

# Experimental data testing the N<sub>2</sub> and CO<sub>2</sub> fixing activity of the UCYN-A/haptophyte symbiosis in nitrate and ammonium rich waters in the California Current from May to October 2017

**Website:** <https://www.bco-dmo.org/dataset/774585>

**Data Type:** experimental

**Version:** 1

**Version Date:** 2023-11-29

## Project

» [Collaborative Research: Biogeochemical significance of the abundant, uncultivated symbiotic cyanobacteria UCYN-A](#) (BSUCS)

Contributors	Affiliation	Role
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## Abstract

Experimental data testing the N<sub>2</sub> and CO<sub>2</sub> fixing activity of the UCYN-A/haptophyte symbiosis in nitrate and ammonium rich waters in the California Current from May to October 2017.

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## Coverage

**Spatial Extent:** N:32.867 E:-115.914 S:28.289 W:-117.531

**Temporal Extent:** 2017-05-03 - 2018-05-12

## Dataset Description

Data are from experiments in Southern California Current waters testing the N<sub>2</sub> and CO<sub>2</sub> fixing activity of the UCYN-A/haptophyte symbiosis in nitrate and ammonium rich waters. Also included are nitrate and ammonium uptake capabilities by the symbiosis. Lastly, CTD sensor data from two cruises on which these experiments were conducted can be found at BCO-DMO dataset CTD sensor (see related datasets).

## Methods & Sampling

A series of onboard incubations were performed in surface waters of the Southern California coastal system

(CCS) to measure bulk community responses (chlorophyll a and POC/PON concentrations), bulk and UCYN-A/haptophyte symbiosis cell-specific N<sub>2</sub> fixation and CO<sub>2</sub> fixation rates, bulk and UCYN-A/haptophyte symbiosis cell-specific NO<sub>3</sub><sup>-</sup>/NH<sub>4</sub><sup>+</sup> uptake rates, and UCYN-A/haptophyte symbiosis growth rates under DIN deplete and replete (NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> amendments) conditions. The experiments were designed to investigate whether UCYN-A continues to fix N<sub>2</sub> when NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> are readily available and if the haptophyte host takes up NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>. Additionally, UCYN-A/haptophyte sublineage-specific responses to NO<sub>3</sub><sup>-</sup>/NH<sub>4</sub><sup>+</sup> additions were investigated.

Four experimental manipulations were conducted during 2017 and 2018; three experiments (NO3.1, NO3.2, NO3.3) were NO<sub>3</sub><sup>-</sup> addition experiments and one was an NH<sub>4</sub><sup>+</sup> addition experiment (NH4.1). NO3.1-3 were conducted at three different stations aboard the R/V Gordon Sproul during two research cruises in 2017 that transited off the coast of Southern California and Baja California Sur, Mexico, while NH4.1 was conducted on the Scripps Institute of Oceanography pier.

For NO3.1-3, surface water was pumped into 40 L carboys, housed in an on-deck laboratory container, using a pneumatic diaphragm pump PVDF and Teflon (Wilden Pump and Engineering, Grand Terrace, CA), to allow mixing of the seawater before being randomly dispensed into acid-cleaned 4 L polycarbonate bottles (Thermo Scientific™ Nalgene™, Waltham, MA). Grazers were removed using 150 µm nitex plankton netting (BioQuip, Rancho Dominguez, CA). The bottles were then incubated in triplicate with or without a 2 µmol L<sup>-1</sup> addition of NO<sub>3</sub><sup>-</sup> at T0. Incubation bottles were placed in a flow-through surface seawater incubator, amended with neutral density screening to attenuate incident light to 20% of the surface irradiance. Incubations lasted 48 h, with initial rate measurements between 0-24h and final rate measurements between 24-48 h. At each time point, bottles were sacrificed and subsampled for measuring chlorophyll a concentration, particulate nutrient concentrations, bulk CO<sub>2</sub> and N<sub>2</sub> fixation rates, inorganic N uptake rates, and UCYN-A/haptophyte symbiosis cell-specific N<sub>2</sub> fixation, CO<sub>2</sub> fixation and NO<sub>3</sub><sup>-</sup> uptake rates (CARD-FISH nanoSIMS). Unlabeled initial samples were used to determine the atom% 15N- and 13C-normal of the unenriched bulk community and UCYN-A/haptophyte symbioses.

