Flow cytometry cell counts obtained during the R/V L'Atalante OUTPACE cruise between New Caledonia and Tahiti from February to April 2015

Website: https://www.bco-dmo.org/dataset/663918
Data Type: Cruise Results
Version:
Version Date: 2016-11-03

Project
» Photoheterotrophy in unicellular cyanobacteria: ecological drivers and significance for marine biogeochemistry (Photoheterotrophy in unicellular cyanobacteria)

Contributors	Affiliation	Role
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Coverage

Spatial Extent: **N**:-17.997 **E**:-159.99 **S**:-22.0008 **W**:159.6432

Dataset Description

This dataset contains abundances of cyanobacteria (*Synechococcus*, and *Prochlorococcus*), picoalgae, non-pigmented picoplankton (bacteria), and heterotrophic protists acquired through flow cytometry. Latitude, longitude, timestamp, and sample depth are also included in this dataset.

Water samples were acquired during the Oligotrophy to UltTra-oligotrophy PACific Experiment (OUTPACE) cruise between New Caledonia and Tahiti from February to April 2015.

Methods & Sampling

Analytical procedure:

Microbial cell abundances were determined using an Influx flow cytometer. Briefly, pigmented groups (Prochlorococcus, Synechococcus, and picoalgae) were enumerated in unstained samples by their chlorophyll and forward scatter signatures (Marie et al. 1999). The high phycoerythrin signal in Synechococcus was used to distinguish this group from Prochlorococcus and picoalgae.

To visualize non-pigmented picoplankton (bacteria) and heterotrophic protists, samples were stained with SYBR Green I DNA dye (SG, 1:10000 final concentration, for 10 min at 4C in the dark) (Zubkov et al. 2007, Christaki et al. 2011). Because bacteria and Prochlorococcus groups exhibit overlapping signal characteristics in surface

samples after SG staining, the abundances of bacteria in surface samples were calculated by subtracting the Prochlorococcus abundance, determined in the unstained aliquot, from the total SG-stained group abundance. An internal standard of 1-um diameter fluorescent microspheres (Fluoresbrite, Polysciences) was added to each sample.

Sampling and Analytical Methodology:

Seawater samples were acquired from bottle samples collected by a CTD rosette during the OUTPACE cruise. 1.8 ml water samples were collected in duplicate, fixed with 0.25% (w/v) paraformaldehyde, flash frozen, and preserved at -80C. One of the duplicate samples was used to count phytoplankton (Prochlorococcus, Synechococcus, picoalgae), and the other was used to count bacteria and protists.

For pigmented groups (Prochlorococcus, Synechococcus, and picoalgae), cells were excited using a combination of two lasers (488+456 nm) focused into one pinhole; while for non-pigmented groups (bacteria and heterotrophic protists), cells were excited using the 488 nm laser only. For calibration, a solution of 1-um diameter fluorescent microspheres (Fluoresbrite, Polysciences) was used.

References:

Christaki U, Courties C, Massana R, Catala P, Lebaron P, Gasol JM, Zubkov MV (2011) Optimized routine flow cytometric enumeration of heterotrophic flagellates using SYBR Green I. Limnology and Oceanography-Methods 9:329-339. https://doi.org/10.4319/lom.2011.9.329

Marie D, Partensky F, Vaulot D, Brussaard C (1999) Enumeration of Phytoplankton, Bacteria, and Viruses in Marine Samples. Current Protocols in Cytometry:11.11.11-11.11.15. https://doi.org/10.1002/0471142956.cy1111s10

Zubkov M, Burkill PH, Topping JN (2007) Flow cytometric enumeration of DNA-stained oceanic planktonic protists. Journal of Plankton Research 29:79-86. https://doi.org/10.1093/plankt/fbl059

Data Processing Description

Flow cytometry data were analyzed using FCS Express Pro (De Novo Software).

BCO-DMO Processing Notes:

* added ISO timestamp from OUTPACE cruise CTD logs
* stripped out_c_ from station column for matching with bottle data
* column station renamed cast to be consistent with CTD log
* added a conventional header with dataset name, PI name, version date
* modified parameter names to conform with BCO-DMO naming conventions

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Data Files

File
flowCyto.csv (Comma Separated Values (.csv), 31.74 KB) MD5:67794f743014e520ebb4e52abb5dee17 Primary data file for dataset ID 663918

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Parameters

Parameter	Description	Units
cast	CTD cast number that acquired sample	unitless
bottle	bottle number	unitless
depth_w	depth of sample	meters
lat	latitude of cast	decimal degrees
lon	longitude of cast; west is negative	decimal degrees
pro	Prochlorococcus cell abundance	cells per milliliter
syn	Synechococcus cell abundance	cells per milliliter
peuk	picoalgae cell abundance	cells per milliliter
bacteria	non-pigmented pico plankton (bacteria) cell abundance	cells per milliliter
protists	heterotrophic (non-pigmented) protist cell abundance	cells per milliliter
ISO_DateTime_UTC	ISO formatted date/time (UTC) in format YYYY-mm-ddTHH:MM:SS[.xx]	unitless

