating with 10 nm Pt/Pd. EDS analysis was performed at the Joint School of Nanoscience and Nanoengineering (Zeiss Auriga SEM, with a Bruker Quantax detector and analysis software) or at North Carolina State University (FEI Verios 460L SEM, with an Oxford Xmax silicon drift EDS detector and AZtec acquisition and analysis software). A minimum of 500,000 counts were collected, between 2,000 – 8,000 cps with an average deadtime < 5%. Standardless quantification was used to determine atomic % and weight % for each element.

Missing Data Value meanings:

Not_Detected = The element was below level of detection for EDS and therefore could not be quantified

Not_Collected = The number of counts was not noted during the acquisition

Processing Description

BCO-DMO data manager processing notes:
* Scientific names were checked using the World Register of Marine Species "Taxa Match" Tool. All names matched accepted names exactly from file "Si Depletion Experiment Dataset.xlsx" sheet name "EDS" column "Species." Species list with the AphiaIDs added as a supplemental file. Data imported with "None" interpreted as a missing data value (discussed with submitter).
* Column names changed to match BCO-DMO naming conventions (e.g. Ca_At% -> Ca_At_prcnt). See https://www.bco-dmo.org/page/bco-dmo-data-processing-conventions
* Data for this Cell counts dataset imported into the BCO-DMO data system from file "Si Depletion Experiment Dataset.xlsx" sheet name "EDS"

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Data Files

File	Version
EDS Spectra Files filename: EDS_Spectra_Files.zip (ZIP Archive (ZIP), 795.68 KB) MD5:c1916d4794fbe5560a8604d8d7d6661a *Energy-dispersive X-ray spectroscopy (EDS) spectra files.*	original
SEM Images filename: SEM_Image_Files.zip (ZIP Archive (ZIP), 6.66 MB) MD5:30cb3e0a30ea470c63c916848658ddf6 *Raw scanning electron microscope (SEM) images.*	original

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Related Datasets

IsRelatedTo

Taylor, A., Meyer, E. M. (2021) **Biogenic silica (BSi) incorporated into phytoplankton cells during Si utilization experiments over 8-day laboratory cultures in 2016 and 2017.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-08-20 http://lod.bco-dmo.org/id/dataset/858830 [view at BCO-DMO]
Relationship Description: Data from the same experiment.

Taylor, A., Meyer, E. M. (2021) **Cell counts from phytoplankton Si utilization experiments during 8-day laboratory cultures in 2016 and 2017.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-08-20 http://lod.bco-dmo.org/id/dataset/858823 [view at BCO-DMO]
Relationship Description: Data from the same experiment.

Taylor, A., Meyer, E. M. (2021) **Dissolved silica (DSi) from phytoplankton Si utilization experiments during 8-day laboratory cultures in 2016 and 2017.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-08-20 http://lod.bco-dmo.org/id/dataset/858835 [view at BCO-DMO]
Relationship Description: Data from the same experiment.

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Parameters

Parameter	Description	Units
Species	The haptophyte and diatom species for which energy-dispersive X-ray spectroscopy (EDS) analysis was performed. For this study those species were: Prymnesium neolepis, Thalasiossira weissflogii, Emiliania huxleyi, Gephyrocapsa oceanica, Calcidiscus leptoporus, Coccolithus braarudii, and Scyphosphaera apsteinii.	unitless
Spectra_Name	The name of the raw spectra file that was collected for each region of interest (ROI) for a specimen. This raw spectra file corresponds to the Atomic % and Weight % values presented in the table.	unitless
SEM_Image_Name	The name of the SEM image file that was taken for each cell. Images were taken at the same time as EDS analysis.	unitless
Date_Imaged	The date that the cells were imaged on an SEM and were analyzed with EDS. Date in ISO 8601 format YYYY-MM-DD	unitless
Cell_Number	Corresponds to the cell replicate number that was imaged for each stub (one stub was created for each species).	unitless
C_At_pcnt	Carbon Atomic percent (At%)	percent (%)
Ca_At_pcnt	Calcium Atomic percent (At%)	percent (%)
O_At_pcnt	Oxygen Atomic percent (At%)	percent (%)
Sr_At_pcnt	Strontium Atomic percent (At%)	percent (%)
Si_At_pcnt	Silicon Atomic percent (At%)	percent (%)
Mg_At_pcnt	Magnesium Atomic percent (At%)	percent (%)
Si_Wt_pcnt	Silicon Weight percent (Wt%)	percent (%)
Sr_Wt_pcnt	Strontium Weight percent (Wt%)	percent (%)
Mg_Wt_pcnt	Magnesium Weight percent (Wt%)	percent (%)
Magnification	The magnification at which the SEM image was taken and EDS analysis was performed.	unitless
Total_Counts	The total amounts of X-rays that were collected for each EDS spectra that were produced.	unitless
KeV	The accelerating voltage of the electron beam during the time of imaging and analysis.	volts (V)
Comments	Any observations that were made during imaging and EDS analysis. Used primarily for analysis on S. apsteinii, which is a dimorphic species. The comments were used to describe whether the spectra collected were for a murolith or a lopadolith.	unitless
SEM_image_link	Link (URL) to the SEM image that was taken for each cell. Images were taken at the same time as EDS analysis.	unitless

