

# Ambient concentrations of acetate, glycerol, and mannitol measured from samples collected during R/V Endeavor cruise EN616 in the northwest Atlantic in July 2018

**Website:** <https://www.bco-dmo.org/dataset/887851>

**Data Type:** Cruise Results

**Version:** 1

**Version Date:** 2023-02-26

## Project

» [Coccolithophore Mixotrophy](#) (Cocco-Mix)

Contributors	Affiliation	Role
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## Abstract

This data set provides ambient concentrations of three dissolved organic compound (acetate, glycerol and mannitol) measured from water samples taken during R/V Endeavor cruise EN616 in the northwest Atlantic in July 2018. These concentrations were derived using new analytical methods described in the below-referenced Science Advances manuscript by Balch et al.

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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 <sup>14</sup>C-acetate, <sup>14</sup>C-mannitol and <sup>14</sup>C-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  $^{14}\text{C}$ -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

The compounds chosen for this study showed high potential for osmotrophy based on previous laboratory experiments (Godrijan et al, 2020). Together, they represent roughly less than a few percent of the total dissolved organic carbon concentration found in the seawater of this particular region, as based on previous field DOC concentration measurements. Mannitol and glycerol are sugar alcohols, whereas acetate is biochemically important and available in marine ecosystems (Wu et al. 1997; Ho et al. 2002). Mannitol occurs in a wide range of living organisms, where it may act as an osmoregulatory substance. It is likely an important source of carbon for marine heterotrophic bacteria. Glycerol is known to passively diffuse into bacterial cells, providing an energy source for cells that have insufficient energy to initiate active transport.

Ambient levels of the compounds were measured from seawater collected in 10L Niskin bottles at four stations during R/V Endeavor cruise EN616 in the northwest Atlantic. Incubation experiments were performed during the cruise, but the ambient concentration of each organic was not known at the time of sampling since DOC concentrations were determined post-cruise. This dataset presents laboratory measurements of ambient concentrations of the three organic compounds that had been used in the radiolabeled DOC uptake and assimilation experiments (Balch et al., 2023 and BCO-DMO dataset <https://www.bco-dmo.org/dataset/887562>)

In order to calculate the uptake of dissolved organic compounds, the ambient concentrations of each compound (unlabeled) were measured in the lab. While at sea, Niskin water samples were poured directly into acid cleaned glass scintillation vials and frozen at -20 degrees Celsius until analysis ashore. The concentrations of acetate, mannitol and glycerol were determined post-cruise using the methods described below. A summary of the limits of detection, accuracy, precision and workable range of each method can be found in Balch et al. (2023).

### Method to determine ambient acetate

An enzymatic approach was adapted to measure ambient concentrations of dissolved acetate (King, 1991). The assay is based on the formation of AMP (adenosine monophosphate) from ATP (adenosine triphosphate), in the presence of acetate, coenzyme A (CoA) and S-acetyl CoA synthetase. Frozen samples (-20°C) from the Niskin bottles were thawed at room temperature prior to subsampling of 1 milliliter into a 2mL clean glass autosampler vial. For both samples and standards, 10 microliters each of S-acetyl-CoA synthetase (~20 U/mL), ATP (10mM), BSA (200ug/L), and CoA (10mM) were added to the 1 mL volume. All reagents were obtained from Sigma-Aldrich, Inc., St. Louis, MO, USA. The mixtures were vortexed for 5 seconds and then incubated on a heating block at 37°C for one hour. Before injection onto the HPLC system for analysis, solutions were removed from the heating block and placed in a boiling water bath for 2 minutes in order to inactivate the S-acetyl CoA synthetase enzyme. For preparation of standards, a 100  $\mu\text{M}$  (micromolar) solution of acetate in filtered aged sea water (FASW) was prepared from 17.4M glacial acetic acid in purified water (10mM) adjusted to pH 8.1 with ammonium hydroxide. This was further diluted in FASW to generate standards of 0.0 $\mu\text{M}$ , 0.1 $\mu\text{M}$ , 0.5 $\mu\text{M}$ , 1.0 $\mu\text{M}$ , 5.0 $\mu\text{M}$ , 10 $\mu\text{M}$ , 15 $\mu\text{M}$ , 25 $\mu\text{M}$ , 30 $\mu\text{M}$ , and 50 $\mu\text{M}$  acetate.

