Coccolithophore survival in darkness from batch growth experiments (Cocco-Mix project)

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Project
» Coccolithophore Mixotrophy (Cocco-Mix)

Contributors	Affiliation	Role
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Abstract
Results for batch growth experiments that lasted for 30 days on two 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, grown in darkness with the addition of acetate, mannitol, and glycerol in final concentrations of 10, 30, 100, 300 and 1000 µmol l−1. We performed these experiments to determine whether coccolithophores (CCMP298 and CCMP3337) can sustain themselves in darkness by using organic compounds as energy and/or carbon sources.

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Coverage

Spatial Extent: **Lat**:43.8597 **Lon**:-69.5802
Temporal Extent: 2018-03-05 - 2018-07-07

Methods & Sampling

Methodology:

We performed these experiments to determine whether coccolithophores (CCMP289 and CCMP3337) can sustain themselves in darkness by using organic compounds as energy and/or carbon sources.

Sampling and analytical procedures:

First, we prepared 350 ml of L1 medium and log phase cells from each strain. The cell concentrations of CCMP289 and CCMP3337 were 5×104 cells L-1 and 1×104 cells L-1, respectively. We then poured 15 mL aliquots into 16 vials were kept in darkness. Our goal was to determine the effect of concentration on growth: one vial was the control with no organics added, and 5 vials with each organic compound in final concentrations at 10, 30, 100, 300, and 1000 µmol L-1. The experiment lasted for approximately 30 days; temperature and irradiance for the illuminated cultures were the same as for culture growth and maintenance,

vials kept in darkness were also kept at the original growth temperature and additionally covered in black aluminum foil, to ensure complete darkness. We sampled the vials for cell counts every 2-3 days, and during sampling we kept light levels corresponding to experimental conditions. This time-course experiment was performed without replicates, however, we took repeated duplicate samples for cell counts (technical duplicates). Cell concentration was determined using a hemocytometer on an American Optical Microscope (Spencer Lens Company, Buffalo, NY, USA) with polarization optics for CCMP289, as well as a Moxi Z Cell Counter (Andwin Scientific, Simi Valley, CA, USA) for CCMP3337. The Moxi Z uses Gaussian curve-fitting with a coincidence correction algorithm of cell count (vs. diameter) histograms to extract precise (>95%) cell count metrics in a sample. The extracted raw data was further used for cellular carbon calculations of CCMP 3337. After the experiment, the vials, which were kept in the dark, were placed in the light, and after 10 days we were able to qualitatively confirm, under the microscope, renewed growth of coccolithophore cells.

Data Processing Description

Researcher processing notes:

We calculated the carbon content of the cells of CCMP3337 according to the equations for cellular elemental content based on nine isolates covering a wide range of coccolithophore cell diameters and representative of the taxonomic diversity of coccolithophores (Villiot et al., 2021). The basis for these calculations were cell diameters measured by Cell Counter Moxi Z, these were averaged from raw data for each sample that was measured and standard deviation of a frequency distribution was calculated.

BCO-DMO processing notes:

- Time zone field removed and added as a part of the column name of related columns
- UTC datetime field added

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Data Files

File	
batch_growth_experiment_new_phyto-1.csv	(Comma Separated Values (.csv), 44.59 KB) MD5:58362613ae277d42463fe1f3ce54b680
Primary data file for dataset ID 868696	

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Related Publications

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

Villiot, N., Poulton, A. J., Butcher, E. T., Daniels, L. R., & Coggins, A. (2021). Allometry of carbon and nitrogen content and growth rate in a diverse range of coccolithophores. Journal of Plankton Research, 43(4), 511–526. doi:10.1093/plankt/fbab038
Related Research

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Parameters

Parameter	Description	Units
CCMP_code	National Center for Marine Algae and Microbiota coccolithophore culture strain	unitless
Substrate	Substrate	unitless
Concentration	Concentration of substrate	µmol/l
Light_conditions	Light conditions in the experiment	unitless
Collection_Site_Lat	Latitude of strain collection site; positive values = North	degrees North
Collection_Site_Long	Longitude of strain collection site; positive values = East	degrees East
ISO_DateTime_UTC	Sampling Datetime in UTC; YYYY-MM-DDTHH:MM:SSZ	MM-DD-YY
Date_EDT	Sampling Date in Eastern Daylight Time (EDT); YYYY-MM-DD	hh:mm:ss
Time_EDT	Sampling Time in Eastern Daylight Time (EDT); HH:MM:SS	unitless
Time_Point	Actual elapsed time	days
Cell_count	Cell count, average of two measurings	cell/l
Cell_size	Mean cell size on effective cell diameter	µm
POC	Organic carbon content per cell	pg
PIC	Inorganic carbon content per cell	pg

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Instruments

Dataset-specific Instrument Name	Moxi Z Cell Counter (Andwin Scientific, Simi Valley, CA, USA)
Generic Instrument Name	Automated Cell Counter
Dataset-specific Description	Cell concentration was determined using a hemocytometer on an American Optical Microscope (Spencer Lens Company, Buffalo, NY, USA) with polarization optics for CCMP289, as well as a Moxi Z Cell Counter (Andwin Scientific, Simi Valley, CA, USA) for CCMP3337. The Moxi Z uses Gaussian curve-fitting with a coincidence correction algorithm of cell count (vs. diameter) histograms to extract precise (>95%) cell count metrics in a sample. The extracted raw data was further used for cellular carbon calculations of CCMP 3337. After the experiment, the vials, which were kept in the dark, were placed in the light, and after 10 days we were able to qualitatively confirm, under the microscope, renewed growth of coccolithophore cells. We calculated the carbon content of the cells of CCMP3337 according to the equations for cellular elemental content based on nine isolates covering a wide range of coccolithophore cell diameters and representative of the taxonomic diversity of coccolithophores (Villiot et al., 2021). The basis for these calculations were cell diameters measured by Cell Counter Moxi Z, these were averaged from raw data for each sample that was measured and standard deviation of a frequency distribution was calculated.
Generic Instrument Description	An instrument that determines the numbers, types or viability of cells present in a sample.

Dataset-specific Instrument Name	American Optical Microscope (Spencer Lens Company, Buffalo, N.Y.) with polarization optics
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Cell concentration was determined using a hemocytometer on an American Optical Microscope (Spencer Lens Company, Buffalo, NY, USA) with polarization optics for CCMP289, as well as a Moxi Z Cell Counter (Andwin Scientific, Simi Valley, CA, USA) for CCMP3337. The Moxi Z uses Gaussian curve-fitting with a coincidence correction algorithm of cell count (vs. diameter) histograms to extract precise (>95%) cell count metrics in a sample. The extracted raw data was further used for cellular carbon calculations of CCMP 3337. After the experiment, the vials, which were kept in the dark, were placed in the light, and after 10 days we were able to qualitatively confirm, under the microscope, renewed growth of coccolithophore cells.
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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