

Energy-dispersive X-ray spectroscopy (EDS) from an experiment examining the effect of Sr on the coccolithophore *Scyphosphaera apsteinii* calcification

Website: <https://www.bco-dmo.org/dataset/866679>

Data Type: experimental

Version: 1

Version Date: 2021-12-10

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

The effect of Sr on the coccolithophore *Scyphosphaera apsteinii* calcification was assessed over a 10 day period. These data resulted from the energy-dispersive X-ray spectroscopy conducted as part of this experiment. The spectra files are also provided in a .zip.

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Coverage

Temporal Extent: 2019-04-19 - 2019-08-05

Methods & Sampling

Experiment Overview:

The effect of strontium (Sr) on the coccolithophore *Scyphosphaera apsteinii* calcification was assessed over a 10 d period. This species of coccolithophore has unusually high levels of Sr in its calcite coccoliths as detected with Energy-dispersive X-ray spectroscopy (EDS), whereas Si levels are undetectable with EDS. The goal of the experiment was to determine whether Sr in seawater plays a significant role in coccolith production and/or coccolith crystal morphology. *S. apsteinii* was acclimated and grown in a range of Sr concentrations (deplete: 0.33 mmol/mol Sr/Ca, ambient: 9 mmol/mol Sr/Ca, and higher than ambient: 36 and 72 mmol/mol Sr/Ca). All treatments had four replicate flasks. Aliquots of cultures for cell counts and Fv/Fm were taken every two days between the start (T0 for cell counts, T4 for Fv/Fm) and end (T10) of the experiment. When cells were at mid-exponential phase (T4-T6) aliquots were collected for scanning electron microscopy (SEM) and EDS analysis to observe morphology and determine Sr incorporation into calcite coccoliths, respectively.

The laboratory experiments were conducted in March 2019 at the University of North Carolina - Wilmington. SEM/EDS analysis was done at the Analytical Instrumentation Facility at NC State from April 19th to May 9th,

2019.

Scanning Electron Microscopy (SEM) and Energy-dispersive X-ray Spectroscopy (EDS):

For EDS analysis, 1-3 mL of culture were filtered onto 13 mm 0.4 um 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 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. The EDS spectra files are provided under Supplemental Files (see spectra_files.zip).

Data Processing Description

Coccolith Scoring:

Forty cells from each treatment were imaged and attached coccoliths were scored. Coccoliths were only scored if the majority of the coccolith could be seen. Murooliths were scored into four categories: normal, incomplete, malformed, and aberrant (descriptions for each can be seen in the Sr Metadata Parameters.xls spreadsheet). Lopadoliths were scored into five categories: normal, expected malformed, incomplete, Type S, Type T, and Type R. Scores for each morphometric category were counted, added, then divided by the total number of murooliths or lopadoliths (example: total # of normal murooliths/total # of murooliths) to determine frequency. Scores for each morphometric category from each replicate flask were then averaged (n = 4).

BCO-DMO Processing:

- removed units from data columns so values can be typed as numeric;
- replaced "Not_Detected" with "nd";
- changed date format to YYYY-MM-DD;
- renamed fields to comply with BCO-DMO naming conventions.

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Data Files

File
sr_EDS.csv (Comma Separated Values (.csv), 66.58 KB) MD5:de6c5d09869b3efdc62ef6840633e03b
Primary data file for dataset ID 866679

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Supplemental Files

File
EDS spectra files filename: spectra_files.zip (ZIP Archive (ZIP), 812.66 KB) MD5:86eae2a63dfd5d199b5babc3a7d40b8e
EDS Spectra files (.txt format)

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Related Datasets

IsRelatedTo

Taylor, A. (2022) **Cell counts from an experiment examining the effect of Sr on the coccolithophore**

Scyphosphaera apsteinii calcification. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-12-10 doi:10.26008/1912/bco-dmo.866456.1 [[view at BCO-DMO](#)]

Taylor, A. (2022) **Coccolith morphology data from an experiment examining the effect of Sr on the coccolithophore Scyphosphaera apsteinii calcification.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-12-10 doi:10.26008/1912/bco-dmo.866738.1 [[view at BCO-DMO](#)]

Taylor, A. (2022) **Quantum yield of photosystem II (Fv/Fm) from an experiment examining the effect of Sr on the coccolithophore Scyphosphaera apsteinii calcification.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-12-10 doi:10.26008/1912/bco-dmo.866648.1 [[view at BCO-DMO](#)]