The instrument used for AMP quantitation was an Agilent 1200 series HPLC with G1315B diode array detector, equipped with a C18 4.6 x 250mm, 5 $\mu\text{m}$  reverse-phase column (Waters™). A 0.1M potassium phosphate, pH 6.0, mobile phase was used in isocratic mode at a flow rate of 1.0 mL min<sup>-1</sup>, with a column temperature of 35°C and total run time of 10min. The injection volume was 100 $\mu\text{L}$ . The diode array detector was set to 260nm with a bandwidth of 4nm and reference wavelength of 360nm. A wash vial containing 0.1 M potassium phosphate, pH 6.0 was used for syringe rinses between injections.

### Method to determine ambient mannitol

A gas chromatographic-mass spectrophotometric approach, previously used to determine mannitol concentration in tissue samples (Kiyoshima et al., 2001), was adapted to quantify ambient mannitol in seawater. Frozen samples (-20°C) from the Niskin bottles were thawed at room temperature prior to subsampling of 5 mL for the analysis of mannitol. Samples and standards were lyophilized and then reconstituted in 1.5mL of ethanol (99.5%). These samples were vortexed for 10 to 15 seconds, sonicated for 30 minutes, vortexed for another 10-15 seconds and then sonicated for a further 30 min. Following transfer to centrifuge tubes and centrifugation for 5 min at 8000 rcf, 1.4mL of the supernatant was removed to cleaned 2mL glass vials. To further concentrate the samples, the ethanol solutions were dried down under a

gentle nitrogen gas stream at 70°C and reconstituted in 100 microliters (μL) of fresh ethanol and sonicated for 120 min. Derivatization of the mannitol was achieved by the addition of 100 μL of butylboronic acid solution (10mg per mL) part way through the sonication step. The derivatized samples were centrifuged again for 5 min at 8000 rcf prior to analysis. Mannitol standards were made up in 5mL filtered aged sea water (FASW) with the addition of 5μL of erythritol (120μg per mL in methanol) as an internal standard, and were processed in the same manner as the seawater samples. The same erythritol internal standard was added to each 5mL seawater sample, prior to lyophilization and derivatization. Mannitol, erythritol, and butylboronic acid were sourced from Sigma-Aldrich, Inc., St. Louis, MO, USA.

Analysis was carried out on a Shimadzu GCMS-QP2010 gas chromatograph-mass spectrometer (GC-MS) equipped with a Shimadzu SHRXI-5MS column, 30m long, 0.25mm ID and 0.24μm df (film thickness). The GC-MS column oven temperature was programmed to start at 100°C and increased at a rate of 30°C until 200°C, then increased at a rate of 20°C to a final temperature of 300°C and held there for 1min. The injection temperature was 280°C, as were the ion source and interface temperatures. The injection volume was 2μL. Helium was used for the carrier gas at a constant pressure of 7.3 psi. For quantification, the major fragment ions were m/z 127 and 253 for mannitol and m/z 127 for erythritol.

### **Method to determine ambient free-glycerol**

To determine dissolved glycerol concentrations in seawater, a coupled enzymatic assay was developed by modification of existing approaches (Garland and Randle, 1962) (Sigma-Aldrich FG0100). Glycerol is phosphorylated by ATP forming glycerol 3-phosphate and ADP in a reaction catalyzed by glycerokinase. Glycerol 3-phosphate is then oxidized by glycerol 3-phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. The basis of the glycerol quantitation was HPLC-based measurement of ATP loss and ADP production.

Glycerol (100mM), ATP disodium salt hydrate (>99%), glycerokinase from *Cellulomonas* sp., and glycerol 3-phosphate oxidase from *Pediococcus* sp., were obtained from Sigma-Aldrich, Inc., St. Louis, MO, USA. A 10.6U mL<sup>-1</sup> enzyme solution of glycerokinase was prepared by adding 0.145mg of 44U mg<sup>-1</sup> glycerokinase to 0.604mL of 50mM Tris-HCl, pH 7.5 solution. A 102U mL<sup>-1</sup> enzyme solution of glycerol 3-phosphate oxidase was prepared by adding 1mL 50mM Tris-HCl, pH 7.5 solution directly to 1.7mg of 60U mg<sup>-1</sup> lyophilized enzyme. Two reaction mixtures were generated: reaction mix #1 (no enzyme) was prepared by adding 1mL of 50mM Tris-HCl, pH 7.5 solution, 250μL of 2μM ATP and 250μL of 180mM MgCl<sub>2</sub> to a 4 mL vial; reaction mix #2 (with enzyme) consisted of 900μL of 50 mM Tris-HCl, pH 7.5 solution, 250μL of 2μM ATP, 250μL of 180 mM MgCl<sub>2</sub>, 50μL of 10.6U mL<sup>-1</sup> glycerokinase, and 50μL of 102U mL<sup>-1</sup> glycerol 3-phosphate oxidase. Glycerol standards of 2.5 to 10nM were prepared in FASW.