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Scanning Electron Microscope
Dataset-specific Description	EDS: Energy-dispersive X-ray spectroscopy (EDS) is a technique that can be performed in a scanning electron microscope (SEM) whereby accelerated electrons interact with the specimen generating x-ray photons that are characteristic for specific elements. Therefore, EDS allows for element identification and quantification. Below are the SEMs used with respective EDS analysis systems: Zeiss Auriga SEM equipped with Bruker Quantax EDS detector and analysis software for elemental analysis. FEI Verios 460L SEM equipped with Oxford Xmax silicon drift EDS detector and AZtec acquisition and analysis software for elemental analysis.
Generic Instrument Description	A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition.

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Project Information

NSFGEO-NERC: An unexpected requirement for silicon in coccolithophore calcification: physiological, ecological and evolutionary implications (Coccolithophore Silicon Requirements)

NSF abstract:

Biomineralization by marine phytoplankton has had a profound impact on our planet. The production of special cell wall material, calcite coccoliths by coccolithophores and silica frustules by diatoms, are major drivers in global biogeochemical cycles, but the underlying cellular processes remain poorly understood. It is widely considered that calcification in coccolithophores occurs through a very different process to silicification in diatoms, however some ecologically important coccolithophore lineages possess diatom-like silicon (Si) transport systems and have an absolute requirement for Si during coccolith formation. Importantly, the abundant bloom-forming coccolithophores such as Emiliania huxleyi exhibit no requirement for Si. There is a clear need to understand how these different physiological requirements for dissolved Si have driven the ecology and evolution of the coccolithophores. The project will yield a more complete understanding of the Si requirements of coccolithophores, its role in the calcification process, and the impacts of Si availability on the biogeography of these important bloom forming phytoplankton. The results are expected to strengthen our ability to predict the responses of coccolithophores to short and long-term environmental change, and therefore the consequences for the marine biogeochemical cycles in which they participate. In addition to the scientific outcomes, the project provides independent research opportunities to a diverse pool of undergraduate students, provide interdisciplinary training for graduate students, and facilitate the professional development of post-doctoral researchers. Public engagement in the research is facilitated through participant involvement in regional science festivals, public outreach

events, production of educational resources, and targeted K-12 summer camp activities.

Calcification in coccolithophores appears to represent a distinct process from silicification in diatoms, another major group of biomineralized phytoplankton. The apparent absence of a requirement for silicon (Si) in coccolithophores has been proposed to play a critical role in their ability to out-compete the otherwise dominant diatoms in areas of low dissolved Si availability. However, the investigators recently demonstrated that some globally important coccolithophores possess diatom-like Si transporters and exhibit an obligate requirement for Si in the calcification process. This discovery has important implications both for phytoplankton ecology and for the evolution of biomineralization. Using a range of physiological, molecular and computational approaches the project will 1) Establish Si requirements of ecologically important coccolithophore groups; 2) Determine the physiological role of Si in coccolithophores; 3) Determine the evolutionary events leading to the differing requirements for Si in calcification; 4) Examine the ecological distribution of Si-requiring coccolithophores, and 5) Determine the impact of the Si requirement on coccolithophore ecology. This project therefore integrates the molecular identification of genes (Si transporters), the physiological role of these transporters, and ecosystem scale models in order to examine how the requirement for Si influences ecosystem functioning and coccolithophore biogeography. The results of this work provides essential data that describes the cellular mechanisms of calcification and the range of physiological diversity between major coccolithophore lineages. The research also explores a previously unforeseen aspect of phytoplankton ecology; examining how the differing requirements for Si in calcifying coccolithophores may have shaped competitive interactions with other phytoplankton over both contemporary and evolutionary timescales. Overall, the research provides novel insights into physiology, ecology and evolution of coccolithophores, including information on how and why coccoliths are produced, which is currently poorly understood. This information is vital in order to understand how coccolithophores have been influenced by past changes in the Earth's climate, and their potential responses to future oceans.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1638838

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