Bulk and cell-specific CO2 fixation and PO4 uptake from Atlantic Explorer cruise AE1524 (BATS validation cruise BV50), September 2015

Website: https://www.bco-dmo.org/dataset/771701
Data Type: Cruise Results
Version: 1
Version Date: 2019-06-24

Project
» Collaborative Research: Role of small-sized protists in the microbial loop with emphasis on interactions between mixotrophic protists and picocyanobacteria (Small protists in microbial loop)

Contributors	Affiliation	Role
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Abstract
Bulk and cell-specific CO2 fixation and PO4 uptake from Atlantic Explorer cruise AE1524 (BATS validation cruise BV50), September 2015. Phosphate uptake rates were measured in Prochlorococcus, Synechococcus, pigmented eukaryotes, and unpigmented eukaryotes. Also reported are CO2 fixation rate by Prochlorococcus, Synechococcus, and pigmented eukaryotes.

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Coverage

Spatial Extent: **N**:33.25 **E**:-64.14 **S**:22.16 **W**:-65.37
Temporal Extent: 2015-09-14 - 2015-09-21

Dataset Description

Bulk and cell-specific CO2 fixation and PO4 uptake from Atlantic Explorer cruise AE1524 (BATS validation cruise BV50), September 2015 in the North Atlantic subtropical gyre (September 2015), along a transect from Bermuda to Puerto Rico extending from 33°N to 22°N. Phosphate uptake rates were measured in Prochlorococcus, Synechococcus, pigmented eukaryotes, and unpigmented eukaryotes. Also reported are CO2 fixation rate by Prochlorococcus, Synechococcus, and pigmented eukaryotes. These data were published by Duhamel et al (2019), Table 3.

Methods & Sampling

Seawater was collected into acid washed, ultra-pure water and sample rinsed, clear polycarbonate incubation bottles. PO4 assimilation rates were measured in triplicate 70-mL samples with ~259 kBq of added 33P-PO4 (Perkin-Elmer #NEZ08000; carrier free), incubated for 30 min to 1h. CO2 fixation rates were measured in duplicate 70-mL samples with ~17 MBq of added 14C-sodium bicarbonate (Perkin Elmer #NEC086H000, 1.6 GBq/mmol), incubated from dawn to dusk. Samples were incubated under simulated light and temperature conditions measured at the sampling site. A killed control sample was also prepared by adding paraformaldehyde (PFA, 2 % final concentration prepared with electron microscopy grade 16 % aqueous solution, Electron Microscopy Sciences) at least 15 minutes before introducing the radioisotope, in order to account for unincorporated radioactivity. At the end of incubation, samples were fixed with PFA (2% final, for 15-min in the dark), and triplicate 20-microliters aliquots were sampled to measure the total radioactivity added (with beta-phenylethylamine for 14C samples). The total microbial activity was determined by filtering a 3-mL aliquot through a 0.2-micron, pore-size polycarbonate membrane filter (Nuclepore). To reduce unincorporated 33P-PO4, the membrane filter was placed onto a filter type HA soaked in 100 mM KH2PO4, then rinsed three times with ~1 mL of 0.2-micron filtered seawater. To remove unincorporated 14C-sodium bicarbonate, the filter was acidified with 0.5 mL of 1N HCl for 24 h. To determine plankton groups specific uptake rates, a 20-mL aliquot was passed through a 0.2-micron polycarbonate membrane filter under gentle vacuum filtration, and the remaining volume from the 70-mL incubation bottle was passed through a 0.8-micron polycarbonate membrane filter. The 0.2-micron and 0.8-micron filters were stored in separate cryovials with 2 mL and 4 mL of the corresponding radiolabeled sample, respectively, vortexed to detach the cells from the filter, then flash frozen for later flow cytometric sorting (see below). The added radioactivity and total microbial activity were assayed by liquid scintillation counting in 7-mL plastic scintillation vials (Simport) with 4 mL of scintillation cocktail (Ultima Gold LLT, Perkin Elmer) added.

Turnover times (TPO4, h) were calculated by dividing the total radioactivity added (Bq L–1) by the rate of radiolabel uptake into the particulate fraction (Bq L–1 h–1). PO4 assimilation rates (nmol P L–1 h–1) were calculated by dividing PO4 concentration by TPO4. We used PO4 concentration estimated from a concentration series bioassays following the method of Wright and Hobbie (1966). Briefly, seawater samples were amended with non-radioactive PO4 to target additions of 0, 5, 10, 25, 50, 75, and 150 nmol PO4 L–1, spiked with 33P-PO4, incubated and sampled as described above. The resulting TPO4 values were plotted against a corresponding concentration of PO4, and extrapolated using linear regression (TPO4 = a x PO4 + b) to estimate the ambient concentration (Sn = b/a), which represents an upper estimate of ambient concentrations as detailed in Zubkov and Tarran (2005). Results from these bioassays were also used to calculate the Michaelis-Menten kinetic parameters for PO4 assimilation rates (Vmax, the maximum rate at saturating substrate concentration and Km, the half-saturation constant).

