Results for radiolabeled acetate, mannitol, and glycerol kinetic uptake and pulse-chase experiments for the species Cruciplacolithus neohelis and Chrysotila carterae (Cocco-Mix project)

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Project
» Coccolithophore Mixotrophy (Cocco-Mix)

Contributors	Affiliation	Role
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Abstract
Results for radiolabeled acetate, mannitol, and glycerol kinetic uptake and pulse-chase experiments for the species Cruciplacolithus neohelis (McIntyre & Bé) Reinhardt strain CCMP298 and Chrysotila carterae (Braarud & Fagerland) Andersen, Kim, Tittley & Yoon (NCMA lists the strain as Pleurochrysis carterae) strain CCMP3337.

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Coverage

Spatial Extent: **Lat**:43.8597 **Lon**:-69.5802
Temporal Extent: 2019-06-27 - 2019-07-08

Methods & Sampling

Methodology:

Radiolabeled DOC kinetic experiments. To understand the mechanism of DOC compound uptake and to see whether species take up different DOC compounds passively or actively, we performed time-course experiments using [14C] labeled DOC compounds. Specific activities of the radiotracers were 52 µCi µmol-1 for [14C]acetate, 57 µCi µmol-1 for [14C]mannitol, and 160 µCi µmol-1 for [14C] glycerol, (PerkinElmer, Waltham, MA, USA).

Pulse-chase experiments. To test if the compounds that were taken up were assimilated within the cell, we performed pulse-chase experiments. This method first takes into account the cellular uptake of radiolabeled compound (“pulse”), that is then exposed to the same unlabeled compound (“cold chase”), at concentrations

far above the labeled one. As an indicator of assimilation, [14C] labeled DOC compound would not be exchanged when “chased” with vastly-higher concentrations of the same unlabeled compound. Unassimilated compounds in intracellular pools, on the other hand, would be released from the cell as a new equilibrium between the intracellular and extracellular DOC concentrations is established (Balch, 1986).

Sampling and analytical procedures:

We prepared a solution of L1 medium and exponentially growing culture of each strain, with final concentrations of 5×104 cells L-1, and left them at their growth temperature in darkness for 24h to adapt. We divided the prepared solution in 45 mL aliquots to 24 vials for each compound and strain. To quadruplicate vials, we added 0.45 mL of unlabeled DOC compound from six stock solutions (1×10-6, 1×10-5, 1×10-4, 1×10-3, 1×10-2, and 1×10-1 mol L-1). The experiment started when we then added 0.02 mL of [14C] labeled DOC compound (2 µCi of added radioactivity) to each of 24 vials giving us final concentrations of organic compounds as stated in the table below. To one vial from those six concentration-quadruplicates we immediately added 1 mL of buffered formaldehyde to act as a killed control. We then incubated the 18 triplicates and six control vials at their growth temperature in darkness for up to 24h, with the sample timing to examine for linear uptake rates at 15 min, 1 h, 3 h, and 24 h. At each sampling, 5 mL of experimental culture were filtered onto a 0.4 µm pore-size, 25 mm diameter polycarbonate filter. We also filtered samples at 24 h for [14C]-microdiffusion analysis, which separates the POC fraction from the PIC fraction (Paasche & Brubak, 1994; Balch et al., 2000). Following the micro-diffusion step to separate acid-labile (PIC) versus acid-stabile (POC) fractions, each filter was then placed in the bottom of a clean scintillation vial, and scintillation cocktail was added (Balch et al., 2000). The radioactivity was measured using a Tri-Carb 3110TR liquid scintillation analyzer (PerkinElmer, Waltham, MA, USA). We calculated the net uptake velocity of [14C] labeled organic compounds using the equations of Parsons et al. (1984):

$v = (Rn – Rf) \times W / R \times T$

where v [mol L-1 h-1] is the net uptake rate, Rn [Bq] is the sample count, Rf [Bq] is the formalin-killed control count, and W [mol L-1] is the total concentration of the organic compound in the sample. R [Bq] is the total activity of the added compound to a sample and T [h] is the number of hours of incubation.

Following the radiolabeled DOC kinetic experiments, after 24h, we added the cold chase as 1 mL of 1 M of unlabeled compound to the remaining 25 mL in vials used in kinetics experiments. The addition of 1 mL of an organic compound could induce a substantial osmotic shock that could lead to short-term osmotic shrinkage of the protoplast before the entry of the organic substance raised the internal osmolarity to the external osmolarity.

