Prochlorococcus N and C uptake from R/V Atlantic Explorer AE1032 in the Western Subtropical North Atlantic from Oct. 2010 (Nitrogen uptake in Prochlorococcus project)

Website: https://www.bco-dmo.org/dataset/542660
Version: 2
Version Date: 2015-05-08

Project
» Prochlorococcus and its contribution to new production in the Sargasso Sea (Nitrogen uptake in Prochlorococcus)

Program
» Ocean Carbon and Biogeochemistry (OCB)

Contributors	Affiliation	Role
Lomas, Michael W.	Bigelow Laboratory for Ocean Sciences	Principal Investigator
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Dataset Description

Nitrogen and carbon uptake rates and cell element quotas for flow-sorted phytoplankton taxa (Prochlorococcus, Synechococcus, eukaryotes). Samples were collected from the Sargasso Sea in Oct-Nov. 2010.

Related References:

Batmalle, C.S., Chiang, H-I, Zhang, K., Lomas, M.W., Martiny, A.C. 2014. Development and bias assessment of a method for targeted metagenomic sequencing of marine Cyanobacteria. Applied and Environmental Microbiology, 80: 1116-1125.

Fawcett, S.E., Lomas, M.W., Casey, J.R., Ward, B.B., Sigman, D.M. 2011. Eukaryotes dominate new production in the Sargasso Sea. Nature Geosciences, 4: 717-722.

Fawcett, S.E., Lomas, M.W., Ward, B.B., Sigman, D.M. 2014. The effect of summer-to-winter mixed layer deepening on eukaryotic new production in the Sargasso Sea. Global Biogeochemical cycles. 28:86-102.

Treibergs, L.A., Fawcett, S.E., Lomas, M.W., Sigman, D.M. 2014. Nitrogen isotopic response of prokaryotic and eukaryotic phytoplankton to nitrate availability in Sargasso Sea surface waters. Limnology and Oceanography. 59:972-985.

Methods & Sampling

Seawater for short 15N incubations was collected from three depths throughout the euphotic zone - the shallow euphotic zone (0-40m) where irradiances are high and nutrients are depleted, the DCM which is in the main NO3- gradient, and ~20m below the DCM where light is very low (<<1%) but NO3- concentrations are high - using clean methods employed by BATS for collecting samples for primary production. Prior to initiating the incubation we measured nutrient concentrations using high-sensitivity colorimetric methods to calculate true tracer (10% ambient) 15N additions. All incubations were carried out under simulated in-situ conditions of irradiance and temperature, which were maintained in screened deckboard incubators. Other water-column samples and data were collected using established protocols (Knap et al. 1997, Sedwick et al. 2005, Steinberg et al. 2001). As much as possible, we collected the seawater for our experiments and corresponding water-column samples and data at the same time of day, in order to minimize the possible confounding effects of diel variations.

15N-uptake measurements: Nitrogen (NH4+, urea, NO2-, and NO3-) uptake rates were measured following the isotopic-tracer procedures described previously (Lipschultz, 2001; Lomas et al., 2002; Slawyk et al., 1977). Incubations were short (4h) and run as a time course (1, 2 and 4h), with replicate bottles sacrificed at each time point. At the final time point from each incubation, seawater samples were taken for isotopic dilution of the added substrate and used in the calculation of uptake rates. Each incubation included control samples with no added 15N, but are taken through the entire incubation/sorting protocol to serve as our 'initial' isotopic value for rate estimations (Lipschultz, 2008). Whole population and cell-specific uptake rates were estimated sensu Dugdale and Goering (1967).

Following incubations, samples were filtered under gentle vacuum (<50 mm Hg), transferred to a vial with filtered seawater and paraformaldehyde (0.5% final concentration), kept at 4°C for ~1 hour, and stored in liquid nitrogen until analysis. Then, we split each sample into two subsamples immediately prior to sorting with one subsample sorted for Prochlorococcus and the other for total autotrophs. Next, we flow sorted the isotopically labeled cells using a previously published method (Casey et al., 2007). All samples were sorted with a Cytopeia Influx Cell Sorter (see facilities) using 0.2 µm filtered 3.6% NaCl solution as the sheath fluid and 488 nm laser excitation (we also have 355nm and 635nm solid state lasers). Sorting was optimized for maximum purity, rather than yield, by triggering on forward scatter, using single drop sorting and 2/16th drop criterion to ensure cells are away from drop boundaries. Purity of sorted samples was routinely assessed by post-sort re-analysis.
Once sorted, cells were filtered (0.2um silver filter) and analyzed on a Europa 20/20 isotope ratio mass spectrometer at the UC Davis isotope facility.

