DOC uptake rates by coccolithophores and scintillation counts from field experiments in the North Atlantic during R/V Endeavor cruise EN616 in July 2018

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Project
» Coccolithophore Mixotrophy (Cocco-Mix)

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Abstract
Field experiments were performed in the NW Atlantic during R/V Endeavor cruise EN616 in July 2018 to determine the potential uptake of dissolved organic carbon (DOC) by coccolithophores to compare with the bicarbonate uptake associated with standard photosynthesis and calcification. Waters from the continental shelf and slope, plus Sargasso Sea were sampled. Natural phytoplankton populations were incubated with radiolabeled dissolved organic carbon compounds (14C-acetate, 14C-mannitol, and 14C-glycerol) as well as 14C-bicarbonate for comparison. At the end of 24-hour incubations, phytoplankton were either bulk filtered onto polycarbonate membrane filters or coccolithophores were concentrated, then sorted, using a flow cytometer, after which sorted cells were washed onto polycarbonate membrane filters and rinsed accordingly. Post-cruise, repeated scintillation counts of each sample were performed using a high sensitivity scintillation counter. The repeated counts were designed to improve the statistical resolution of the low-level radioactivity measurements. Post-cruise, ambient concentrations of acetate, mannitol, and glycerol also were measured and cellular-DOC uptake rates were calculated.

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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 ^{14}C-acetate, ^{14}C-mannitol and ^{14}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 related dataset: https://www.bco-dmo.org/dataset/858771). The design of these experiments used radiochemical and single cell/flow cytometer methods to distinguish osmotrophy by coccolithophores from that of other naturally-occurring microalgae.
- **Goal #2**: test for the fixation of ^{14}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

Coccolithophores are typically thought of as photoautotrophs yet they are known to inhabit sub-euphotic environments with insufficient light for photosynthesis. Field experiments were performed in the NW Atlantic to determine the potential uptake of dissolved organic carbon (DOC) by coccolithophores to compare with the bicarbonate uptake associated with standard photosynthesis and calcification. The cruise sampled portions of the Gulf of Maine, Georges Bank, continental shelf and slope waters of the northern part of the mid-Atlantic Bight plus Sargasso Sea, during *R/V Endeavor* cruise EN616 in July 2018. Natural phytoplankton populations were incubated with radiolabeled DOC compounds, ^{14}C-acetate, ^{14}C-mannitol and ^{14}C-glycerol. Following the 24 hour incubations at simulated *in situ* conditions of light and temperature, coccolithophores were sorted from these natural populations using flow cytometry (based on their chlorophyll fluorescence and birefringence signatures). The sorts were collected and the uptake rate of these compounds into both organic and inorganic fractions was subsequently measured using the microdiffusion technique coupled with high-precision scintillation counting. Bulk samples were also filtered from these incubated natural populations in which radiolabeled DOC was taken-up and assimilated into the particulate organic carbon of general phytoplankton (not just coccolithophores) as well as the PIC of the broader assemblage of calcifiers (likely dominated by coccolithophores based on the small sample volumes). The bulk samplings had higher signal-to-noise for radioactivity measurements of DOC uptake into coccolithophore PIC due to the increased sample size, whereas the flow cytometer sorts had higher specificity for isolating coccolithophores, which allowed better discerning the fixation of ^{14}C-labeled DOC into both organic tissues and calcium carbonate (particulate inorganic carbon) of coccolithophores, despite the lower signal-to-noise. DOC uptake rates were measurable but slow relative to bicarbonate uptake rates and the resultant growth rates on these compounds were low, suggesting osmotrophy plays more of a survival strategy in low-light situations. Notably, a significant amount of the assimilated DOC was found in both particulate organic carbon and calcite coccoliths (particulate inorganic carbon) within 24 hours, suggesting that osmotrophic uptake of dissolved organics into calcite of coccolithophores is a small but notable part of both the biological carbon pump and alkalinity pump paradigms.

Incubation experiments

A total of nine stations were visited during *R/V Endeavor* cruise EN616 in the northwest Atlantic during July 2018, but only four were used for incubation experiments (stations 1, 3, 5, and 9). The depth of maximum coccolithophore concentration was determined by sampling at eight depths and performing filter transfer freeze (FTF) coccolithophore counts (Hewes & Holm-Hansen, 1983; Balch et al., 2023). The FTF technique is a semi-quantitative technique for microscopy and enumeration of phytoplankton onboard the ship to determine rough depth profiles of phytoplankton concentration (different from the more-quantitative birefringence counts done ashore, postcruise. See Related Dataset below).

