

# Bulk and cell-specific CO<sub>2</sub> fixation and PO<sub>4</sub> 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)

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## Abstract

Bulk and cell-specific CO<sub>2</sub> fixation and PO<sub>4</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> fixation and PO<sub>4</sub> 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 CO<sub>2</sub> 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. PO<sub>4</sub> assimilation rates were measured in triplicate 70-mL samples with ~259 kBq of added <sup>33</sup>P-PO<sub>4</sub> (Perkin-Elmer #NEZ08000; carrier free), incubated for 30 min to 1h. CO<sub>2</sub> fixation rates were measured in duplicate 70-mL samples with ~17 MBq of added <sup>14</sup>C-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 <sup>14</sup>C 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 <sup>33</sup>P-PO<sub>4</sub>, the membrane filter was placed onto a filter type HA soaked in 100 mM KH<sub>2</sub>PO<sub>4</sub>, then rinsed three times with ~1 mL of 0.2-micron filtered seawater. To remove unincorporated <sup>14</sup>C-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 (TPO<sub>4</sub>, h) were calculated by dividing the total radioactivity added (Bq L<sup>-1</sup>) by the rate of radiolabel uptake into the particulate fraction (Bq L<sup>-1</sup> h<sup>-1</sup>). PO<sub>4</sub> assimilation rates (nmol P L<sup>-1</sup> h<sup>-1</sup>) were calculated by dividing PO<sub>4</sub> concentration by TPO<sub>4</sub>. We used PO<sub>4</sub> concentration estimated from a concentration series bioassays following the method of Wright and Hobbie (1966). Briefly, seawater samples were amended with non-radioactive PO<sub>4</sub> to target additions of 0, 5, 10, 25, 50, 75, and 150 nmol PO<sub>4</sub> L<sup>-1</sup>, spiked with <sup>33</sup>P-PO<sub>4</sub>, incubated and sampled as described above. The resulting TPO<sub>4</sub> values were plotted against a corresponding concentration of PO<sub>4</sub>, and extrapolated using linear regression (TPO<sub>4</sub> = a x PO<sub>4</sub> + b) to estimate the ambient concentration (S<sub>n</sub> = 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 PO<sub>4</sub> assimilation rates (V<sub>max</sub>, the maximum rate at saturating substrate concentration and K<sub>m</sub>, 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 <sup>14</sup>C-labeled samples were acidified with 1 mL of 2 mol L<sup>-1</sup> HCl for 24 h to remove any unincorporated <sup>14</sup>C-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<sup>-1</sup>). Radioactivity in sorted cells from the PFA-killed control samples (dpm cell<sup>-1</sup>) was deduced from radioactivity in the sorted cells from the respective samples (dpm cell<sup>-1</sup>). The cell-specific assimilation rate (nmol cell<sup>-1</sup> h<sup>-1</sup>) was calculated by dividing the radioactivity per cell (dpm cell<sup>-1</sup>) by the total microbial activity (dpm L<sup>-1</sup>) measured in the same sample, and then multiplied by the total microbial assimilation rate at ambient substrate concentration (nmol L<sup>-1</sup> h<sup>-1</sup>).

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

## Data Files

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

## 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](https://doi.org/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](https://doi.org/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](https://doi.org/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](https://doi.org/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](https://doi.org/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](https://doi.org/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](https://doi.org/10.3354/ame040241)

*Methods*

## 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 <sup>-1</sup> )
PO4_33P	Bulk phosphate uptake rate (>0.2 microns)	nanomol Phosphate/liter/hour (nmol P L <sup>-1</sup> h <sup>-1</sup> )
Vmax	Phosphate uptake kinetic parameter Vmax	nanomol Phosphate/liter/hour (nmol P L <sup>-1</sup> h <sup>-1</sup> )
Km	Phosphate uptake kinetic parameter Km	nanomol Phosphate/liter (nmol P L <sup>-1</sup> )
CO2_14C	Bulk CO2 fixation rate (>0.2 microns)	milligrams Carbon/meter <sup>3</sup> /day (mg C m <sup>-3</sup> d <sup>-1</sup> )
PO4_Pro_33P	Phosphate uptake rate by Prochlorococcus	attomole/cell/hour (amol cell <sup>-1</sup> h <sup>-1</sup> )
PO4_Syn_33P	Phosphate uptake rate by Synechococcus	attomole/cell/hour (amol cell <sup>-1</sup> h <sup>-1</sup> )
PO4_P_Euk_33P	Phosphate uptake rate by pigmented eukaryotes	attomole/cell/hour (amol cell <sup>-1</sup> h <sup>-1</sup> )
PO4_NP_Euk_33P	Phosphate uptake rate by non-pigmented eukaryotes	attomole/cell/hour (amol cell <sup>-1</sup> h <sup>-1</sup> )
CO2_Pro_14C	CO2 fixation rate by Prochlorococcus	femtogram Carbon/cell/hour (fg C cell <sup>-1</sup> h <sup>-1</sup> )
CO2_Syn_14C	CO2 fixation rate by Synechococcus	femtogram Carbon/cell/hour (fg C cell <sup>-1</sup> h <sup>-1</sup> )
CO2_P_Euk_14C	CO2 fixation rate by pigmented eukaryotes	femtogram Carbon/cell/hour (fg C cell <sup>-1</sup> h <sup>-1</sup> )

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## Instruments

<b>Dataset-specific Instrument Name</b>	BD Influx flow cytometer
<b>Generic Instrument Name</b>	Flow Cytometer
<b>Dataset-specific Description</b>	Used for flow cytometry analyses
<b>Generic Instrument Description</b>	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: <a href="http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm">http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm</a> )

<b>Dataset-specific Instrument Name</b>	Epifluorescence microscope
<b>Generic Instrument Name</b>	Fluorescence Microscope
<b>Dataset-specific Description</b>	Used to count calibration beads.
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	Horiba FluoroMax-4 spectrofluorometer
<b>Generic Instrument Name</b>	Fluorometer
<b>Dataset-specific Description</b>	Used to measure fluorescence
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	Packard Tri-Carb 3110 TR liquid scintillation counter with ultra-low-level option kit
<b>Generic Instrument Name</b>	Liquid Scintillation Counter
<b>Dataset-specific Description</b>	Used to assay sample radioactivity.
<b>Generic Instrument Description</b>	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 to quantify the activity of particulate emitting ( $\beta$ and $\alpha$ ) radioactive samples, it can also detect the Auger electrons emitted from $^{51}\text{Cr}$ and $^{125}\text{I}$ 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.

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## Deployments

### AE1524

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/743183">https://www.bco-dmo.org/deployment/743183</a>
<b>Platform</b>	R/V Atlantic Explorer
<b>Start Date</b>	2015-09-24
<b>End Date</b>	2015-09-29
<b>Description</b>	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 picocyanobacteria *Prochlorococcus* and *Synechococcus*.

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1458070</a>

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