Results from 14C-labeled uptake experiments determining uptake of specific dissolved organic compounds which showed high potential for osmotrophy

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Version: 2
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Project
» Coccolithophore Mixotrophy (Cocco-Mix)

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Abstract
This dataset includes results for 14C-labeled uptake experiments determining uptake of specific dissolved organic compounds which showed high potential for osmotrophy. Experiments used the BioLog Eco-plates (BioLog, Haywood, CA, U.S.A.) and were conducted at Bigelow Laboratory for Ocean Sciences, East Boothbay, ME.

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Coverage

Spatial Extent: **Lat**:43.8597 **Lon**:-69.5802
Temporal Extent: 2017-09-06 - 2018-01-27

Methods & Sampling

We investigated the uptake of specific dissolved organic compounds, which showed high potential for osmotrophy. We selected five ^{14}C-labeled-DOC compounds based on results of the BioLog Eco-plates survey as well as commercial availability of radiotracer-labeled compounds. The selected compounds included sugar alcohols (glycerol and mannitol), carbohydrate (xylose), and amino-acid (arginine). Additionally, we selected acetate due to its biochemical importance and availability in marine ecosystems (Ho et al. 2002; Wu et al. 1997). Specific activities of the radiotracers were: glycerol - 160 µCi $µmol^{-1}$, mannitol - 57 µCi $µmol^{-1}$, xylose - 200 µCi $µmol^{-1}$, arginine - 338 µCi $µmol^{-1}$, and acetate - 52 µCi $µmol^{-1}$ (acetic acid sodium salt) (PerkinElmer, Inc. Waltham, MA). As a reference uptake compound we used ^{14}C-bicarbonate (56 µCi µmol) (MP Biomedicals, LLC, Santa Ana, CA, USA) incubations in photosaturated light conditions. We performed radiolabel uptake experiments on axenic coccolithophore strains, CCMP289 *Cruciplacolithus neohelis* and CCMP3337 *Chrysotila carterae* (NCMA lists the strain as *Pleurochrysis carterae*). We maintained the cultures in media and light

conditions as described above, and at 22°C (CCMP289) and at 16°C (CCMP3337).

For the survey of arginine and xylose net uptake in darkness, we prepared two 70 mL master samples (concentration of 1×10^{5} cells L^{-1}) of CCMP289 and CCMP3337 cultures in log phase growth. We measured cell concentrations using a haemocytometer on an American Optical Microscope (Spencer Lens Company, Buffalo, N.Y.) with polarization optics. We added unlabeled arginine or xylose to each strain's master sample up to a 20 µM final concentration. From each master sample, 10 mL were then removed into separate borosilicate vials that were kept in the dark for subsequent cell counts over the duration of the experiment. To the remaining 60 mL culture samples containing unlabeled arginine or xylose, we added ^{14}C-arginine or ^{14}C-xylose, to a final concentration (labeled and unlabeled) of 20.25 µM and 20.83 µM, respectively. We withdrew 45 mL of the 60 mL sample and divided that into three 15 mL replicate vials. We transferred the remaining 15 mL into a fourth vial with buffered formalin as a formalin-killed control. Due to logistical issues in sample manipulation, the actual time of addition of the first ^{14}C-labeled compound was 10±5 min after addition of formalin to the labeled control. We then subsampled and filtered all 16 vials (12 samples (triplicates of the two ^{14}C-labeled compounds x two strains) and 4 formalin samples (two compounds x two strains)). After the first time point, we placed samples in the dark incubators at 22°C for CCMP289 and 16°C for CCMP3337. Subsampling for each time course experiment was performed at 3 h, 6 h, 24 h, and 48 h. For subsampling, we performed filtration of each 2 mL of culture subsamples onto each 0.4 µm pore-size, 25 mm diameter polycarbonate filter. Following filtration, filters were carefully rinsed three times with ASW (including a careful rim rinse) to remove any ^{14}C-labeled, dissolved compound left on the filter. Each filter was then placed in the bottom of a clean scintillation vial, and scintillation cocktail was added (Balch et al., 2000).

We also examined the net uptake of ^{14}C-arginine and ^{14}C-xylose uptake in illuminated cultures over 24 hours. We added these ^{14}C- labeled compounds to axenic cultures (CCMP289 or CCMP3337) to a final concentration of 0.37 µM for ^{14}C-arginine and 1 µM for ^{14}C-xylose. We sampled at T15 min and T24 h, stopping the incubation by filtration, and measured the ^{14}C uptake as described above.

