Extracted chlorophyll data, particulate carbon and nitrogen, flow cytometry and 15N2/13C rate measurements collected during EN596 from April 2017 in the North Atlantic

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Project
» EAGER: Collaborative Research: Detection limit in marine nitrogen fixation measurements - Constraints of rates from the mesopelagic ocean (EAGER NitFix)

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Table of Contents

Methods & Sampling

15N Nitrogen Fixation, 13C Primary Production:
Standard protocols were used to measure in vitro primary productivity via the 13C radiotracer method [Lengendre et al., 1996] and N2-fixation rate measurements via a modified bubble release 15N2 method modified from Montoya et al [1996]. Briefly, water samples were collected before dawn in 4.3L polycarbonate bottles, a plug of 4ml of 15N2 gas was injected through a septa cap using a gas tight syringe. Bottles were then inverted for 15 minutes using a gas sample tumbler to assist in bringing the gas into solution. Following mixing the bubble was released and a Tinitial was collected for 15N2 enrichment. Bottles were then topped off using water for the same depth, sealed with caps fitted with septum and injected with 0.5ml of 47mM 13C bicarbonate stock and gently mixed by inversion. Samples for 13C-DIC to test the 13C enrichment concentration were taken by removing 1ml of enriched sample volume and injecting it into an evacuated exetainer pre-loaded with 85% phosphoric acid. Bottles were incubated on deck in incubators screened to 48% of surface irradiance and plumbed with flow through surface seawater for temperature regulation. All bottles were incubated in the incubator for ~ 24hr. Duplicate light bottles were paired with dark bottles and time final bottles that received no enrichment. Time zero bottles were also collected and sacrificed for each station. Natural abundance of dissolved 15N2 samples were also collected at each station. Following dawn to dawn incubations, a sample for Tpost 15N2 enrichment was collected from each enriched bottle and the remaining volume was filtered onto a combusted 25mm glass fiber filter, filters were folded in half and placed in combusted aluminum foil and stored at -80C for later analysis. Post-cruise, all filters were thawed and dried overnight at 60C. Samples were then balled into Ag and Sn boats and plated for analysis. The carbon and nitrogen isotopic composition (13C and 15N, respectively) were analyzed by continuous-flow isotope ratio mass spectrometry using a Carlo Erba elemental analyzer (EA) connected to a Thermo DeltaPlus isotope ratio mass spectrometer (IRMS). For all samples the international standards USGS40 (d13C = -26.389 permil vs VPDB, d15N = -4.52 permil vs air) and USGS41 (d13C = +37.626permil vs VPDB, d15N = +47.6 permil vs air) were used to calibrate each run of samples and the international standard IAEA-600 (d13C = -27.771 permil vs

VPDB, d15N = +1.0 permil vs air) was used as a check standard (i.e., not used to calibrate data).13C-DIC samples were sent to UC Davis for analysis, resultant data was used to correct the 13C DIC addition for each bottle.

Diazotroph Abundance:
At select stations where N2 Fixation measurements were conducted, the entire volume of a Niskin bottle at 25m was gravity filtered through a 47-mm diameter, 10 micron pore size, black polycarbonate filter with a polyester drain disk as a backing filter. If the volume was not filtered after 2 hours, filtration was terminated and the remaining volume of the carboy was measured in order to calculate volume filtered. Following filtration, filter holders were fit with a short section of tubing and a syringe leur fitting on once side and a 2-way valve on the outflow side. For each filter, 5-ml of 2% glutaraldehyde was slowly injected onto the filter and samples were allowed to fix for 30 minutes. Fixative was drained after this time and 60ml of air was used to flush all filters. Polycarbonate filters were then mounted onto 3x2 glass slides with immersion oil, cover slides were added and the edges of each cover slip was sealed with quick dry nail polish. All slides were stored at -20C and counted within 30 days. Enumeration of diazotrophic taxa was performed using epifluorescence microscopy.The entire slide was counted for DDAs and Trichodesimum abundance. Endosymbiont bearing diatoms of the following genus were enumerated: Rhizosolenia, Hemiaulus, Climacodium and Chaetoceras. Free Richelia intracellularis were also counted. Trichodesmium filaments were counted and the length of each filament was recorded. Trichodesmium cell number was then calculated by dividing the filament length by the mean cell length (9.9 micron +/-2.5 micron).

