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

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Project
» Coccolithophore Mixotrophy (Cocco-Mix)

Contributors	Affiliation	Role
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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

Acquisition Description

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.

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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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. doi:10.1016/s0967-0645(99)00145-9 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	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.

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	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

Coccolithophores are unicellular haptophyte algae generally thought of as photoautotrophs. They are covered with scales or "coccoliths" (made of calcium carbonate (particulate inorganic carbon, PIC)). Recent observations suggest that globally, haptophytes contribute more biomass than ubiquitous *Prochlorococcus* and *Synechococcus*. Coccolithophores can affect the draw-down of atmospheric CO2 and are involved in two fundamental "pump paradigms": (1) The alkalinity pump (also known as the carbonate, PIC, or CaCO3 pump) lowers total alkalinity (TA) and dissolved inorganic carbon (DIC) in the euphotic zone during calcification, and increases upper ocean and atmospheric CO2. Coccoliths eventually sink below the ocean's lysocline (the depth where calcium carbonate dissolves), where they release the bicarbonate back into deep water. Thus, they essentially "pump" bicarbonate alkalinity from surface to benthic waters, where it remains isolated in the deep sea for thousands of years. (2) The biological pump in which the ballasting effect of the heavy coccoliths on sinking particulate organic carbon (POC) increases the magnitude of the soft tissue (POC) pump, which ultimately decreases surface CO2. The soft-tissue and alkalinity pumps reinforce each other in maintaining a vertical gradient in DIC but they oppose each other in terms of the air-sea exchange of CO2. Thus, the net effect of coccolithophores on atmospheric CO2 depends on the balance of their CO2-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 the PIC found in coccoliths originates exclusively from DIC, not dissolved organic carbon (DOC). However, there is an increasing body of evidence that coccolithophores are mixotrophic (defined as a combination of growth fueled by autotrophy, uptake of DOC and phagotrophy of small particles (POC). This proposal is to describe the potential uptake and assimilation of an array of DOC compounds in the sea, the kinetics of their uptake and potential incorporation of organic carbon by coccolithophores into PIC coccoliths (which could significantly alter the alkalinity pump paradigm since calcite production in the surface ocean would not be at the expense of bicarbonate).

This work is fundamentally directed at quantifying coccolithophore mixotrophy in lab of technological advances to address this issue, all of which we will apply in this work. We will: (a) screen axenic coccolithophore cultures for the uptake and oxidation of a large array of potential DOC substrates, (b) perform radiolabel-uptake experiments with these molecules using high-specific activity substrates in order to provide the basic kinetic response at environmentally-realistic concentrations, (c) measure radio-labelled carbon fixed into organic tissue, separate from that fixed into PIC, (d) sort 14C-labelled coccolithophores free of the other free-living phytoplankton and bacteria using flow cytometry and e) distinguish the modes of nutrition in these sorted coccolithophore cells. This work will advance the state of knowledge of coccolithophore mixotrophy in the marine environment 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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