Once the depth of maximum coccolithophore concentration was found, the Niskin bottles from that depth were drained and combined into a single, acid-cleaned polycarbonate carboy for the 24 hour incubation experiments with natural populations. At each station, 1 to 2 liters of ambient seawater from the combined carboy water sample were poured into 16 intravenous or “IV” flexible plastic bags (Thermo Fisher LabTainer BioProcess Container). Four bags each were used for incubations with each of the four radiolabeled compounds (16 bags total):

- ^{14}C-acetate (specific activity = 1.924 MBq μmol-1 544; inoculum = 120μL per bag at 1.923 mM stock concentration),
- ^{14}C-mannitol (specific activity = 2.146 MBq μmol-1; inoculum = 120μL per bag at 1.724 mM stock concentration),

- ^{14}C-glycerol (specific activity = 5.92 M Bq mol-1 547; inoculum = 120µL per bag at 0.625mM stock concentration), and
- ^{14}C-bicarbonate (specific activity =2.146 MBq µmol-1 548; inoculum = 200µL per bag at 17.24mM stock concentration) (PerkinElmer, Waltham, MA, USA).

See Balch et al. (2023) for detailed methodology, as well as final concentrations of radiolabeled and ambient DOI compounds for each experiment.

Following inoculation, all bags were gently mixed to disperse the radiolabeled compound. The bags were placed in an incubator with neutral-density screening to achieve the ambient light level from the collection depth and maintained at the collection temperature with day-night cycle adjusted for the collection location. One of each of the four replicate bags received 5% final concentration of buffered formalin and served as a killed control. Bags were incubated for 24 hours then brought into the darkened radioisotope van (with only dim red light). Bags were hung and 100-200mL were withdrawn and immediately filtered as "bulk samples" on a 25mm diameter, 0.4µm pore-size polycarbonate filter and subsequently processed using the microdiffusion technique (Balch et al., 2000) to determine the C14 activity in POC and PIC of the particles (Balch et al., 2023).

Bulk phytoplankton measurements

Bulk water samples from the I.V. bags were filtered onto a 0.4um-poresize polycarbonate filter, held in a 12-place Millipore filter tub under <5mm Hg vacuum. Following filtration, each filter was rinsed three times with filtered seawater and then given a gentle "rim rinse" following the removal of the top filter holder, in order to remove any remaining dissolved ^{14}C activity from the moist filter. Each filter was then removed for the microdiffusion protocol (Balch et al., 2000; Paasche and Brubak, 1994). Briefly, each filter was placed on the bottom of a clean scintillation vial, sealed with a rubber septum which also held a suspended bucket containing a GFA filter saturated with 0.2 mL of phenethylamine (PEA is a CO_2 absorbent organic compound). One mL of 1% (by volume) phosphoric acid was injected through the rubber septum, past the suspended bucket, onto the original sample filter on the bottom of the scintillation vial to dissolve the PIC, converting it to CO_2 gas, which diffused into the headspace. This ^{14}C-CO_2 was absorbed onto the filter in the suspended bucket over the next 24 hours as the sealed scintillation vials were gently shaken on a shaker table. After 24 hours shaking, the vials were removed, septa opened within a fume hood, and the bucket containing the GFA filter with absorbed ^{14}C-CO_2 (originally ^{14}C-PIC) was snipped into a new, clean scintillation vial, to which scintillation cocktail was added (Ecolume; MP Biomedicals). Sample radioactivity was measured within two months following the cruise.

Sample radioactivity measurements

The radioactivity of the filters was measured using a Tri-Carb 3110TR time-resolved liquid scintillation counter (PerkinElmer), set for transformed spectral index of external standards. The instrument was coupled to automatic efficiency correction as well as background subtraction from each of the three spectral counting regions of the counter. Static electricity associated with each vial was eliminated by the counter. True decay events from the sample were defined as being within 18 ns (nanosecond) time difference for the two PMTs (photomultiplier tubes) monitoring a sample vial. The time period that the detector looks for additional pulses after the initial pulse (termed "after pulses") was set to 75ns. Beyond this "after-pulse" time, the scintillation events were considered to be associated with background counts.   To increase the precision of the radioactivity measurements, ten replicate counts were performed for each sample. Replicate sample counts continued until the final average count rate had an overall 95% confidence limit <= ±0.5%. This was achieved after a total of 160,000 accumulated scintillation counts. If the accumulated counts did not reach that level in 10 minutes, counting of that replicate was terminated (but the count was still tabulated along with its confidence limit). This ten-replicate approach allowed an increase in precision, reducing the standard errors of the radioactivity measurements (in units of disintegrations per minute or DPMs) by a factor of 3 (=square root of (10-1)).

