Plankton cell abundances and APA data from RV Atlantic Explorer BATS cruise BV50 in the North Atlantic subtropical gyre during September 2015.

Website: https://www.bco-dmo.org/dataset/739353
Data Type: Cruise Results
Version: 1
Version Date: 2018-06-27

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
Plankton cell abundances and APA data from RV Atlantic Explorer BATS cruise BV50 in the North Atlantic subtropical gyre during September 2015.

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Coverage

Spatial Extent: **N**:33.4468 **E**:-63.2169 **S**:21.8378 **W**:-66.2052
Temporal Extent: 2015-09-24 - 2015-09-29

Dataset Description

Plankton cell abundances and APA data collected on BV50 in the North Atlantic subtropical gyre (September 2015), along a transect from Bermuda to Puerto Rico extending from 33°N to 22°N.

Methods & Sampling

Flow cytometry analyses

Duplicate 1.8-ml samples were fixed with paraformaldehyde (electron microscopy grade, 0.25% final concentration) for 15–20 min in the dark at room temperature, flash frozen in liquid nitrogen and preserved at -80°C until analysis. The sheath fluid consisted in a sodium chloride solution filtered in-line through a 0.22 µm Sterivex filter unit. Reference beads (Fluoresbrite, YG, 1-µm) were added to each sample to maintain proper alignment and focus of the instrument. *Prochlorococcus*, *Synechococcus* and pigmented protists (P-Protists) were discriminated in unstained samples based on their chlorophyll (red) fluorescence and forward scatter (FSC, size) signatures. The high phycoerythrin (orange) signal in *Synechococcus* was used to distinguish them from *Prochlorococcus* and P-Protists. Using a forward scatter detector with small particle option and focusing a 488 plus a 457 nm (200 and 300 mW solid state, respectively) laser into the same pinhole permitted the resolution of dim surface *Prochlorococcus* group from background noise (Duhamel et al., 2014). Additionally, non-pigmented protists (NP-Protists) were discriminated from P-Protists in SYBR Green stained samples using a chlorophyll (red) vs SYBR Green (green) fluorescence plot, as described in Christaki et al. (2011) and Bock et al. (2018).

Microscopy

Ten-ml aliquots of the formalin-fixed plankton samples were centrifuged to sediment the plankton, and the overlying supernatant removed leaving 0.5 to 1 ml. The sedimented pellet was gently resuspended in the remaining supernatant and stained with Lugol's iodine. Samples of the thoroughly suspended stained sample (20 µl) were taken with a micropipette and deposited as a droplet in the base of an Uttermohl viewing chamber and examined using an inverted compound microscope with phase contrast optics. The contents of each 20-µl droplet was scanned field-by-field exhaustively at 400x, and each individual microplankton observed was measured in the micron range, using an ocular reticule, and assigned to a broad taxonomic morphogroup (e.g., coccolithophore, diatom, dinoflatellate). Examples of individuals within each morphogroup were photographed using a digital camera, and representative images of each morphogroup were assembled in a plate of micrographs illustrating the range of microplankton that were observed.

Alkaline phosphatase activity

Alkaline phosphatase activity (APA) was measured following a modification of the Dyhrman and Ruttenberg (2006) protocol. Seawater samples were collected onto two separate filters with 0.2- and 0.8-µm porosities, chosen to tease apart activity by the bacterioplankton (i.e. bacteria and cyanobacteria) and the protists enriched fractions (0.2–0. 8 µm and >0.8 µm, respectively). Filters were stored frozen at –20°C until analysis (<1 month). Samples were processed using the fluorogenic phosphatase substrate 4-methylumbelliferyl phosphate (MUF-P, Sigma-Aldrich) at saturating concentration (10 µmol l–1, Casey et al. (2009)). Fluorescence was measured at several time points within the linear range of the assay. A standard curve using 4-methylumbelliferone (MUF, Sigma-Aldrich) was used to calculate MUF-P hydrolysis rates (Duhamel et al 2011).

Data Processing Description

For cell enumeration, cytograms were analyzed using FCS Express 6 Flow Cytometry Software (De Novo Software, CA, US). Data were analyzed using MS Excel.

BCO-DMO Data Processing Notes:

-Changed N.D. to nd
-Reformatted column names to meet BCO-DMO standards
-Changed points in column names to underscores
-Added Cruise column

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Data Files

File
BV50_cell.csv (Comma Separated Values (.csv), 352 bytes) MD5:c95e37c2ff62c8f84c64534526cf9965 Primary data file for dataset ID 739353

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Related Publications

Bock, N., Van Wambeke, F., Dion, M., & Duhamel, S. (2018). Microbial community structure in the Western Tropical South Pacific. Biogeosciences Discussions, 1–24. doi:10.5194/bg-2017-562
General

Casey, J., Lomas, M., Michelou, V., Dyhrman, S., Orchard, E., Ammerman, J., & Sylvan, J. (2009). Phytoplankton taxon-specific orthophosphate (Pi) and ATP utilization in the western subtropical North Atlantic. Aquatic Microbial Ecology, 58, 31–44. doi:10.3354/ame01348
General

Christaki, U., Courties, C., Massana, R., Catala, P., Lebaron, P., Gasol, J. M., & Zubkov, M. V. (2011). Optimized routine flow cytometric enumeration of heterotrophic flagellates using SYBR Green I. Limnology and Oceanography: Methods, 9(8), 329–339. doi:10.4319/lom.2011.9.329
General

Duhamel, S., Björkman, K. M., Van Wambeke, F., Moutin, T., & Karl, D. M. (2011). Characterization of alkaline phosphatase activity in the North and South Pacific Subtropical Gyres: Implications for phosphorus cycling. Limnology and Oceanography, 56(4), 1244–1254. doi:10.4319/lo.2011.56.4.1244
General

Duhamel, S., Björkman, K., Doggett, J., & Karl, D. (2014). Microbial response to enhanced phosphorus cycling in the North Pacific Subtropical Gyre. Marine Ecology Progress Series, 504, 43–58. doi:10.3354/meps10757
General

Dyhrman, S. T., & Ruttenberg, K. C. (2006). Presence and regulation of alkaline phosphatase activity in eukaryotic phytoplankton from the coastal ocean: Implications for dissolved organic phosphorus remineralization. Limnology and Oceanography, 51(3), 1381–1390. doi:10.4319/lo.2006.51.3.1381
General

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Parameters

Parameter	Description	Units
Stn	Station number	unitless
Lat	Latitude	decimal degrees
Long	Longitude	decimal degrees
Pro	Prochlorococcus cell abundance	x10^3 cell per milliliter
Syn	Synechococcus cell abundance	x10^3 cell per milliliter
P_Prot	Pigmented protist cell abundance	x10^3 cell per milliliter
NP_Prot	Non-Pigmented protist cell abundance	x10^3 cell per milliliter
Phyto	Phytoplankton cell abundance	x10^3 cell per liter
Dino	Dinoflagellate cell abundance	x10^3 cell per liter
APA_0_2_0_8	Alkaline phosphatase activity in the 0.2 to 0.8 um size fraction	nmol L -1 h -1
APA_greaterThan_0_8	Alkaline phosphatase activity in the size fraction greater than 0.8 um	nmol L -1 h -1
Cruise	Cruise ID	unitless

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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	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	Nikon Diaphot inverted compound microscope
Generic Instrument Name	Inverted Microscope
Dataset-specific Description	Used in microscopy analyses
Generic Instrument Description	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

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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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