Furthermore, we examined the net uptake of ^{14}C-acetate, ^{14}C-glycerol, and ^{14}C-mannitol in darkness over 24 h and compared it with ^{14}C-bicarbonate net uptake (in light). Prior to addition of radiolabeled compounds, axenic cultures (CCMP289 or CCMP3337) were divided into separate vials and 5 mL of log-phase culture were removed for the enumeration of cell concentration. To correct for any effects due to EtOH solvent in the ^{14}C-acetate, in one 5 mL sample we added only 0.0125 mL of EtOH as a control. We added ^{14}C- labeled compounds to each separate vial to a final concentration of 4.81 µM of ^{14}C-acetate, 1.49 µM of ^{14}C-glycerol, 4.18 µM of ^{14}C-mannitol, and for comparison we used ^{14}C-bicarbonate to a final concentration of 2.6 mM of labeled and unlabeled form. Triplicate samples for uptake measurements were filtered after 15 min and 24 h of darkness.

Data Processing Description

Data Processing:
We calculated the ^{14}C-labeled-compound net uptake rates following the equations of Parsons et al. (1984)

$v = ((R_n – R_f) \times W) / (R \times T)$

Where v is the net uptake rate [mol L^{-1} h^{-1}], R_n is the sample count [dpm] at time T, R_f is the formalin-killed control count [dpm], and W [mol L^{-1}] is the concentration of available compound in the sample. R is the total activity [dpm] of the added compound to a sample and T [h] is the number of hours of incubation.

BCO-DMO Processing:
- added column for species name;
- converted dates to YYYY-MM-DD format;
- created date-time field in ISO8601 format (UTC).

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Data Files

File
14C_uptake.csv (Comma Separated Values (.csv), 21.00 KB) MD5:536038fe668339064f4912965d1fd054 Primary data file for dataset ID 858771

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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

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
Results

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
Methods

Parsons, T. R., Maita, Y., & Lalli, C.M. (1984). A manual of chemical and biological methods for seawater analysis. Pergamon Press. doi:10.1016/c2009-0-07774-5 https://doi.org/10.1016/C2009-0-07774-5
Methods

Wu, H., Green, M., & Scranton, M. (1997). Acetate Cycling in the Water Column and Surface Sediment of Long Island Sound Following a Bloom. Limnology and Oceanography, 42(4), 705-713. Retrieved August 20, 2021, from http://www.jstor.org/stable/2839116
Methods

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Parameters

Parameter	Description	Units
CCMP_code	Strain code (CCMP) from the National Center for Marine Algae and Microbiota (NCMA)	unitless
Species	Species name	unitless
Substrate	Substrate	unitless
Light_conditions	Light conditions of the experiment	unitless
Latitude	Latitude; positive values = North	decimal degrees North
Longitude	Longitude; positive values = East	decimal degrees East
Date	Sampling date; format: YYYY-MM-DD	unitless
Time	Sampling Time; format: hh:mm:ss	unitless
Time_zone	Time zone	unitless
Time_Point	Actual elapsed time	hours
Cell_count	Cell count	cells per milliliter (cell/ml)
Uptake	Uptake of 14C-labeled compounds	moles per liter per hour (mol/L*h)
Avg_uptake	Calculated average of uptake	moles per liter per hour (mol/L*h)
Stdev_uptake	Calculated standard deviation of uptake	moles per liter per hour (mol/L*h)
Avg_Net_uptake	Calculated average of net uptake	picomoles per cell per hour (pmol/cell*h)
Stdev_Net_uptake	Calculated standard deviation of net uptake	picomoles per cell per hour (pmol/cell*h)
ISO_DateTime_UTC	Date and time converted to ISO8601 format (UTC): YYYY-MM-DDThh:mm:ssZ	unitless

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Instruments

Dataset-specific Instrument Name	haemocytometer
Generic Instrument Name	Hemocytometer
Generic Instrument Description	A hemocytometer is a small glass chamber, resembling a thick microscope slide, used for determining the number of cells per unit volume of a suspension. Originally used for performing blood cell counts, a hemocytometer can be used to count a variety of cell types in the laboratory. Also spelled as "haemocytometer". Description from: http://hlsweb.dmu.ac.uk/ahs/elearning/RITA/Haem1/Haem1.html.

Dataset-specific Instrument Name	Tri-Carb 3110TR liquid scintillation analyzer
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	Tri-Carb 3110TR liquid scintillation analyzer (PerkinElmer, Waltham, MA, USA)
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. Liquid scintillation counters are instruments assaying alpha and beta radiation by quantitative detection of visible light produced by the passage of rays or particles through a suitable scintillant incorporated into the sample.

Dataset-specific Instrument Name	American Optical Microscope
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	American Optical Microscope (Spencer Lens Company, Buffalo, N.Y.) with polarization optics
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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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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