For cell sorting of Prochlorococcus, Synechococcus, pigmented and non-pigmented protists, the Influx flow cytometer was set at the highest sorting purity (1.0 drop single mode) and potential attached cells were discarded using a pulse width vs. forward scatter plot. The drop delay was calibrated using Accudrop Beads (BD Biosciences, USA) and verified manually by sorting a specified number of reference beads onto a glass slide and counting the beads under an epifluorescence microscope. Performance was validated as described in Duhamel et al. (2018). Three to four proportional numbers of cells from the same incubation sample were sorted for each target population. Sorted cells were assessed by liquid scintillation analysis following previously published protocols (Talarmin et al. 2011; Duhamel et al. 2012; Rii et al. 2016). The 14C-labeled samples were acidified with 1 mL of 2 mol L-1 HCl for 24 h to remove any unincorporated 14C-sodium bicarbonate.

For each group, at least three samples were sorted and regression analysis between the number of cells sorted and the radioactivity taken up by the sorted cells was used to calculate the per cell activity (dpm cell−1). Radioactivity in sorted cells from the PFA-killed control samples (dpm cell−1) was deduced from radioactivity in the sorted cells from the respective samples (dpm cell−1). The cell-specific assimilation rate (nmol cell-1 h-1) was calculated by dividing the radioactivity per cell (dpm cell−1) by the total microbial activity (dpm L−1) measured in the same sample, and then multiplied by the total microbial assimilation rate at ambient substrate concentration (nmol L−1 h−1).

Michaelis– Menten kinetic parameters were determined using the Michaelis–Menten model in Prism 6.

Data Processing Description

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- added cruise column

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Data Files

File
BV50_CO2_PO4.csv (Comma Separated Values (.csv), 508 bytes) MD5:cb9f03d3ed64a5c2e93bae06030a373d Primary data file for dataset ID 771701

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Related Publications

Duhamel, S., Björkman, K., & Karl, D. (2012). Light dependence of phosphorus uptake by microorganisms in the subtropical North and South Pacific Ocean. Aquatic Microbial Ecology, 67(3), 225–238. doi:10.3354/ame01593
Methods

Duhamel, S., Kim, E., Sprung, B., & Anderson, O. R. (2019). Small pigmented eukaryotes play a major role in carbon cycling in the P-depleted western subtropical North Atlantic, which may be supported by mixotrophy. Limnology and Oceanography. doi:10.1002/lno.11193
Results

Duhamel, S., Van Wambeke, F., Lefevre, D., Benavides, M., & Bonnet, S. (2018). Mixotrophic metabolism by natural communities of unicellular cyanobacteria in the western tropical South Pacific Ocean. Environmental Microbiology, 20(8), 2743–2756. doi:10.1111/1462-2920.14111
Methods

Rii, Y. M., Duhamel, S., Bidigare, R. R., Karl, D. M., Repeta, D. J., & Church, M. J. (2016). Diversity and productivity of photosynthetic picoeukaryotes in biogeochemically distinct regions of the South East Pacific Ocean. Limnology and Oceanography, 61(3), 806–824. doi:10.1002/lno.10255
Methods

Talarmin, A., Van Wambeke, F., Duhamel, S., Catala, P., Moutin, T., & Lebaron, P. (2011). Improved methodology to measure taxon-specific phosphate uptake in live and unfiltered samples. Limnology and Oceanography: Methods, 9(10), 443–453. doi:10.4319/lom.2011.9.443
Methods

Wright, R. R., & Hobbie, J. E. (1966). Use of Glucose and Acetate by Bacteria and Algae in Aquatic Ecosystems. Ecology, 47(3), 447–464. doi:10.2307/1932984
Methods

Zubkov, M., & Tarran, G. (2005). Amino acid uptake of Prochlorococcus spp. in surface waters across the South Atlantic Subtropical Front. Aquatic Microbial Ecology, 40, 241–249. doi:10.3354/ame040241
Methods

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Parameters

Parameter	Description	Units
cruise	cruise identifier	unitless
station	station identifier	unitless
lat	station latitude; north is positive	decimal degrees
lon	station longitude; east is positive	decimal degrees
PO4	Phosphate concentration estimate	nanomol Phosphate/liter (nmol P L-1)
PO4_33P	Bulk phosphate uptake rate (>0.2 microns)	nanomol Phosphate/liter/hour (nmol P L-1 h-1)
Vmax	Phosphate uptake kinetic parameter Vmax	nanomol Phosphate/liter/hour (nmol P L-1 h-1)
Km	Phosphate uptake kinetic parameter Km	nanomol Phosphate/liter (nmol P L-1)
CO2_14C	Bulk CO2 fixation rate (>0.2 microns)	milligrams Carbon/meter^3/day (mg C m-3 d-1)
PO4_Pro_33P	Phosphate uptake rate by Prochlorococcus	attomole/cell/hour (amol cell-1 h-1)
PO4_Syn_33P	Phosphate uptake rate by Synechococcus	attomole/cell/hour (amol cell-1 h-1)
PO4_P_Euk_33P	Phosphate uptake rate by pigmented eukaryotes	attomole/cell/hour (amol cell-1 h-1)
PO4_NP_Euk_33P	Phosphate uptake rate by non-pigmented eukaryotes	attomole/cell/hour (amol cell-1 h-1)
CO2_Pro_14C	CO2 fixation rate by Prochlorococcus	femtogram Carbon/cell/hour (fg C cell-1 h-1)
CO2_Syn_14C	CO2 fixation rate by Synechococcus	femtogram Carbon/cell/hour (fg C cell-1 h-1)
CO2_P_Euk_14C	CO2 fixation rate by pigmented eukaryotes	femtogram Carbon/cell/hour (fg C cell-1 h-1)

