Coccolithophore counts from polarized microscopy birefringence measurements of samples collected in the Northwest Atlantic during R/V Endeavor cruise EN616 in July 2018

Website: https://www.bco-dmo.org/dataset/887863
Data Type: Cruise Results
Version: 1
Version Date: 2023-02-05

Project
» Coccolithophore Mixotrophy (Cocco-Mix)

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Abstract
This dataset presents polarized microscopy-derived concentration data for coccolithophores and detached coccoliths in samples collected from stations in the Northwest Atlantic during R/V Endeavor cruise EN616 in July 2018. Counts are based on image analysis of dark-field, cross-polarized views of filtered particulate matter. These counts take advantage of the birefringence property of calcium carbonate (particulate inorganic carbon) that it rotates the plane of linearly polarized incident light by 90 degrees. Incident light directed upwards, towards the microscope slide, is polarized 90 degrees with a linear polarizer. Particles are viewed from above the slide, through a second, linear polarizer filter held between the microscope stage and the camera which only accepts light that is polarized orthogonal to the lower polarizer. Calcium carbonate particles in the beam appear as bright dots of light. Image analysis software then analyzes the pattern of birefringence and enumerates only those particles with size and shape of coccolithophores or detached coccoliths.

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Coverage

Spatial Extent: **N**:43.71835 **E**:-66.51748 **S**:36.98572 **W**:-72.92708
Temporal Extent: 2018-07-05 - 2018-07-13

Dataset Description

This dataset is part of a larger study with the following goals:

- **Goal #1**: measure the mixotrophic uptake and assimilation of 14C-acetate, 14C-mannitol and 14C-glycerol as a carbon source by natural assemblages of coccolithophores and compare it to their autotrophic uptake and assimilation of DIC. These three organics were chosen due to their high potential for significant osmotrophy by coccolithophores as seen in previous culture studies (Godrijan et al., 2020). The design of these experiments used radiochemical and single cell/flow cytometer methods to distinguish osmotrophy of coccolithophores from that by other naturally-occurring microalgae.
- **Goal #2**: test for the fixation of 14C-labeled organics into both POC and PIC fractions in natural populations of coccolithophores, in order to examine the potential role of coccolithophore osmotrophy in the biological carbon pump and alkalinity carbon pump paradigms.

Acquisition Description

Inherent optical properties of coccolithophores can be used for estimating particulate inorganic carbon (PIC) distributions in the water column. Polarized microscopy was used to determine the concentration of coccolithophores and detached coccoliths in samples collected in the Northwest Atlantic during R/V Endeavor cruise EN616 in July 2018. Water samples were collected using CTD casts from nine stations encompassing New England Shelf, Slope, and Sargasso Sea waters.

At eight depths, three 10L Niskin samples were taken for discrete measurements of:

1. Chlorophyll, nutrients including nitrate, nitrite, ammonium, phosphate, and silicate
2. Particulate organic carbon (POC) plus particulate organic nitrogen (PON)
3. Particulate inorganic carbon (PIC)
4. Biogenic silica
5. Birefringence counts of coccolithophores (done ashore)
6. Shipboard Yokogawa Fluid Imaging Technologies FlowCam imaging cytometer, in order to enumerate the major microalgal classes and estimate the particle size distribution function

Measurements 1 to 4 are part of BCO-DMO dataset 837074 (See https://www.bco-dmo.org/dataset/837074, and the Related Datasets section below).
Measurement 5 of birefringence counts data is detailed here on this dataset page
Measurement 6 is BCO-DMO dataset 887787. (See https://www.bco-dmo.org/dataset/887787, and the Related Datasets section below)

Quantitative light microscope counts of birefringent coccolithophores and detached coccoliths were also required for determining their concentration in the field incubation sample. A volume of 200mL was filtered onto 0.4µm-pore size, 25mm diameter polycarbonate filter then processed according to Balch & Utgoff (2009).

Processing Description

The "CCC algorithm" (Count Coccolithophores and Coccoliths) from Balch & Utgoff (2009) was implemented. The CCC algorithm takes advantage of the distinct birefringence patterns of coccoliths to analyze polarized microscope images for individual coccoliths as well as plated coccolithophores.

