Cell counts from phytoplankton Si utilization experiments during 8-day laboratory cultures in 2016 and 2017

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

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Abstract
Cell counts 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

Acquisition Description

The methodology below describes all data collected from this experiment. This dataset landing page serves the "Si Depletion Experiment: Cell Counts" 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 NaSiO3 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 104 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 100oC 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.

Processing Description

Cell Counts:
The formulas for growth rate and doublings per day were captured as the image "cell_counts_processing.png" which is displayed below and can also be found in the "Supplemental Files" section.

Data processing:

Cell Counts: Cell counts (Cells mL^{-1}) were used to determine growth rate (r) and doublings per day (k) for each species using the following equations:

$$\text{Rate of increase (r)} = \frac{(LN_{average\ cells\ t0}) - (LN_{average\ cells\ t1})}{(t_1 - t_0)}$$

$$\text{Doublings per day (k)} = \frac{r}{0.6931}$$

LN is the natural log of the average cell numbers per L. t is the timepoint.

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 for this Cell counts dataset imported into the BCO-DMO data system from file "Si Depletion Experiment Dataset.xlsx" sheet name "Cell_Counts"

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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) **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.

Taylor, A., Meyer, E. M. (2021) **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.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-08-20 http://lod.bco-dmo.org/id/dataset/858840 [view at BCO-DMO]
Relationship Description: Data from the same experiment.

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Parameters

Parameter	Description	Units
Sample	The sample of cells that were being counted. Name is in the following order: Species_Replicate flask number. The species used were Prymnesium neolepis, Thalassiosira weissflogii, Emiliania huxleyi, Gephyrocapsa oceanica, Calcidisdcus leptoporus, Coccolithus braarudii, and Scyphosphaera apsteinii.	unitless
Sampling_Day	Day aliquots were taken from flasks and cells were counted. Numeric day of the experiment for the sample starting at day 0.	days (d)
Cells_per_mL	The amount of cells of each species in 1 mL of growth media on the day of sampling. Cells were counted with either a Sedgwick-Rafter or Hemocytometer.	Cells per milliliter (Cells/mL)

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	light microscope with either a Hemocytometer or Sedgewick-Rafter chamber
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

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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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