# FlowCAM enumeration of phytoplankton classes from samples taken during R/V Endeavor cruise EN616 in July 2018

**Website**: https://www.bco-dmo.org/dataset/887787
**Data Type**: Cruise Results
**Version**: 1
**Version Date**: 2023-01-27

**Project**
» Coccolithophore Mixotrophy (Cocco-Mix)

| Contributors | Affiliation | Role |
|---|---|---|
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**Abstract**
This dataset presents imaging cytometer data from water samples collected during R/V Endeavor cruise EN616. Niskin bottle samples were taken at nine stations and eight depths in the northwest Atlantic in July 2018. A Yokogawa FlowCAM imaging cytometer was used to enumerate the major microalgal classes, and the particle size distribution function was estimated.

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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) and BCO-DMO dataset: https://www.bco-dmo.org/dataset/858771). 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.

**Methods & Sampling**

**At sea collections**
Nine stations were visited during R/V Endeavor EN616 cruise in the northwest Atlantic.
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 BCO-DMO dataset 887863 (See https://www.bco-dmo.org/dataset/887863, and the Related Datasets section below).
Measurement 6 is flow cytometer data presented in **this** dataset.

**FlowCAM enumeration of various phytoplankton classes**
A Yokogowa FlowCAM imaging cytometer was used to enumerate different classes of phytoplankton. The instrument was keyed on particle backscattering and fluorescence properties. Samples were first filtered through 100um Nitex mesh to make sure the 100um diameter flow chamber did not clog. The instrument was run with a 10X objective in order to reliably count particles bigger than 4-5um diameter. Samples were processed according to Poulton and Martin (2010).

Concentrations (per mL), percent contribution with respect to total particles, and biomass are presented. Carbon biomass was determined based on the Menden-Deuer & Lessard (2000) method.

**Data Processing Description**

**BCO-DMO processing**
- Data is from columns P through AT on the original source file titled "EN616_master_datasheet_bottle_and_discrete_organics_updated_ccc_BCODMO.csv"
- FlowCAM cytometry data extracted from combined "master datasheet" into a separate file called "Flow_cytometer_EN616.csv"
- Modified parameter (column) names to conform with BCO-DMO naming conventions.
- Converted date format to ISO Date 8601 format
- Missing data identifier of -999 was removed to keep value from being mistaken for true data

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## Data Files

| File | |
|---|---|
| **flow_cytometer_en616.csv** | (Comma Separated Values (.csv), 23.40 KB)<br>MD5:a5c5a16b1679ac208f39487b1cb86bfe |
| FlowCAM imaging cytometer data enumerating phytoplankton classes from samples taken during R/V Endeavor cruise EN616 in July 2018 | |

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## Related Publications

Balch, W. M., Drapeau, D. T., Poulton, N., Archer, S. D., Cartisano, C., Burnell, C., & Godrijan, J. (2023). Osmotrophy of dissolved organic compounds by coccolithophore populations: Fixation into particulate organic and inorganic carbon. Science Advances, 9(21). https://doi.org/10.1126/sciadv.adf6973
*Results*

Menden-Deuer, S., & Lessard, E. J. (2000). Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnology and Oceanography, 45(3), 569–579. doi:10.4319/lo.2000.45.3.0569
*Methods*

Poulton, N. J. and Martin, J.L. (2010). Imaging flow cytometry for quantitative phytoplankton analysis — FlowCAM. In: Intergovernmental Oceanographic Commission of ©UNESCO. Karlson, B., Cusack, C. and Bresnan, E. (editors). Microscopic and molecular methods for quantitative phytoplankton analysis. Paris, UNESCO. (IOC Manuals and Guides, no. 55.) (IOC/2010/MG/55), 110 pages. Available from: https://unesdoc.unesco.org/ark:/48223/pf0000187824
*Methods*

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## Related Datasets

### IsRelatedTo

Balch, W. M., Archer, S. D., Drapeau, D. T., Godrijan, J. (2023) **Ambient concentrations of acetate, glycerol, and mannitol measured from samples collected during R/V Endeavor cruise EN616 in the northwest Atlantic in July 2018.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-02-26 doi:10.26008/1912/bco-dmo.887851.1 [view at BCO-DMO]

Balch, W. M., Archer, S. D., Drapeau, D. T., Godrijan, J. (2023) **Coccolithophore counts from polarized microscopy birefringence measurements of samples collected in the Northwest Atlantic during R/V Endeavor cruise EN616 in July 2018.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-02-05 doi:10.26008/1912/bco-dmo.887863.1 [view at BCO-DMO]