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Instruments

Dataset-specific Instrument Name	BD Influx flow cytometer
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Used for flow cytometry analyses
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	Epifluorescence microscope
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Used to count calibration beads.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	Horiba FluoroMax-4 spectrofluorometer
Generic Instrument Name	Fluorometer
Dataset-specific Description	Used to measure fluorescence
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	Packard Tri-Carb 3110 TR liquid scintillation counter with ultra-low-level option kit
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	Used to assay sample radioactivity.
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.

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Deployments

AE1524

Website	https://www.bco-dmo.org/deployment/743183
Platform	R/V Atlantic Explorer
Start Date	2015-09-24
End Date	2015-09-29
Description	North Atlantic subtropical gyre, September 2015, along a transect from Bermuda to Puerto Rico extending from 33°N to 22°N

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Project Information

Collaborative Research: Role of small-sized protists in the microbial loop with emphasis on interactions between mixotrophic protists and picocyanobacteria (Small protists in microbial loop)

Coverage: North Pacific subtropical gyre (Station ALOHA) and Northwestern Mediterranean Sea (Station DYFAMED)

This project is an NSF Collaborative Research Project.

Description from NSF award abstract:
Protists are mostly single-celled, eukaryotic microorganisms, including algae and protozoans. They are ubiquitous, diverse, and major contributors in oceanic food webs. Determining their taxonomic identity and the extent to which they contribute to carbon and nutrient cycles (whereby carbon and minerals are continuously changed chemically in the environment and reincorporated in living organisms) are among the major goals of this study. Moreover, the investigators will study how they respond to environmental change, one of the most important and challenging current problems in oceanography. Answering these questions is fundamental to understanding how living organisms in the ocean environment interact with one another and contribute to the health and productivity of the ocean. The main goal of the project is to investigate biotic interactions of small-sized protists with very tiny cyanobacteria also known as picocyanobacteria, which represent the most

abundant photosynthetic organisms in the ocean. These studies will be done both in ocean environments and in laboratory experimental settings. Considering the limited knowledge on this topic, the work planned in this project promises important and exciting discoveries. Two early career female scientists will lead this project. In addition, one postdoctoral scholar, one graduate student, and at least three undergraduate summer interns will participate in the proposed research activities. The principal investigators will create a strong public outreach program that will engage middle school students in hands-on activities related to ocean sciences, and will produce a video in collaboration with the Education Department at the American Museum of Natural History. The video will summarize the major findings of the proposed research. It can be used in schools and in informal learning settings, including access by the public on the Internet through the Museum's Science Bulletins web page.

Single-celled eukaryotic microorganisms or protists, though largely outnumbered by picocyanobacteria (*Prochlorococcus* and *Synechococcus*), contribute significantly to ocean carbon biomass and primary productivity, partially by virtue of their larger cell size. In addition, small planktonic protists can regulate picocyanobacteria abundance through grazing. The main goal of this project is to investigate biotic interactions of planktonic pico- and nano-sized eukaryotes with picocyanobacteria, both in the field and in laboratory settings. A set of field- and culture-based experiments will be conducted, using state-of-the-art methodologies, including fluorescence-activated cell sorting, isotope and fluorescent stain labeling, and next-generation molecular sequencing to address the research objectives.

Operationally, this project is structured around two objectives:

Objective 1 is to assess the contribution of small protists to carbon and nutrient cycling through measurement of primary production, bacterivory, mixotrophy and phosphorus uptake in major microbial groups, and evaluate the role of nutrient availability in controlling mixotrophy.

Objective 2 will focus on assessing the distribution and diversity of small-sized protists that feed on picocyanobacteria and further evaluate the role of nutrient availability among the protists that are mixotrophic.

To reach these objectives field-based experiments will be conducted in contrasted environments: the North Pacific subtropical gyre (phosphorus replete, dominated by *Prochlorococcus* at Sta. ALOHA) and the North West Mediterranean sea (phosphorus deplete, dominated by *Synechococcus* at Sta. DYFAMED). Complementary experiments using model protists and picocyanobacteria will be conducted using controlled cultures in the lab. The work will provide critical new information on the phylogenetic diversity and function of marine microbial eukaryotes, with emphasis on their ecological role as predators (phagotrophy, mixotrophy) on, and competitors with, the picoyanobacteria *Prochlorococcus* and *Synechococcus*.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1458070

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