Uptake was calculated as the difference between the sample average count and its associated formalin-killed blank. Uptake was calculated as:

$$Uptake = [(DPM_{(s)}-DPM_{(b)}) \times 1.05] /[DPM_{(tot)} \times (V_{(s)}/V_{(tot)}) \times T_{(elap)}]$$

where 1.05 was the isotope discrimination factor for ^{14}C compared to ^{12}C, $DPM_{(tot)}$ was the radioactivity of the total counts added to the experimental sample, $V_{(s)}$ and $V_{(tot)}$ were the volumes of the experimental sample and the subsample for determination of the total activity, respectively, and $T_{(elap)}$ was the elapsed time

between the moment the isotope was added until the sample was filtered. The radioactivity for the three experimental bags was then averaged within a treatment and standard deviation calculated. Cellular uptake into PIC for bulk phytoplankton samples was calculated by dividing the uptake rates into PIC (i.e. calcification rate) from the equation above by the concentration of coccolithophores in the sample. For flow cytometer samples, the coccolithophore-specific POC and PIC production rates were calculated by taking the results of the above equation and dividing by the numbers of coccolithophores sorted by the flow cytometer for each sample. For flow cytometry results, see the Related Datasets section below.

Data Processing Description

Designations for experiments and blanks

- P designates incorporation of radiolabeled compound into cellular organic matter
- C designates incorporation of radiolabeled compound into particulate inorganic carbon (calcium carbonate)
- TOT designates the total radioactivity of the compound added to the incubation at time-zero (usually sampled in a 100 microliter volume)
- TOP/BOT designate radioactive counts in 1) the upper bucket (TOP) with PEA CO2 trap or 2) bottom (BOT) of the scintillation vial (as organic carbon)
- PEA designates the radioactive counts associated with a 0.2 mL addition of phenethylamine (placed in the upper hanging bucket as a CO2 trap). This is done as a type of procedural blank.
- PEA TIP designates the radioactive counts associated with the tip of the PEA dispenser, typically measured at the end of the experiment. This was simply a check to make sure that ^{14}C-CO2 within the fume hood had not been adsorbed onto the tip of the PEA dispenser, thereby becoming a source of potential contamination in the low-level scintillation counts.
- BLANK designates a blank filter

BCO-DMO processing
- Data is concatenated from the 'raw counts and calc' tabs on each of the four separate files of EN616 isotope results.
- Modified parameter (column) names to conform with BCO-DMO naming conventions.
- Added latitudes and longitudes to match Stations 1, 3, 5, and 9.
- Added sampling datetimes for the four stations from 'reduced' tab on the isotope results files which indicated times of inoculation, filtration, and sorting of the incubation samples
- Converted datetimes to UTC ISO8601 format.
- Added columns for identification numbers and processes captured within the Sample ID
- Separated statistics portion from the scintillation counts and served as a Supplemental document

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Data Files

File
scintillation_counts.csv (Comma Separated Values (.csv), 638.34 KB) MD5:ea18659f4ca1c0f31fdc9663aefce5ce Scintillation counts for incubation experiments

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Supplemental Files

File	
statistics_scint_counts.csv	(Comma Separated Values (.csv), 85.90 KB) MD5:9c2d522ac61d9954a1abca11fde63d05

Statistics of 10 count averages for incubation experiments

Parameters for the file are described below:
Parameter, Description, Units, Data_type,,
Start_Time_UTC,Start datetime of incubation experiment (time of inoculation) in UTC with ISO8601 format,unitless,Datetime,%Y-%m-%dT%H:%M:%SZ
End_Time_UTC,End datetime of incubation experiment (time of filtration or sorting) in UTC with ISO8601 format,unitless,Datetime,%Y-%m-%dT%H:%M:%SZ
Latitude, Latitude of incubation station, decimal degrees, Float,,
Longitude, Longitude of incubation station, decimal degrees, Float,,
Experiment_ID, Experiment ID, unitless, String,,
Sample_num, Sample number, unitless, Integer,,
Compound, "Organic compound being measured (acetate, mannitol, glycerol, bicarbonate)", unitless, String,,
Incubation, Incubation number where 1 to 3 are replicates and 4 is formalin-treated incubation, unitless, Integer,,
Type, Type of sample (bulk or flow cytometer sorted), unitless, String,,
SampleID, Sample ID, unitless, String,,
Average_10ct, Ten-count average of the disintegrations per minute (DPM) , DPM, Float,,
StdDev_10ct, Standard deviation of the ten-count average, DPM, Float,,
CI_95pct_10ct,95% Confidence Interval for ten-count average, DPM, Float,,
Sample_DPM_minus_blank_DPM, Ten-count average minus the formalin blank, DPM, Float,,
StdDev_sample_minus_blank, "Standard deviation of the 'sample minus blank' values, for ten-count average and calculated using standard error propagation", DPM, Float,,
Incub_reps_avg, Average of three replicate incubations with no blank subtracted, DPM, Float,,
StdDev_incub_3reps, Standard deviation of the three replicate incubations, DPM, Float,,
CI_95pct_3reps,95% Confidence Interval for replicate counts based on the standard deviation of replicate incubations and three x 10-replicate counts within each experiment, DPM, Float,,
Coeff_Var, Coefficient of Variation, Percent, Float,,
Calcification,Indicator for calcification process; Entries with no C indicate incorporation into organic matter,unitless,String,
Comments,"Comments about sample, experiment, or other relevant information",unitless,String,
Start_Time_EDT,Start datetime of incubation experiment (time of inoculation) in EDT time zone,unitless,Datetime,%Y-%m-%d %H:%M:%S
End_Time_EDT,End datetime of incubation experiment (time of filtration or sorting) in EDT time zone,unitless,Datetime,%Y-%m-%d %H:%M:%S