Prochlorococcus enumeration: Separate subsamples for total Prochlorococcus counts - by flow cytometry - were fixed in para-formaldehyde (0.5% final concentration), and stored in liquid nitrogen (Sieracki et al., 1995). Red fluorescence histogram of the total Prochlorococcus population, normalized to 0.53 µm bead fluorescence, will be used as a qualitative criteria to distinguish between 'low-light' and 'high-light' sub-populations (Campbell and Vaulot, 1993).

High sensitivity nutrient analysis and isotopic composition of dissolved substrates: Samples for NH4+, urea, NO2-, and NO3- were filtered through 0.8 µm polycarbonate filters in duplicate and analyzed immediately prior to the incubations in order to calibrate the 15N tracer addition. All analyses were colorimetric and run on an Alpkem Flow Solution IV autoanalyzer that will be modified as part of this project with fiber-optic flow cells to improve methodological sensitivity to the nanomolar level. Relevant methods are: NO3- and NO2- (Lomas et al., 2009), NH4+ (Li et al., 2005), urea (Cozzi, 2004) and PO4- (Li and Hansell, 2008; Li et al., 2008).

Methods References:

Campbell, L., Vaulot, D., 1993. Photosynthetic picoplankton community structure in the subtropical north Pacific ocean near Hawaii (station ALOHA). Deep-Sea Research (Part 1, Oceanographic Research Papers) 40, 2043-2060.

Casey, J.R., Lomas, M.W., Mandecki, J., Walker, D.E., 2007. Prochlorococcus contributes to new production in the Sargasso Sea deep chlorophyll maximum. Geophysical Research Letters 34 (10), L10604, doi:10.1029/2006GL028725.

Cozzi, S., 2004. A new application of the diacetyl monoxime method to the automated determination of dissolved urea in seawater. Marine Biology 145 (4), 843-848.

Dugdale, R.C., Goering, J.J., 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnology and Oceanography 12, 196-206.

Knap, A., A. Michaels, D. Steinberg, et al. 1997. BATS Methods Manual Version 4. U.S. JGOFS Planning Office.

Li, Q.P., Hansell, D.A., 2008. Intercomparison and coupling of magnesium-induced co-precipitation and long-path liquid-waveguide capillary cell techniques for trace analysis of phosphate in seawater. Analytica Chimica Acta 611 (1), 68-72.

Li, Q.P., Hansell, D.A., Zhang, J.Z., 2008. Underway monitoring of nanomolar nitrate plus nitrite and phosphate in oligotrophic seawater. Limnology and Oceanography-Methods 6, 319-326.

Li, Q.P., Zhang, J.Z., Millero, F.J., Hansell, D.A., 2005. Continuous colorimetric determination of trace ammonium in seawater with a long-path liquid waveguide capillary cell. Marine Chemistry 96 (1-2), 73-85.

Lipschultz, F., 2001. A time-series assessment of the nitrogen cycle at BATS. Deep-Sea Research 48 (8-9), 1897-1924.

Lipschultz, F., 2008. Isotope Tracer Methods for Studies of the Marine Nitrogen Cycle. In: Mulholland, M.R., Bronk, D.A., Capone, D.G., Carpenter, E.J. (Eds.), Nitrogen in the marine environment. Academic Press, New York, pp. 1345-1384.

Lomas, M.W., Lipschultz, F., Nelson, D.M., Bates, N.R., 2009. Biogeochemical responses to late-winter storms in the Sargasso Sea. I. Pulses of new and primary production. Deep Sea Research 56:843-860.

Lomas, M.W., Trice, T.M., Glibert, P.M., Bronk, D.A., McCarthy, J.J., 2002. Temporal and spatial dynamics of urea uptake and regeneration rates and concentrations in Chesapeake Bay. Estuaries 25 (3), 469-482.

Sedwick, P. N., T. M. Church, A. R. Bowie, et al. 2005. Iron in the Sargasso Sea (Bermuda Atlantic Time-series Study region) during summer: Eolian imprint, spatiotemporal variability, and ecological implications. Global Biogeochemical Cycles 19: GB4006, doi:10.1029/2004GB002445.

Sieracki, M.E., Haugen, E.M., Cucci, T.L., 1995. Overestimation of Heterotrophic Bacteria in the Sargasso-Sea - Direct Evidence by Flow and Imaging Cytometry. Deep-Sea Research Part I-Oceanographic Research Papers 42 (8), 1399.