For the reaction, samples were removed from the -20°C freezer and allowed to thaw to room temperature before use. Samples, or standards, were combined in a 1:1 ratio with each reaction mixture and incubated at room temperature for 10 minutes before injection onto the HPLC. For the analytical sequence, 150 μL of the FASW blank was added to a 300μL autosampler vial followed immediately by 150 μL of reaction mix #1 (no enzyme). The mixture was mixed several times by pipette aspiration. After 10 minutes, the blank was injected onto the HPLC. The next incubation using another 150 μL of FASW blank and 150μL of reaction mix #2 (enzyme) occurred in a separate autosampler vial.

An Agilent 1200 series HPLC with G1315B diode array detector was used to analyse ATP and ADP concentrations at the end of the 10 min reactions. The HPLC was equipped with a C18 4.6 x 250mm, 5μm reverse-phase column (Waters™), run with a mobile phase of 0.1 M potassium phosphate, pH 6.0 at a flow rate of 1.0mL min<sup>-1</sup> and column temperature of 35°C. The sample injection volume was 100μL. The chromatography run time was 10 min. The diode array detector was set to 260 nm with reference wavelength of 360 nm. A wash of 0.1 M potassium phosphate, pH 6.0 was used as a syringe rinse between samples. ATP and ADP concentrations in reaction mix #2 were corrected relative to the no enzyme-control in reaction mix #1, in order to estimate glycerol concentrations.

## **Data Processing Description**

### **BCO-DMO processing**

- Data is from columns BL through BQ on the original source file titled "EN616\_master\_datasheet\_bottle\_and\_discrete\_organics\_updated\_ccc\_BCODMO.csv"
- Data extracted from combined "master datasheet" into a separate file called "ambient\_nutrients\_EN616.csv"
- Modified parameter (column) names to conform with BCO-DMO naming conventions.

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## Data Files

File	
<b>ambient_nutrients_en616.csv</b>	(Comma Separated Values (.csv), 2.91 KB) MD5:51b18308e0da37463b1e09fa2e1aa4bb
Ambient concentrations of acetate, glycerol, and mannitol compounds from seawater collected during cruise EN616	

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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*

Garland, P. B., & Randle, P. J. (1962). A Rapid Enzymatic Assay for Glycerol. *Nature*, 196(4858), 987–988. <https://doi.org/10.1038/196987a0>  
*Methods*

Godrijan, J., Drapeau, D. T., & Balch, W. M. (2021). Osmotrophy of dissolved organic carbon by coccolithophores in darkness. *New Phytologist*, 233(2), 781–794. doi:[10.1111/nph.17819](https://doi.org/10.1111/nph.17819)  
*Related Research*

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](https://doi.org/10.1002/lno.11396)  
*Related Research*

Ho, T.-Y., Scranton, M. I., Taylor, G. T., Varela, R., Thunell, R. C., & Muller-Karger, F. (2002). Acetate cycling in the water column of the Cariaco Basin: Seasonal and vertical variability and implication for carbon cycling. *Limnology and Oceanography*, 47(4), 1119–1128. doi:[10.4319/lo.2002.47.4.1119](https://doi.org/10.4319/lo.2002.47.4.1119)  
*Related Research*

King, G. M. (1991). Measurement of Acetate Concentrations in Marine Pore Waters by Using an Enzymatic Approach. *Applied and Environmental Microbiology*, 57(12), 3476–3481. <https://doi.org/10.1128/aem.57.12.3476-3481.1991>  
*Methods*

Kiyoshima, A., Kudo, K., Hino, Y., & Ikeda, N. (2001). Sensitive and simple determination of mannitol in human brain tissues by gas chromatography–mass spectrometry. *Journal of Chromatography B: Biomedical Sciences and Applications*, 758(1), 103–108. [https://doi.org/10.1016/s0378-4347\(01\)00145-1](https://doi.org/10.1016/s0378-4347(01)00145-1)  
*Methods*