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Related Publications

Balch, W. M., Drapeau, D. T., & Fritz, J. J. (2000). Monsoonal forcing of calcification in the Arabian Sea. Deep Sea Research Part II: Topical Studies in Oceanography, 47(7–8), 1301–1337. https://doi.org/10.1016/s0967-0645(99)00145-9
Methods

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

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

Hewes, C. D., & Holm-Hansen, O. (1983). A method for recovering nanoplankton from filters for identification with the microscope: The filter-transfer-freeze (FTF) technique. Limnology and Oceanography, 28(2), 389–394. Portico. https://doi.org/10.4319/lo.1983.28.2.0389
Related Research

Hewes, C. D., & Holm-Hansen, O. (1983). A method for recovering nanoplankton from filters for identification with the microscope: The filter-transfer-freeze (FTF) technique1. Limnology and Oceanography, 28(2), 389–394. Portico. https://doi.org/10.4319/lo.1983.28.2.0389
Related Research

Paasche, E., & Brubak, S. (1994). Enhanced calcification in the coccolithophorid Emiliania huxleyi (Haptophyceae) under phosphorus limitation. Phycologia, 33(5), 324–330. https://doi.org/10.2216/i0031-8884-33-5-324.1
Methods

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Related Datasets

IsRelatedTo

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

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) **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
Start_Time_UTC	Start datetime of incubation experiment (time of inoculation) in UTC with ISO8601 format	unitless
End_Time_UTC	End datetime of incubation experiment (time of filtration or sorting) in UTC with ISO8601 format	unitless
Latitude	Latitude of incubation station	decimal degrees
Longitude	Longitude of incubation station	decimal degrees
Experiment_ID	Experiment ID	unitless
Sample_num	Sample number	unitless

Compound	Organic compound being measured (acetate, mannitol, glycerol, bicarbonate)	unitless
Incubation	Incubation number where 1 to 3 are replicates and 4 is formalin-treated incubation	unitless
Type	Type of sample (bulk or flow cytometer sorted)	unitless
SMPL_ID	Sample ID	unitless
Count_Time	Scintillation counting time	minutes
CPMA	Counts per minute of activity	counts per minute
DPM1	Disintengrations per minute calculated after taking counting efficiency into consideration	disintegrations per minute (DPM)
Count_Precision	A:2S%; Count precision as + or - percent for two sigma error	percent
Coeff_Var	%CV:DPM1; Coefficient of variation for counts	percent
Calcification	Indicator for calcification process. Entries with no C indicate incorporation into organic matter.	unitless
Comments	Comments about sample, experiment, or other relevant information	unitless
Start_Time_EDT	Start datetime of incubation experiment (time of inoculation) in EDT time zone	unitless
End_Time_EDT	End datetime of incubation experiment (time of filtration or sorting) in EDT time zone	unitless

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Instruments

Dataset-specific Instrument Name	Tri-Carb 3110TR time-resolved liquid scintillation counter
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	The radioactivity of the filters was measured using a Tri-Carb 3110TR time-resolved liquid scintillation counter (PerkinElmer), set for transformed spectral index of external standards.
Generic Instrument Description	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used the quantify the activity of particulate emitting (ß and a) radioactive samples, it can also detect the auger electrons emitted from 51Cr and 125I samples.

Dataset-specific Instrument Name	shaker table
Generic Instrument Name	Shaker
Dataset-specific Description	The sealed scintillation vials were gently shaken on a shaker table for 24 hours
Generic Instrument Description	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.

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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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