Slawyk, G., Collos, Y., Auclair, J.C., 1977. Use of C-13 and N-15 Isotopes for Simultaneous Measurement of Carbon and Nitrogen Turnover Rates in Marine-Phytoplankton. Limnology and Oceanography 22 (5), 925-932.

Steinberg, D. K., C. A. Carlson, N. R. Bates, et al. 2001. Overview of the US JGOFS Bermuda Atlantic Time-series Study (BATS): a decade-scale look at ocean biology and biogeochemistry. Deep Sea Research Part II, 48:1405-1447.

Data Processing Description

Uptake rates are calculated using the following formula:

P = (At%sample - At%std)/(At%enrich) x Time x PN

PN = particulate nitrogen (or carbon) in units of nmol/L
Time = is incubation duration in hours
At%sample/At%std = isotopic values measured on particulate material
At%enrich = atom % rerichment of the source pool calculated from ambient concentration and added isotopic tracer.]

BCO-DMO Processing:

- added conventional header with dataset name, PI name, version date
- renamed parameters to BCO-DMO standard
- shortened C:N significant digits from 14 to 4
- reduced number of significant digits of C_to_N from 14 to 4

Version 2014-11-29 had incorrect station locations and was replaced with version 2 (2015-05-08) on 5/22/2015.

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Data Files

File
Prochloro_NC_uptake.csv (Comma Separated Values (.csv), 18.64 KB) MD5:8c53bc9cf376b2bfe778df7ba3b3131e Primary data file for dataset ID 542660

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Parameters

Parameter	Description	Units
cruiseid	cruise ientification	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
population	phytoplankton population sorted	unitless
N_substrate	particular nitrogen substrate added for the incubation	unitless
Nmass_corr	Nitrogen mass of the sample corrected for blanks	ug/sample
Cmass_corr	Carbon mass of the sample corrected for blanks	ug/sample
num_cells	number of cells sorted for analysis	#
N_per_cell	calculation of N per cell as quotient of "Nmass corr" and "cells sorted"	fmol/cell
C_per_cell	calculation of C per cell as quotient of "Cmass corr" and "cells sorted"	fmol/cell
C_to_N	mass C:N ratio	mol:mol
N15_At_pcent_sample	15N atom percent for the sample	%
C13_At_pcent_sample	13C atom percent for the sample	%
N15_At_pcent_xs	15N atom percent excess for the sample corrected for natural abundance of 15N	%
C13_At_pcent_xs	13N atom percent excess for the sample corrected for natural abundance of 13N	%
time_inc	incubation duration	h
N_tracer_add	concentration of added 15N tracer	nM
C_tracer_add	concentration of added 13C tracer	uM
N_ambient	ambient N concentration	nM
C_ambient	ambient C concentration	uM
N_At_pcent_enrich	atom percent 15N enrichment of substrate pool; calculated as (tracer)/(tracer+ambient)	%
C_At_pcent_enrich	atom percent 13C enrichment of substrate pool; calculated as (tracer)/(tracer+ambient)	%
N_uptake_rate	N uptake rate	fmol/cell/h
C_uptake_rate	C uptake rate	fmol/cell/h

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Instruments

Dataset-specific Instrument Name	Flow Cytometer
Generic Instrument Name	Flow Cytometer
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	Nutrient Autoanalyzer
Generic Instrument Name	Nutrient Autoanalyzer
Dataset-specific Description	Alpkem Flow Solution IV autoanalyzer
Generic Instrument Description	Nutrient Autoanalyzer is a generic term used when specific type, make and model were not specified. In general, a Nutrient Autoanalyzer is an automated flow-thru system for doing nutrient analysis (nitrate, ammonium, orthophosphate, and silicate) on seawater samples.

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Deployments

AE1032

Website	https://www.bco-dmo.org/deployment/542529
Platform	R/V Atlantic Explorer
Start Date	2010-10-18
End Date	2010-11-03
Description	Bermuda Atlantic Time-series Study (BATS) Validation stations, Panulirus Hydrographic Stations, Prochlorococcus in the Sargasso Sea

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Project Information

Prochlorococcus and its contribution to new production in the Sargasso Sea (Nitrogen uptake in Prochlorococcus)

Coverage: Western Subtropical North Atlantic; ~32N to ~19N, ~65W

Description from NSF award abstract:

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The cyanobacterium *Prochlorococcus marinus* is ubiquitous in the oligotrophic subtropical and tropical oceans and can contribute up to 82% of the primary productivity in certain regions. In contrast to most other phytoplankton, cultured Prochlorococcus isolates cannot assimilate NO3-. However, Lomas' group has used flow cytometry and stable isotope tracers to demonstrate direct NO3- assimilation by Prochlorococcus in the Sargasso Sea. In support of these findings, Martiny and colleagues have shown that Prochlorococcus cells residing in the mixed layer carry genes for NO2- and NO3- assimilation, and that these genes are functional and expressed in field populations. The combined results suggest that uncultured lineages of Prochlorococcus are capable of NO3- assimilation and can contribute to new production in many oceanic regions - but the overall significance is yet unknown.