For NH4.1, surface water was pumped into 40 L carboys from the waters surrounding the SIO Pier using a pneumatic diaphragm pump PVDF and Teflon (Wilden Pump and Engineering), then randomly dispensed into acid-cleaned 2 L polycarbonate bottles (Thermo Scientific™ Nalgene™). Grazers were removed using 150 µm nitex plankton netting (BioQuip, Rancho Dominguez, CA). The bottles were then incubated with or without a 2 µmol L<sup>-1</sup> addition of NH<sub>4</sub><sup>+</sup> at T0. Incubation bottles were placed in a flow-through surface seawater incubator, amended with neutral screening to attenuate incident light to 20% of the surface irradiance. Incubations lasted 48 h, with N<sub>2</sub> fixation initial rate measurements between 0-24h and final rate measurements between 24-48 h. For NH<sub>4</sub><sup>+</sup> uptake rates, initial rates were measured between 0-6 h, and final rates in NH<sub>4</sub><sup>+</sup>-treatments were measured between 45-51 h. Incubation times (6 h) were chosen to ensure detection of isotope enrichments while minimizing isotope dilution. At each time point, bottles were sacrificed and subsampled for chlorophyll a concentration, particulate nutrient concentrations, bulk CO<sub>2</sub> and N<sub>2</sub> fixation rates, inorganic N uptake rates, and UCYN-A/haptophyte symbiosis cell-specific N<sub>2</sub> fixation, CO<sub>2</sub> fixation and NO<sub>3</sub><sup>-</sup> uptake rates (CARD-FISH nanoSIMS). Unlabeled initial samples were used to determine the atom% 15N- and 13C-normal of the unenriched bulk community and UCYN-A/haptophyte symbioses.

## Data Processing Description

NanoSIMS images were processed using Look@nanosims software.

BCO-DMO Processing Notes:

- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- combined sheets "Bulk Rate measurements", "Bulk\_Biomass", and "Single Cell Rate Measurements"
- added a column for sheet name
- extracted start\_date and end\_date columns from Date column.

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## Data Files

File
<b>774585_v1_arrigo_ucyna.csv</b> (Comma Separated Values (.csv), 10.27 KB) MD5:16093b87a6ee65c30f7c351f464e2499
Primary data file for dataset ID 774585, version 1

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## Related Datasets

### IsRelatedTo

Arrigo, K. R., Zehr, J. P. (2023) **CTD sensor data from two cruises from R/V Robert Gordon Sproul SP1714 in the California Current waters off the coast of Southern California and Baja California from 2017-2018**. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-11-29 doi:10.26008/1912/bco-dmo.774459.1 [[view at BCO-DMO](#)]  
*Relationship Description: CTD sensor data from two cruises on which experiments were conducted.*

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## Parameters

Parameter	Description	Units
Experiment	experiment identifier	unitless
Cruise	cruise identifier	unitless
Date	date coverage of experiment	unitless
lat	latitude with positive values indicating North	decimal degrees
lon	longitude with negative values indicating West	decimal degrees
Treatment	treatment description	unitless
n	count	count
iso_sub	Isotopic Substrate	unitless
bulk_fix_uptake_rate_bulk_avg	Average Bulk N2 fixation rate or DIN uptake rate	micromole Nitrogen per meter cubed per day (umol N/m3/d)
bulk_fix_uptake_rate_bulk_sd	Standard deviation of Bulk N2 fixation rate or DIN uptake rate	micromole Nitrogen per meter cubed per day (umol N/m3/d)