Balch, W. M., Godrijan, J., Drapeau, D. T., Archer, S. D. (2023) **DOC uptake rates by coccolithophores and scintillation counts from field experiments in the North Atlantic during R/V Endeavor cruise EN616 in July 2018.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-03-22 doi:10.26008/1912/bco-dmo.887562.1 [view at BCO-DMO]

### IsSupplementedBy

Balch, W. M., Archer, S. D., Drapeau, D. T., Godrijan, J. (2023) **Hydrography and environmental conditions measured with CTD at nine stations during R/V Endeavor cruise EN616 in July 2018.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-02-24 doi:10.26008/1912/bco-dmo.887800.1 [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 |
| ParticleSizeDistFunc_Slope_5um | PDF Slope logABD>0.75 (only particles >5um); Particle size Distribution Function slope of the plot of log cell abundance (particles per mL) versus Area Based Diameter (micrometers) calculated for particles of >= 5 micrometers diameter using a Yokogowa FlowCAM. Area Based Diameter (ABD) is defined as the diameter measured by the number of grey scale pixels of the binary image converted to a circle with the same number of pixels. | unitless |
| StdErr_of_PDFslope | Std Err of PDF Slope logABD>0.75 (for particles >5um); Standard error of the above particle size distribution slope for only particles of 5 micrometers diameter or larger using a Yokogowa FlowCAM | unitless |
| Y_int_of_PDFslope | Y-int of PDF Slope logABD>0.75 (only particles >5um); the Y intercept of above PDF for only particles of 5um diameter or larger using a Yokogowa FlowCAM | unitless |
| R2_of_PDFslope | R2of PDF Slope logABD>0.75; squared correlation coefficient of above PDF for only particles of >= 5um diameter or larger using a Yokogowa FlowCAM | unitless |
| F_statistic_of_PDFslope | F-statistic of PDF Slope logABD>0.75 (only particles >5um); the F statistic of the above PDF for only particles of >=5um diameter or larger using a Yokogowa FlowCAM | unitless |

| | | |
|---|---|---|
| Total | Concentration of total particles measured by Yokogowa measured by Yokogowa FlowCAM imaging cytometer | cells per milliliter (cells/mL) |
| Small_0_to_4um | Concentration of small particles with diameters of 0 to 4 micrometers measured by Yokogowa FlowCAM imaging cytometer | cells per milliliter (cells/mL) |
| Round_4_to_12um | Concentration of round particles with diameters of 4 to 12 micrometers measured by Yokogowa FlowCAM imaging cytometer | cells per milliliter (cells/mL) |
| Ovoid_4_to_12um | Concentration of ovoid particles with long axis of 4 to 12 micrometers measured by Yokogowa FlowCAM imaging cytometer | cells per milliliter (cells/mL) |
| Dinoflagellates | Concentration of dinoflagellates measured by Yokogowa FlowCAM imaging cytometer | cells per milliliter (cells/mL) |
| Ciliates | Concentration of ciliates measured by Yokogowa FlowCAM imaging cytometer | cells per milliliter (cells/mL) |
| Diatoms | Concentration of pennate and centric diatoms measured by Yokogowa FlowCAM imaging cytometer | cells per milliliter (cells/mL) |
| Silicoflagellates | Concentration of silicoflagellates measured by Yokogowa FlowCAM imaging cytometer | cells per milliliter (cells/mL) |
| Other_Cells | Concentration of unidentified other particles measured by Yokogowa FlowCAM imaging cytometer | cells per milliliter (cells/mL) |
| Pct_Small_0_to_4um | Percent of total particles contributed by small (0-4 um) particles as measured by Yokogowa FlowCAM imaging cytometer | percent |
| Pct_Round_4_to_12um | Percent of total particles contributed by round (4-12 um diameters) particles as measured by Yokogowa FlowCAM imaging cytometer | percent |
| Pct_Ovoid_4_to_12um | Percent of total particles contributed by ovoid (long axis 4-12 um) particles as measured by Yokogowa FlowCAM imaging cytometer | percent |
| Pct_Dinoflagellates | Percent of total particles contributed by dinoflagellates as measured by Yokogowa FlowCAM imaging cytometer | percent |