Flow Cytometry Bacteria:
Flow cytometry samples were collected from rosette nisken bottles at specific depths where 3ml of sample volume was pipette into cryovials, preserved by adding 60ul of 2% paraformaldehyde, allowed to sit in the dark for 10 minutes then stored in a -80C until analysis. Post cruise, the samples were thawed in the dark, a 250ul aliquot of sample was removed and added to 250ul Deionized water and 45ul 300mM Potassium citrate, 5ul of SYBR Green stain and 25ul of 1um beads were added, samples were run on a Becton Dickinson FACSCalibur flow cytometer using the methods adpated from Marie, et al 1997. All values are reported in cells/L, NaN are no data.

Flow Cytometry-Phytoplankton:
Flow cytometry samples were collected from rosette nisken bottles at specific depths where 3ml of sample volume was pipette into cryovials, preserved by adding 60ul of 2% paraformaldehyde, allowed to sit in the dark for 10 minutes then stored in a -80C until analysis. Post cruise, the samples were thawed in the dark, a 500ul aliquot was removed and 3um beads were added post run, samples were run for 3mins on a Becton Dickinson FACSCalibur flow cytometer using the methods outlined in Sherr, et al 2002.

Fluorometric Chlorophyll:
Fluorometric Chlorophyll were collected from rosette nisken bottles at specific depths and filtered at low vacuum through a glass fiber filter. Samples were then flash frozen in liquid nitrogen and stored in a histoprep capsule at -80C until analysis. Post cruise, the filters were extracted using 90% acetone for a total of 48 hours in a -20C freezer, covered to prevent the photodegradation of pigments. Upon extraction samples were centrifuged to settle filter particulates and gently poured into a glass cuvette. Samples were then run on a Turner 10-AU Fluorometer. Data was corrected for average glass fiber filter blank values and where duplicate samples were taken the average and standard deviation are reported. NaN are no data, duplicates not taken.

Particulate Carbon (PC) and Particulate Nitrogen (PN):
The carbon, and nitrogen content of particulate material (PC, PN) was collected from rosette nisken bottles at specific depths and filtered at low vacuum through a combusted glass fiber filter (PCPN). Following filtration, filters for PCPN were folded in half and placed in combusted aluminum foil and stored at -80C for later analysis. Post-cruise, all filters were thawed, acidified for a minimum of 12 hours and dried overnight at 60C. Samples were then balled into Ag and Sn boats and plated for analysis. Organic carbon and total nitrogen content was analyzed using high-temperature combustion (1020 C) on a ThermoQuest NC 2500 elemental analyzer. Three standards with known %C and %N were weighed, folded in tin boats, and analyzed with the samples to develop the calibration equation for each run. The calibration equation was then used to calculate the mass (mg) of carbon and nitrogen in each sample using EAS Clarity Chromatography Station software. The standards are Cystine (Elemental Microanalysis, B2035, 11.66 %TN, 29.99 %TC), Atropine (Elemental Microanalysis, B2002, 4.84 %TN, 70.56%TC), and GoniLab Secondary Standard (low %C)-A1 (an internal standard of sediment from the Gulf of Mexico, MV0803, with low carbon - 0.12 %N, 1.3 %C).

[table of contents | back to top]

Parameters

Parameter	Description	Units
Julian_day	Julian day of station and cast	unitless
Station	station number	unitless
Cast	cast number	unitless
Depth	sample depth	meters
Nisken	nisken bottle number	unitless
chl_avg	average chlorophyll concentration	micrograms per liter (ug/L)
chl	chlorophyll concentration	micrograms per liter (ug/L)
chl_std	standard deviation of chlorophyll concentration	micrograms per liter (ug/L)
fix_rate_13C_avg	Average 13C Fixation Rate	milligrams Carbon per cubic meter per day (mg C/m3/day)
fix_rate_15N2_avg	Average 15N Fixation Rate	nanomole per liter per day (nmol/L/day)
fix_rate_13C_std_dev	standard deviation of 13C Fixation Rate	milligrams Carbon per cubic meter per day (mg C/m3/day)
fix_rate_15N2_std_dev	standard deviation of 15N Fixation Rate	nanomole per liter per day (nmol/L/day)
HNA	High Nucleic Acid	cells per liter (cells/L)
LNA	Low Nucleic Acid	cells per liter (cells/L)
tot_bact	sum of High Nucleic Acid and Low Nucleic Acid	cells per liter (cells/L)
PC_avg	Particulate Carbon	micromole Carbon (umol C)
PC_std	Particulate carbon standard deviation	micromole Carbon (umol C)