C_fix_rate_bulk_avg	Average Carbon fixation rate	millimole Carbon per meter cubed per day (mmolC/m3/d)
C_fix_rate_bulk_sd	Standard Deviation of Carbon fixation rate	millimole Carbon per meter cubed per day (mmolC/m3/d)
LOD_bulk	Limit of detection, bulk values	umol N/m3/d or mmolC/m3/d depending on column it references
MQR_bulk	Minimum quantifiable rate, bulk values	umol N/m3/d or mmolC/m3/d depending on column it references
sheet	name of the original data sheet from which the data was extracted	unitless
chl_a_n	count for chlorophyll a measurements	count
chla_avg	Average Chlorophyll a concentration	micrograms per liter (ug/L)
chla_sd	Standard deviation of Chlorophyll a concentration	micrograms per liter (ug/L)
PON_n	Count of Particulate Organic Nitrogen	count
PON_avg	Average Particulate Organic Nitrogen concentration	micromole per liter (umol/L)
PON_sd	Standard deviation of Particulate Organic Nitrogen concentration	micromole per liter (umol/L)
POC_n	Count of Particulate Organic Carbon	count
POC_avg	Average Particulate Organic Carbon concentration	micromole per liter (umol/L)
POC_sd	Standard deviation of Particulate Organic Carbon concentration	micromole per liter (umol/L)
Sublineage	sublineage	unitless
sub_enrich_atom_pcnt	substrate enrichment Atom percent	percent
sub_enrich_atom_pcnt_sd	standard deviation of substrate enrichment Atom percent	percent

fix_uptake_rate_sc_avg	Average N2 fixation rate or DIN uptake Rate for single cell rate measurements	femtomole Nitrogen per cell per day (fmol N/cell/day)
fix_uptake_rate_sc_sd	Standard deviation of N2 fixation rate or DIN uptake Rate for single cell rate measurements	femtomole Nitrogen per cell per day (fmol N/cell/day)
C_fix_rate_sc_avg	Average Carbon fixation rate	femtomole Carbon per cell per day (fmol C/cell/day)
C_fix_rate_sc_sd	Standard deviation of Carbon fixation rate	femtomole Carbon per cell per day (fmol C/cell/day)
LOD_sc	Limit of detection, single cell values	fmol N/cell/day or fmol C/cell/day depending on column it references
MQR_sc	Minimum quantifiable rate, single cell values	unknown
comment	additional comments related to the data	unitless
start_date	start date of experiment	unitless
end_date	end date of experiment	unitless

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## Instruments

<b>Dataset-specific Instrument Name</b>	Cameca nanoSIMS 50L
<b>Generic Instrument Name</b>	Mass Spectrometer
<b>Dataset-specific Description</b>	Samples for single cell N2 fixation and CO2 fixation rates were analyzed using a Cameca nanoSIMS 50L ( <a href="https://www.cameca.com/products/sims/nanosims">https://www.cameca.com/products/sims/nanosims</a> ) located at Stanford University's nano shared facilities (SNSF, <a href="https://snsf.stanford.edu/equipment/xsa/nanosims.html">https://snsf.stanford.edu/equipment/xsa/nanosims.html</a> ).
<b>Generic Instrument Description</b>	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

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## Deployments

SP1714

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/699986">https://www.bco-dmo.org/deployment/699986</a>
<b>Platform</b>	R/V Robert Gordon Sproul
<b>Start Date</b>	2017-05-03
<b>End Date</b>	2017-05-11
<b>Description</b>	R/V Robert Gordon Sproul Cruise SP1714 May 3 - 11, 2017 Chief Scientist - Matthew Mills ( <a href="mailto:mmmills@stanford.edu">mmmills@stanford.edu</a> ) See more cruise information from R2R: <a href="https://www.rvdata.us/search/cruise/SP1714">https://www.rvdata.us/search/cruise/SP1714</a>

## SP1727

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/774496">https://www.bco-dmo.org/deployment/774496</a>
<b>Platform</b>	R/V Robert Gordon Sproul
<b>Start Date</b>	2017-10-04
<b>End Date</b>	2017-10-11
<b>Description</b>	R/V Robert Gordon Sproul Cruises SP1727 October 4 - 11, 2017 Chief Scientist - Matthew Mills ( <a href="mailto:mmmills@stanford.edu">mmmills@stanford.edu</a> ) See more cruise information from R2R: <a href="https://www.rvdata.us/search/cruise/SP1727">https://www.rvdata.us/search/cruise/SP1727</a>

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## Project Information

### Collaborative Research: Biogeochemical significance of the abundant, uncultivated symbiotic cyanobacteria UCYN-A (BSUCS)