CCC analysis involves the following steps:

- Quantification (and elimination) of background light scattering in the image.
- Estimation of the birefringence of non-PIC particles in the image (which typically are more diffuse than PIC particles, with lower contrast).
- Enumeration of free coccoliths in the image (clusters of bright, birefringent pixels, arranged in distinctive patterns within a prescribed proximity of each other).
- Partioning of the remaining birefringence patterns into (a) plated coccolithophores (having coccoliths, arranged in spherical patterns with known diameters of plated coccolithophore cells and within a specific proximity of each other) or (b) randomly shaped coccolith aggregates (statistically distinct from spherical coccolithophore cells)

BCO-DMO processing
- Data is from columns I through O on the original source file called "EN616_master_datasheet_bottle_and_discrete_organics_updated_ccc_BCODMO.csv"
- Birefringence data extracted from combined "master datasheet" into a separate file called "birefringence_EN616.csv"
- Modified parameter (column) names to conform with BCO-DMO naming conventions.
- Converted date format to ISO Date 8601 format

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Data Files

File	Version
birefringence_en616.csv filename: birefringence_en616.csv (Comma Separated Values (.csv), 8.03 KB) MD5:81d19d91289fb8206e159a4fd293b2dc Polarized microscopy birefringence data of coccolithophores sampled during cruise EN616.	1

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Related Publications

Balch, W., & Utgoff, P. (2009). Potential Interactions Among Ocean Acidification, Coccolithophores, and the Optical Properties of Seawater. Oceanography, 22(4), 146–159. https://doi.org/10.5670/oceanog.2009.104
Methods

Balch, W.M., Drapeau, D.T., Poulton, N., Archer, S.D., Cartisano, C., Burnell, C., Godrijan, J. (2023) Coccolithophore osmotrophy of dissolved organic compounds into particulate organic carbon and calcium carbonate. Science Advances (in press).
Results

Godrijan, J., Drapeau, D., & Balch, W. M. (2020). Mixotrophic uptake of organic compounds by coccolithophores. Limnology and Oceanography, 65(6), 1410–1421. doi:10.1002/lno.11396
Related Research

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Related Datasets

IsRelatedTo

Balch, W. M. (2021) **Measurements of Chlorophyll, NO2, NO3, PO4, Silicate, NH4, PIC, POC, PON, BSi from CTD casts on R/V Endeavor cruise EN616 in July 2018.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-03-08 doi:10.26008/1912/bco-dmo.837074.1 [view at BCO-DMO]

Balch, W. M., Drapeau, D. T., Archer, S. D., Godrijan, J. (2023) **Ambient concentrations of acetate, glycerol, and mannitol measured from samples collected during R/V Endeavor EN616 cruise in the northwest Atlantic in July 2018.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-02-26 http://lod.bco-dmo.org/id/dataset/887851 [view at BCO-DMO]

Balch, W. M., Drapeau, D. T., Archer, S. D., Godrijan, J. (2023) **FlowCAM imaging cytometer data from EN616 cruise.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-01-27 http://lod.bco-dmo.org/id/dataset/887787 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
Cruise	Cruise identification	unitless
Station	Station number for EN616 cruise for water sample collection	unitless
Type	Type of sample. B = discrete bottle sample	unitless
Longitude	Longitude of water sample collection	decimal degrees
Latitude	Latitude of water sample collection	decimal degrees
Depth	Depth of water sample	meters
ISO_DateTime_UTC	Date and time of sample collection	unitless
Gear	Gear used to collect the water and coccolithophore samples	unitless
Balch_Sample_num	Consecutive unique numbers assigned to each water sample for all analyses done for a given station	unitless
Singlet_Lith	Concentration of birefringent particles under polarized light microscope that show one cross-polarized point of light per particle	particles per milliliter (particles/mL)
Doublet_Lith	Concentration of birefringent particles under polarized light microscope that show two cross-polarized points of light per particle	particles per milliliter (particles/mL)
Triplet_Lith	Concentration of birefringent particles under polarized light microscope that show three cross-polarized points of light per particle	particles per milliliter (particles/mL)
Quadruplet_Lith	Concentration of birefringent particles under polarized light microscope that show four cross-polarized points of light per particle	particles per milliliter (particles/mL)
Total_Lith	Concentration of all birefringent particles under polarized light microscope that show single, double, triple, or quadruple cross-polarized points of light per particle	particles per milliliter (particles/mL)
Cell_plus_Aggregates	Concentration of plated coccolithophores, empty coccospheres, and coccolith aggregates enumerated by image-analysis software (CCC) that was used to analyze all polarized microscope samples	particles per milliliter (particles/mL)

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Instruments

Dataset-specific Instrument Name	Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset-specific Description	At eight depths, three 10L Niskin bottle samples were taken
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	Quantitative light microscope (Olympus BH-2 microscope equipped with polarization optics)
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Quantitative light microscope (Olympus BH-2 microscope equipped with polarization optics) was used to determine counts of coccolithophores and detached coccoliths.
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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Deployments

EN616

Website	https://www.bco-dmo.org/deployment/837075
Platform	R/V Endeavor
Start Date	2018-07-03
End Date	2018-07-15
Description	See additional cruise information from the Rolling Deck to Repository (R2R): https://www.rvdata.us/search/cruise/EN616