| | | |
|---|---|---|
| Pct_Ciliates | Percent of total particles contributed by ciliates as measured by Yokogowa FlowCAM imaging cytometer | percent |
| Pct_Diatoms | Percent of total particles contributed by diatoms as measured by Yokogowa FlowCAM imaging cytometer | percent |
| Pct_Silicoflagellates | Percent of total particles contributed by silicoflagellates as measured by Yokogowa FlowCAM imaging cytometer | percent |
| Pct_Other_Cells | Percent of total particles contributed by unidentified other particles as measured by Yokogowa FlowCAM imaging cytometer | percent |
| Total_C_biomass | Total carbon biomass (based on Menden-Deuer and Lessard, 2000) for total particles measured by Yokogowa FlowCAM imaging cytometer | micrograms per liter (ug/L) |
| Small_C_biomass | Carbon biomass (based on Menden-Deuer and Lessard, 2000) for small (0-4 um) particles measured by Yokogowa FlowCAM imaging cytometer | micrograms per liter (ug/L) |
| Round_C_biomass | Carbon biomass (based on Menden-Deuer and Lessard, 2000) for round-(4-12 um) particles measured by Yokogowa FlowCAM imaging cytometer | micrograms per liter (ug/L) |
| Ovoid_C_biomass | Carbon biomass (based on Menden-Deuer and Lessard, 2000) for ovoid (4-12 um long axis) particles measured by Yokogowa FlowCAM imaging cytometer | micrograms per liter (ug/L) |
| Dinoflagellates_C_biomass | Carbon biomass (based on Menden-Deuer and Lessard, 2000) for dinoflagellates measured by Yokogowa FlowCAM imaging cytometer | micrograms per liter (ug/L) |
| Ciliates_C_biomass | Carbon biomass (based on Menden-Deuer and Lessard, 2000) for ciliates measured by Yokogowa FlowCAM imaging cytometer | micrograms per liter (ug/L) |
| Diatoms_C_biomass | Carbon biomass (based on Menden-Deuer and Lessard, 2000) for the sum of pennate and centric diatoms measured by Yokogowa FlowCAM imaging cytometer | micrograms per liter (ug/L) |
| Silicoflagellates_C_biomass | Carbon biomass (based on Menden-Deuer and Lessard, 2000) for silicoflagellates measured by Yokogowa FlowCAM imaging cytometer | micrograms per liter (ug/L) |

| | | |
|---|---|---|
| Other_Cells_C_biomass | Carbon biomass (based on Menden-Deuer and Lessard, 2000) for unidentified other particles measured by Yokogowa FlowCAM imaging cytometer | micrograms per liter (ug/L) |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | Yokogowa FlowCAM imaging cytometer |
| **Generic Instrument Name** | Yokogawa Fluid Imaging Technologies FlowCam VS particle imaging system |
| **Dataset-specific Description** | Particles were measured by Yokogowa FlowCAM imaging cytometer. Imaging cytometers are automated instruments that quantify properties of single cells, one cell at a time. They combine some aspects of flow cytometry with particle imaging capabilities in an automated device to classify small particles, including phytoplankton and protozoa. They can measure a variety of properties: cell size, cell granularity, cell aspect ratio, equivalent spherical diameter (ESD) and area-based diameter (ABD) [to estimate bio-volume, which is used to estimate cell carbon biomass]. Particle images are digitally recorded and sorted into different classes according to training libraries using a support vector machine (supervised learning methods). The instruments particle-size is calibrated using different sizes of latex beads. |
| **Generic Instrument Description** | Imaging cytometers are automated instruments that quantify properties of single cells, one cell at a time. They combine some aspects of flow cytometry with particle imaging capabilities in an automated device to classify small particles, including phytoplankton and protozoa. They can measure a variety of properties: cell size, cell granularity, cell aspect ratio, equivalent spherical diameter (ESD) and area-based diameter (ABD) [to estimate bio-volume, which is used to estimate cell carbon biomass]. Particle images are digitally recorded and sorted into different classes according to training libraries using a support vector machine (supervised learning methods). The instruments particle-size is calibrated using different sizes of latex beads. The FlowCam VS series are automated imaging-in-flow instruments that generate high-resolution digital images for measuring size and shape of microscopic particles. The sample introduced in the system is attracted by a peristaltic or a syringe pump into a flow cell (or flow chamber) with known dimensions, located in front of a microscope objective which is connected to a camera video. The benchtop model is ideally suited to a typical laboratory environment with applications in oceanographic research, municipal water, biopharmaceutical formulations, chemicals, oil and gas, biofuels, and many other markets. FlowCam VS is available in four models, from the imaging-only VS-I (i.e. without excitation wavelength or fluorescence emission wavelengths) to the top-of-the-line VS-IV with two channels of fluorescence measurement and scatter triggering capabilities. The instrument can measure particles between 2µm and 2mm; can analyse in vivo or fixed samples; has a flow rate between 0.005 ml/minute and 250 ml/minute (dependant upon magnification, flow cell depth, camera frame rate, efficiency desired, etc.). It can produce either 8-bit Grayscale (Monochrome Camera) or 24-bit Colour (Colour Camera) images, depending on the model. |

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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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