**Coverage:** California Current waters off the Southern California shelf

#### NSF Award Abstract:

Nitrogen is a nutrient whose availability limits growth and productivity of ecosystems. Nitrogen is extremely abundant in the atmosphere in the inert form of gaseous N<sub>2</sub>, but most organisms cannot reduce N<sub>2</sub> into a biologically available form. In all environments, including agricultural soils, there are microorganisms that can make available the N from gaseous N<sub>2</sub> by reducing it to the biologically available form, ammonium. In the vast expanses of the open ocean, few organisms are known to have this ability, and recently a unique symbiosis between a single-celled cyanobacterium and a single-celled algae was discovered, which appears to be very widely distributed and likely of global biogeochemical significance. The cyanobacterium in this symbiotic partnership has very unusual metabolism and genomic streamlining. Little is known of the symbiosis because it is not detectable except by modern molecular biological techniques. Recent work has shown this symbiosis to be very widely spread through the oceans, and that there is previously unrecognized diversity in both the cyanobacterial and algal hosts. This research will examine the environmental distributions and the biogeochemical significance of this diversity in coastal US waters. The investigators will engage the public in ocean sciences through internship programs at local high schools and for undergraduate students at Stanford, and by documenting their field research in a 'virtual cruise' blog.

In the marine environment, the contribution of N<sub>2</sub> fixation to the fixed nitrogen (N) pool is poorly quantified, in part due to an incomplete understanding on the abundance, activity, and physiology of diazotrophs. The symbiotic unicellular cyanobacteria (UCYN-A) is a poorly characterized, yet globally important, group of marine diazotrophs. UCYN-A is widely distributed in the marine environment, and lives symbiotically with a picoeukaryotic prymnesiophyte alga. We now know that there are multiple ecotypes of UCYN-A, which may be adapted to specific locations in the water-column and different oceanic provinces. Typically N<sub>2</sub> fixation was considered unimportant in coastally influenced and non-tropical waters, however recent data shows that multiple subclades of UCYN-A are present. The distribution and rate of N<sub>2</sub> fixation by UCYN-A subclades in coastal/nearshore environments is a major unknown in the oceanic N cycle. Its presence in nearshore waters may change the paradigm of the balance between basin N sources (N<sub>2</sub> fixation) and sinks (denitrification).

Likewise, significant N<sub>2</sub> fixation by UCYN-A will need to be considered when determining estimates of new production in coastally influenced waters. This project aims to quantify the significance of different UCYN-A subclades to coastal/nearshore N budgets. It tackles the issue of determining N<sub>2</sub> fixation rates by different UCYN-A subclades in coastal waters through rigorous fieldwork off the west coast of North America. The temporal and spatial distribution of UCYN-A subclades, as well as the rates of N<sub>2</sub> fixation, will be determined by coupling N<sub>2</sub> fixation measurements of bulk communities and individual cells (nanoSIMS) with molecular assays to study these widespread, but dilute, diazotrophic symbionts and their hosts. Additionally the investigators will conduct experiments aimed at constraining the effects of light and nutrient ratios (N/P) on UCYN-A N<sub>2</sub> fixation rates, and the prymnesiophyte host's rate of carbon fixation. They will conduct this work through seasonal sampling of a coastal site in the Southern California Bight (Scripps Pier) and on two process cruises in the coastal waters between central California and the Baja Peninsula. The cruise work will provide an opportunity to understand the temporal dynamics of the UCYN-A/prymnesiophyte associations over larger spatial scales. Finally, evidence suggests that unidentified UCYN-A subclades and hosts exist and the investigators have developed a strategy to identify and quantify their temporal and spatial distributions as well as their N<sub>2</sub> fixation activities. Data on the coastal distribution, ecology and activity of UCYN-A is critical for obtaining a better understanding of their contribution to fixed N to the marine environment. The group-specific and bulk rates of N<sub>2</sub> fixation measured in this study of coastally influenced waters, will provide data for future modeling efforts, which will make an important contribution to constraining oceanic N<sub>2</sub> fixation inputs.

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1559165</a>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1559152</a>

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