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Parameters

Parameter	Description	Units
Sr_Concentration	The Sr concentration of the growth media that <i>S. apsteinii</i> was exposed to. The concentrations were 3.4, 90, 360, and 720 micromoles Sr.	micromoles per liter Si (umol/L)
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; 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 flask replicate per treatment).	unitless
Coccolith_Type	Identification of what type of coccolith was sampled (muralith or lopadolith, M and L respectively). If the coccolith was a lopadolith, it was either sampled at the base (B), middle (M), or tip (T). Coccolith description is formatted as follows: Coccolith type - lopadolith location (if applicable).	unitless
Ca_At_pcnt	Calcium Atomic %	Atomic % (At%)
C_At_pcnt	Carbon Atomic %	Atomic % (At%)
O_At_pcnt	Oxygen Atomic %	Atomic % (At%)
Sr_At_pcnt	Strontium Atomic %	Atomic % (At%)
Ca_Wt_pcnt	Calcium Weight %	Weight % (Wt%)

C_Wt_pcnt	Carbon Weight %	Weight % (Wt%)
O_Wt_pcnt	Oxygen Weight %	Weight % (Wt%)
Sr_Wt_pcnt	Strontium Weight %	Weight % (Wt%)
Magnification	The magnification at which the SEM image was taken and EDS analysis was performed.	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.	unitless

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Instruments

Dataset-specific Instrument Name	FEI Verios 460L SEM
Generic Instrument Name	Scanning Electron Microscope
Dataset-specific Description	SEM and 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 is the SEM used with respective EDS analysis system: 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 Award Abstract:

Biominingalization 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 *Emiliana 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.

This is a project jointly funded by the National Science Foundation's Directorate for Geosciences (NSF/GEO) and the National Environment Research Council (NERC) of the United Kingdom (UK).

NERC Award Abstract:

The oceans cover more than three quarters of the surface of the Earth and tiny algae in our seas are responsible for half of all photosynthesis on our planet. These single celled organisms, known as phytoplankton, form the basis of marine food webs and their activities can have an enormous impact on the geology of our planet. One group of phytoplankton known as the coccolithophores produce a covering of calcium carbonate plates (coccoliths) and can form vast blooms in the oceans. When the coccolithophores die, their coccoliths settle to the ocean floor, leading to the formation of sedimentary rocks, such as chalks and limestones. In many parts of the ocean the low availability of nutrients (such as nitrogen and phosphorus) limits phytoplankton growth. Competition for nutrients plays an important role in determining which phytoplankton species can grow in different environments. One of the most successful phytoplankton groups in modern oceans is the diatoms, which are fast-growing, making it difficult for many other phytoplankton types to compete with them. However, diatoms need lots of dissolved silicon to make their silica cell walls. In some marine environments, the availability of silicon limits the growth of diatoms, allowing other phytoplankton (which do not need silicon) to grow in their place. It is commonly thought that the calcifying coccolithophores have no requirement for silicon. However, we have recently discovered that some important coccolithophore species actually possess silicon transporters that are similar to those used by diatoms. Remarkably, we found that these coccolithophores use silicon to make their calcium carbonate coccoliths. Therefore the processes of silica formation in diatoms and calcite production in coccolithophores, which were previously believed to be distinct processes, show a completely unexpected link. These findings have important implications for the evolution of biomineralisation in phytoplankton and for the competitive interactions between coccolithophores and diatoms. Not all coccolithophores show a requirement for silicon. We found that the species responsible for the massive coccolithophore blooms, *Emiliana huxleyi*, does not possess silicon transporters and exhibits

no need for silicon in the calcification process. The absence of a requirement for silicon may have enabled bloom-forming species to grow better in areas where silicon is low (e.g. after a diatom bloom). There is therefore a clear need to understand the role of silicon in coccolithophore biology. In this proposal we will address this issue using a combination of laboratory experiments and computational modelling approaches. Firstly, we will use molecular genetic and laboratory experiments to determine which of the major coccolithophore species exhibit a requirement for silicon. We will then select species for detailed physiological analysis, to determine how silicon contributes to the formation of coccoliths and how coccolithophores take up silicon from the surrounding seawater. These studies will allow us to examine the evolutionary history of the requirement for silicon and determine when certain lineages appear to have lost this trait. Using parameters on Si uptake and usage derived from our experimental work, we will use computer simulations to model global coccolithophore distributions and identify environments where the requirement for Si appears to be playing an important role in coccolithophore ecology. The research will provide novel insight into physiology, ecology and evolution of coccolithophores, including information on how and why coccoliths are produced, which is currently poorly understood. The research will also inform us on the evolution of coccolith formation, which will be vitally important if we are to understand how coccolithophores have been influenced by past changes in the Earth's climate and how they may respond to changes in the future.

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Funding

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

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