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Instruments

Dataset-specific Instrument Name	Sea-Bird CTD 9+
Generic Instrument Name	CTD Sea-Bird
Dataset-specific Description	This CTD-Rosette was called "classique" to differentiate it from the trace metal CTD-Rosette. For more information on the CTD configuration see the OUTPACE site.
Generic Instrument Description	A Conductivity, Temperature, Depth (CTD) sensor package from SeaBird Electronics. This instrument designation is used when specific make and model are not known or when a more specific term is not available in the BCO-DMO vocabulary. Refer to the dataset-specific metadata for more information about the specific CTD used. More information from: http://www.seabird.com/

Dataset-specific Instrument Name	Influx Flow cytometer
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Instrument Type: Flow cytometer (Manufacturer: BD) Model: Influx
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)

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Deployments

OUTPACE

Website	https://www.bco-dmo.org/deployment/663891
Platform	R/V L'Atalante
Start Date	2015-02-18
End Date	2015-04-03
Description	Oligotrophy to UltRa-oligotrophy PACific Experiment (OUTPACE) cruise DOI: http://dx.doi.org/10.17600/15000900 For more information see the cruise website: https://outpace.mio.univ-amu.fr/?lang=en South west Pacific between New Caledonia (166°28' E; 22°14' S) and Tahiti (149°36' W; 17°34' S) 0-2000 m **Methods & Sampling** OUTPACE (Oligotrophy to UltRa-oligotrophy PACific Experiment) cruise onboard the RV L'Atalante (France) https://outpace.mio.univ-amu.fr/ OUTPACE cruise DOI: http://dx.doi.org/10.17600/15000900

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Project Information

Photoheterotrophy in unicellular cyanobacteria: ecological drivers and significance for marine biogeochemistry (Photoheterotrophy in unicellular cyanobacteria)

Coverage: Southwest Pacific

Description from NSF award abstract:
Unicellular cyanobacteria are major contributors to primary production and carbon export in the open ocean. They also play an important role in the control of nutrient availability. The ability of these microbes to harvest light energy benefits a range of physiological functions, but the effect of light on their metabolism (other than for photosynthesis) is poorly known and controversial. This project will investigate the role of light in uptake of organic substrates (carbon and nutrients) by unicellular cyanobacteria and elucidate the importance of photoheterotrophy. The ability of these organisms to assimilate organic compounds and its modulation by light

and nutrients will provide additional hints about the ecological success of unicellular cyanobacteria in the ocean. The proposed work will involve field experiments in the southwest Pacific Ocean, complemented by laboratory experiments in controlled cultures of ecologically relevant cyanobacteria. The study will employ innovative methods, including single cell assays and molecular tools that target individual cyanobacteria and evaluate their response to light for the assimilation of organic substrates. This research project will lead to an increased understanding of the microbial adaptations to light and nutrient gradients and the role these adaptations play in elemental cycling in oceanic habitats.

Unicellular cyanobacteria inhabit the surface ocean (generally <150 m deep), and use solar energy to compete for a limited supply of available nutrients. Therefore, they are expected to utilize light energy not only for photosynthesis but also to enhance their metabolism of dissolved organic compounds. Yet, the role of light in the uptake of organic compounds (both carbon and nutrients) and the importance of photoheterotrophy are still poorly understood. This proposal seeks to investigate the ecological drivers and significance of photoheterotrophy in the unicellular cyanobacteria *Prochlorococcus* and *Synechococcus*, the most abundant groups of primary producers in the ocean, and *Crocosphaera* an important nitrogen fixing organism. This proposal argues that adaptations to specific light regimes must shape spatiotemporal partitioning of resources among microbial groups in the ocean. Field experiments along a west-east transect in the southwest Pacific Ocean will cover a range of nutrient conditions and cyanobacterial abundances. Radioactive substrate incubations combined with flow cytometry cell sorting and microautoradiography paired to catalyzed reporter deposition fluorescence in situ hybridization (MICRO-CARD-FISH) will allow the separation of unicellular cyanobacteria from non-pigmented bacterioplankton and evaluation of their response to light for the uptake of different organic substrates. These experiments will be complemented by laboratory tests in controlled cultures of axenic strains representative of different ecologically relevant functional groups of cyanobacteria. Lastly, the capacity of the important nitrogen fixer *Crocosphaera watsonii* to feed on glucose will be tested, taking advantage of the sequenced genome of the representative strain WH8501 in targeting the expression of genes involved in glucose metabolism in situ.

Project investigators will participate in a Southwest Pacific cruise, the OUTPACE (Oligotrophy to UlTraoligotrophy PACific Experiment) expedition. The cruise will sample stations along a West-East transect between New Caledonia and Tahiti.

More information:
* OUTPACE cruise (doi: http://dx.doi.org/10.17600/15000900)
* OUTPACE website: https://outpace.mio.univ-amu.fr/?lang=en

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1434916

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