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Project Information

Coccolithophore Mixotrophy (Cocco-Mix)

Coverage: Partially lab-based, with field sites in Gulf of Maine and NW Atlantic between the Gulf of Maine and Bermuda

NSF Award Abstract
Coccolithophores are single-cell algae that are covered with limestone (calcite) plates called coccoliths. They may make up most of the phytoplankton biomass in the oceans. Coccolithophores are generally considered to be autotrophs, meaning that they use photosynthesis to fix carbon into both soft plant tissue and hard minerogenic calcite, using sunlight as an energy source ("autotrophic"). However, there is an increasing body of evidence that coccolithophores are "mixotrophic", meaning that they can fix carbon from photosynthesis as well as grow in darkness by engulfing small organic particles plus taking up other simple carbon molecules from seawater. The extent to which Coccolithophores engage in mixotrophy can influence the transfer of carbon into the deep sea. This work is fundamentally directed at quantifying coccolithophore mixotrophy -- the ability to use dissolved and reduce carbon compounds for energy -- using lab and field experiments plus clarifying its relevance to ocean biology and chemistry. This work will generate broader impacts in three areas: 1) Undergraduate training: Two REU undergraduates will be trained during the project. The student in the second year will participate in the research cruise. 2) Café Scientifique program: This work will be presented in Bigelow Laboratory's Café Scientifique program. These are free public gatherings where the public is invited to join in a conversation about the latest ideas and issues in ocean science and technology. 3) Digital E-Book: We propose to make a digital E-book to specifically highlight and explain mixotrophy within coccolithophores. Images of mixotrophic coccolithophores would be the primary visual elements of the book. The E-book will be publicly available and distributed to our educational affiliate, Colby College. The goal of the book is to further communicate the intricacies of the microbial world, food web dynamics, plus their relationship to the global carbon cycle, to inspire interest, education, and curiosity about these amazing life forms.

Coccolithophores can significantly affect the draw-down of atmospheric CO2 and they can transfer CO2 from the surface ocean and sequester it in the deep sea via two carbon pump mechanisms: (1) The "alkalinity pump" (also known as the calcium carbonate pump), where coccolithophores in the surface ocean take up dissolved inorganic carbon (DIC; primarily a form called bicarbonate, a major constituent of ocean alkalinity). They convert half to CO2, which is either fixed as plant biomass or released as the gas, and half is synthesized into their mineral coccoliths. Thus, coccolithophore calcification can actually increase surface CO2 on short time scales (i.e. weeks). However, over months to years, coccoliths sink below thousands of meters, where they dissolve and release bicarbonate back into deep water. Thus, sinking coccoliths essentially "pump" bicarbonate alkalinity from surface to deep waters, where that carbon remains isolated in the abyssal depths for thousands of years. (2) The "biological pump", where the ballasting effect of the dense limestone coccoliths speeds the sinking of organic, soft-tissue debris (particulate organic carbon or POC), essentially "pumping" this soft carbon tissue to depth. The biological pump ultimately decreases surface CO_2. The soft-tissue and alkalinity pumps reinforce each other in maintaining a vertical gradient in DIC (more down deep than at the surface) but they oppose each other in terms of the air-sea exchange of CO_2. Thus, the net effect of coccolithophores on atmospheric CO2 depends on the balance of their CO_2-raising effect associated with the alkalinity pump and their CO2-lowering effect associated with the soft-tissue biological pump. It is virtually always assumed that coccolith particulate inorganic carbon (PIC) originates exclusively from dissolved inorganic carbon (DIC, as bicarbonate), not dissolved organic carbon (DOC). The goal of this proposal is to describe a) the potential uptake and assimilation of an array of DOC compounds by coccolithophores, b) the rates of uptake, and potential incorporation of DOC by coccolithophores into PIC coccoliths, which, if true, would represent a major shift in the alkalinity pump paradigm. This work is fundamentally directed at quantifying coccolithophore mixotrophy using lab and field experiments plus clarifying its relevance to ocean biology and chemistry. There have been a number of technological advances to address this issue, all of which will be applied in this work. The investigators will: (a) screen coccolithophore cultures for the uptake and assimilation of a large array of DOC molecules, (b) perform tracer experiments with specific DOC molecules in order to examine uptake at environmentally-realistic concentrations, (c) measure fixation of DOC into organic tissue, separately from that fixed into PIC coccoliths, (d) separate coccolithophores from other phytoplankton and bacteria using flow cytometry and e) distinguish the modes of nutrition in these sorted coccolithophore cells. This work will fundamentally advance the state of knowledge of coccolithophore mixotrophy in the sea and address the balance of carbon that coccolithophores derived from autotrophic versus heterotrophic sources.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1635748

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