Wu, H., Green, M., & Scranton, M. I. (1997). Acetate cycling in the water column and surface sediment of Long Island Sound following a bloom. *Limnology and Oceanography*, 42(4), 705–713. Portico. <https://doi.org/10.4319/lo.1997.42.4.0705>  
*Related Research*

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## Related Datasets

### IsSupplementTo

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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](#)]

#### IsRelatedTo

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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., Archer, S. D., Drapeau, D. T., Godrijan, J. (2023) **FlowCAM enumeration of phytoplankton classes from samples taken during R/V Endeavor cruise EN616 in July 2018.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-01-27 doi:10.26008/1912/bco-dmo.887787.1 [[view at BCO-DMO](#)]

#### IsSupplementedBy

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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
Acetate_conc	Average ambient concentration of acetate	micromolar (uM)
Acetate_stddev	Standard deviation for replicate acetate samples	micromolar (uM)
Glycerol_conc	Average ambient concentration of glycerol	nanomolar (nM)
Glycerol_stddev	Standard deviation for replicate glycerol samples	nanomolar (nM)
Mannitol_conc	Average ambient concentration of mannitol	micromolar (uM)
Mannitol_stddev	Standard deviation for replicate mannitol samples	micromolar (uM)

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## Instruments

<b>Dataset-specific Instrument Name</b>	centrifuge
<b>Generic Instrument Name</b>	Centrifuge
<b>Dataset-specific Description</b>	Following transfer to centrifuge tubes and centrifugation for 5min at 8000rcf, 1.4mL of the supernatant was removed
<b>Generic Instrument Description</b>	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

<b>Dataset-specific Instrument Name</b>	Shimadzu GCMS-QP2010 gas chromatograph-mass spectrometer
<b>Generic Instrument Name</b>	Gas Chromatograph Mass Spectrometer
<b>Dataset-specific Description</b>	Analysis was carried out on a Shimadzu GCMS-QP2010 gas chromatograph-mass spectrometer (GC-MS) equipped with a Shimadzu SHRXI-5MS column
<b>Generic Instrument Description</b>	Instruments separating gases, volatile substances or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay by a mass spectrometer.

<b>Dataset-specific Instrument Name</b>	Agilent 1200 series HPLC with G1315B diode array detector
<b>Generic Instrument Name</b>	High-Performance Liquid Chromatograph
<b>Dataset-specific Description</b>	An Agilent 1200 series HPLC with G1315B diode array detector was used to analyse ATP and ADP concentrations at the end of the 10 min reactions. The HPLC was equipped with a C18 4.6 x 250mm, 5µm reverse-phase column (Waters™),
<b>Generic Instrument Description</b>	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

<b>Dataset-specific Instrument Name</b>	Niskin
<b>Generic Instrument Name</b>	Niskin bottle
<b>Dataset-specific Description</b>	While at sea, Niskin water samples were poured directly into acid cleaned glass scintillation vials and frozen for subsequent analysis
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	shaker table
<b>Generic Instrument Name</b>	Shaker
<b>Dataset-specific Description</b>	The sealed scintillation vials were gently shaken on a shaker table for 24 hours
<b>Generic Instrument Description</b>	A Shaker is a piece of lab equipment used to mix, blend, or to agitate substances in tube(s) or flask(s) by shaking them, which is mainly used in the fields of chemistry and biology. A shaker contains an oscillating board which is used to place the flasks, beakers, test tubes, etc.

<b>Dataset-specific Instrument Name</b>	sonicator
<b>Generic Instrument Name</b>	ultrasonic cell disrupter (sonicator)
<b>Dataset-specific Description</b>	To further concentrate the samples, the ethanol solutions were dried down under a gentle nitrogen gas stream at 70°C and reconstituted in 100µL of fresh ethanol and sonicated for 120min.
<b>Generic Instrument Description</b>	Instrument that applies sound energy to agitate particles in a sample.

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## Deployments

EN616

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

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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 CO<sub>2</sub> and they can transfer CO<sub>2</sub> 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 CO<sub>2</sub>, 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 CO<sub>2</sub> 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<sub>2</sub>. 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<sub>2</sub>. Thus, the net effect of coccolithophores on atmospheric CO<sub>2</sub> depends on the balance of their CO<sub>2</sub>-raising effect associated with the alkalinity pump and their CO<sub>2</sub>-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
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1635748</a>

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