The overarching hypothesis of this project is that cell-specific NO3- assimilation rate is a function of both the ambient nutrient concentrations and the metabolic potential of the cell (i.e. presence of genes encoding for NO2- and NO3- assimilation). The specific research questions of this project are:

1) Is NO3- a quantitatively important nutrient source for Prochlorococcus and does Prochlorococcus contribute to new production?

2) What is the influence of seasonal and vertical variation in nitrogen substrates (NH4+, urea, NO2-, andNO3-) on the genome content of Prochlorococcus and oxidized nitrogen uptake rates?

To answer these questions, PIs will use the combination of high-sensitivity nutrient measurements, a flow cytometric assay developed by Lomas to quantify nitrogen assimilation in specific taxonomic groups, and metagenomics and a qPCR assay to determine the occurrence of nitrite (nirA) and nitrate reductase (narB) genes associated with Prochlorococcus. Using these tools, they will quantify NO3- assimilation and the distribution of NO3- assimilation genes in Prochlorococcus through three full seasonal cycles and over the entire euphotic zone. In addition, these direct measurements will be augmented by manipulative mesocosm experiments (reciprocal transplant and nutrient addition experiments) to explicitly test aspects of their hypotheses. The PIs hope to achieve a mechanistic understanding of direct (variations in the concentration of nitrogen species) and indirect controls (genomic adaptation in Prochlorococcus) on NO3- assimilation rates. One of the most exciting outcomes from this project will be a more complete understanding of the nutritional ecology of Prochlorococcus in field assemblages. The PIs have selected to conduct this study in the Sargasso Sea, because of the wealth of necessary supporting data and logistical infrastructure that this site provides, and because they have already shown that Prochlorococcus is capable of nitrate assimilation in this region.

Related Background Publications:
Casey, J. R., M. W. Lomas, J. Mandecki, et al. 2007. Prochlorococcus contributes to new production in the Sargasso Sea deep chlorophyll maximum. Geophysical Research Letters 34:-

Martiny, A. C., S. Kathuria, and P. Berube. 2009. Widespread metabolic potential for nitrite and nitrate assimilation among *Prochlorococcus* ecotypes.

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Program Information

Ocean Carbon and Biogeochemistry (OCB)

Website: http://us-ocb.org/

Coverage: Global

The Ocean Carbon and Biogeochemistry (OCB) program focuses on the ocean's role as a component of the global Earth system, bringing together research in geochemistry, ocean physics, and ecology that inform on and advance our understanding of ocean biogeochemistry. The overall program goals are to promote, plan, and coordinate collaborative, multidisciplinary research opportunities within the U.S. research community and with international partners. Important OCB-related activities currently include: the Ocean Carbon and Climate Change (OCCC) and the North American Carbon Program (NACP); U.S. contributions to IMBER, SOLAS, CARBOOCEAN; and numerous U.S. single-investigator and medium-size research projects funded by U.S.

federal agencies including NASA, NOAA, and NSF.

The scientific mission of OCB is to study the evolving role of the ocean in the global carbon cycle, in the face of environmental variability and change through studies of marine biogeochemical cycles and associated ecosystems.

The overarching OCB science themes include improved understanding and prediction of: 1) oceanic uptake and release of atmospheric CO2 and other greenhouse gases and 2) environmental sensitivities of biogeochemical cycles, marine ecosystems, and interactions between the two.

The OCB Research Priorities (updated January 2012) include: ocean acidification; terrestrial/coastal carbon fluxes and exchanges; climate sensitivities of and change in ecosystem structure and associated impacts on biogeochemical cycles; mesopelagic ecological and biogeochemical interactions; benthic-pelagic feedbacks on biogeochemical cycles; ocean carbon uptake and storage; and expanding low-oxygen conditions in the coastal and open oceans.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0928544
NSF Division of Ocean Sciences (NSF OCE)	OCE-0927567

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