PN_avg	Particulate Nitrogen	N/L
PN_std	Particulate Nitrogen standard deviation	N/L
Picoeukaryotes	Picoeukaryotes	cells per liter (cells/L)
Prochlorococcus	Prochlorococcus	cells per liter (cells/L)
Richella	Free Richella intracellularis	heterocysts per liter (heterocysts/L)
SAL	salinity	psu
SST	sea surface temperature	degrees Celsius
Synechococcus	Synechococcus	cells per liter (cells/L)
tot_diaz	abundance of large diazotrophs	cells per liter (cells/L)
trico	Trichodesmium cell counts	cells per liter (cells/L)
cells_m3	total cell count	cells per meter cubed (clls/m3)
Latitude	latitude in decimal degrees. Positive incated north.	decimal degrees
Longitude	longitude in decimal degrees. Positive indicates East.	decimal_degrees

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Carlo Erba elemental analyzer (EA)
Generic Instrument Name	Carlo-Erba NA-1500 Elemental Analyzer
Dataset-specific Description	The carbon and nitrogen isotopic composition (13C and 15N, respectively) were analyzed by continuous-flow isotope ratio mass spectrometry using a Carlo Erba elemental analyzer (EA) connected to a Thermo DeltaPlus isotope ratio mass spectrometer (IRMS).
Generic Instrument Description	A laboratory instrument that simultaneously determines total nitrogen and total carbon from a wide range of organic and inorganic sediment samples. The sample is completely and instantaneously oxidised by flash combustion, which converts all organic and inorganic substances into combustion products. The resulting combustion gases pass through a reduction furnace and are swept into the chromatographic column by the carrier gas which is helium. The gases are separated in the column and detected by the thermal conductivity detector which gives an output signal proportional to the concentration of the individual components of the mixture. The instrument was originally manufactured by Carlo-Erba, which has since been replaced by Thermo Scientific (part of Thermo Fisher Scientific). This model is no longer in production.

Dataset-specific Instrument Name	ThermoQuest NC 2500 elemental analyzer
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	Organic carbon and total nitrogen content was analyzed using high-temperature combustion (1020 C) on a ThermoQuest NC 2500 elemental analyzer.
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	epifluorescence microscopy
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Enumeration of diazotrophic taxa was performed using epifluorescence microscopy.
Generic Instrument Description	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.

Dataset-specific Instrument Name	Thermo DeltaPlus isotope ratio mass spectrometer (IRMS)
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	The carbon and nitrogen isotopic composition (13C and 15N, respectively) were analyzed by continuous-flow isotope ratio mass spectrometry using a Carlo Erba elemental analyzer (EA) connected to a Thermo DeltaPlus isotope ratio mass spectrometer (IRMS)
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	nisken bottles
Generic Instrument Name	Niskin bottle
Dataset-specific Description	rosette nisken bottles
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

[table of contents | back to top]

Deployments

EN596

Website	https://www.bco-dmo.org/deployment/774354
Platform	R/V Endeavor
Start Date	2017-04-25
End Date	2017-04-30

[table of contents | back to top]

Project Information

EAGER: Collaborative Research: Detection limit in marine nitrogen fixation measurements - Constraints of rates from the mesopelagic ocean (EAGER NitFix)

Coverage: North Atlantic Ocean, Pacific Ocean

NSF Award Abstract:
The availability of nitrogen is required to support the growth and production of organisms living in the surface

of our global ocean. This element can be scarce. To alleviate this scarcity, a special class of bacteria and archaea, called nitrogen fixers, can derive the nitrogen needed for growth from nitrogen gas. This project would carefully examine one specific method for measuring nitrogen fixation that has been used recently to suggest the occurrence of small amounts of nitrogen fixation in subsurface ocean waters. If these reports are verified, then a revision of our understanding of the marine nitrogen cycle may be needed. The Ocean Carbon and Biogeochemistry program will be used as a platform to develop community consensus for best practices in nitrogen fixation measurements and detection of diversity, activity, and abundances of the organisms responsible. In addition, a session will be organized in a future national/international conference to communicate with the broader scientific community while developing these best practices.

The goal of this study is to conduct a thorough examination of potential experimental and analytical errors inherent to the 15N2-tracer nitrogen fixation method, in tandem with comprehensive molecular measurements, in mesopelagic ocean waters. Samples will be collected and experimental work conducted on a cruise transect in the North Atlantic Ocean, followed by analytical work in the laboratory. The specific aims of this study are to (1) determine the minimum quantifiable rates of 15N2 fixation based on incubations of mesopelagic waters via characterization of sources of experimental and analytical error, and (2) seek evidence of presence and expression of nitrogen fixation genes via comprehensive molecular approaches on corresponding samples. The range of detectable rates and diazotroph activity from the measurements made in this study will be informative for the understanding of the importance of nitrogen fixation in the oceanic nitrogen budget.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1732206

[table of contents | back to top]