We therefore measured particulate cellular radioactivity using the procedure described above at 5 min, 20 min, and 3 h post-chase, which provided three different time scales to evaluate how exchangeable the intracellular compounds were.

Data Processing Description

Processing notes from researcher:

We calculated the 14C-labeled-compound net uptake rates following the equations of Parsons et al. (1984)

$v = ((Rn – Rf) \times W) / (R \times T)$

Where v is the net uptake rate [mol L-1 h-1], Rn is the sample count [dpm] at time T, Rf 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.

Problem report:

The calculation procedure was to subtract the radioactivity of the formalin-killed control from the radioactivity of each treatment; in a few cases where the formalin-killed number was higher, we assumed zero net incorporation (nd). In addition, the forceps used to handle the filters were placed in acid between sampling to avoid transfer of radioactivity between samples. However, if the forceps were not subsequently neutralized in water (MQ), this affected the result of that sample (nd).

BCO-DMO Processing Notes:

- Date formating changed from mm/dd/yy to yyyy-mm-dd
- Added a UTC datetime column
- Removed timezone column and added _ETD to Date and Time column names
- Merged acetate, mannitol, and Glycerol into one data table
- Removed unit row from data table

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Data Files

File
kinetics_pulse_chase_new_phyto-1.csv (Comma Separated Values (.csv), 120.68 KB) MD5:b4dc77a6100ede65c9a39d2d33b38905 Primary data file for dataset ID 870815

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Related Publications

Balch, W. M. (1986). Exploring the mechanism of ammonium uptake in phytoplankton with an ammonium analogue, methylamine. Marine Biology, 92(2), 163–171. https://doi.org/10.1007/bf00392833 https://doi.org/10.1007/BF00392833
Related Research

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. 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
Results

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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Parameters

Parameter	Description	Units
CCMP_code	National Center for Marine Algae and Microbiota coccolithophore culture strain	unitless
Substrate	Labled DOC compound; either acetate, mannitol, or glycerol	unitless
Concentration	Concentration of substrate	µmol/l
Light_conditions	Light conditions of the experiment	unitless
Latitude	latitude, in decimal degrees, North is positive, negative denotes South	degrees North
Longitude	longitude, in decimal degrees, East is positive, negative denotes West	degrees East
Date_EDT	Sampling date in EDT; YYYY-MM-DD	unitless
Time_EDT	Sampling Time in EDT; HH:MM:SS	unitless
Primary_ISO_Datetime	Sampling Datetime in UTC; YYYY-MM-DDTHH:MM:SSZ	unitless
Time_Point	Actual elapsed time	days
Cell_count	Cell count	cell/l
net_cellular_uptake_of_organics	Uptake of recorded 14C-label per cell	mol/cell
uptake_into_PIC	Uptake of recorded 14C-label per cell (radioactivity recorded at 24 h in the inorganic carbon fraction)	mol/cell
Avg_uptake	Calculated average of uptake	mol/cell
Stdev_uptake	Calculated standard deviation of uptake	mol/cell
velocity_of_organics_uptake	Velocity of uptake of recorded 14C-label per cell	mol/cell*d
velocity_of_uptake_into_PIC	Velocity of uptake of recorded 14C-label per cell (radioactivity recorded at 24 h in the inorganic carbon fraction)	mol/cell*d
Avg_velocity_of_uptake	Calculated average of uptake	mol/cell*d
Stdev_velocity_of_uptake	Calculated standard deviation of uptake	mol/cell*d

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Instruments

Dataset-specific Instrument Name	Tri-Carb 3110TR liquid scintillation analyzer (PerkinElmer, Waltham, MA, USA)
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	Following the micro-diffusion step to separate acid-labile (PIC) versus acid-stabile (POC) fractions, each filter was then placed in the bottom of a clean scintillation vial, and scintillation cocktail was added (Balch et al., 2000). The radioactivity was measured using a 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	American Optical Microscope (Spencer Lens Company, Buffalo, N.Y.) with polarization optics
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	The American Optical Microscope was used in the preparation stage of the experiment for the assessment of the initial cell concentration.
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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