Hemiaulus-Richelia physiological response to different nitrogen sources

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Project
» META-DDA: METabolic Activities of Diatom-Diazotroph Associations (META-DDA)

Contributors	Affiliation	Role
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Abstract
Diatom-diazotroph associations (DDAs) play an important role at the base of the food web by fixing both carbon dioxide and nitrogen gas into organic matter. The ability of DDAs to fix nitrogen allows the relatively large diatom host to survive under nitrogen-deplete conditions and, thus, contribute significantly to organic carbon export in oligotrophic waters. Yet, relatively little is known about this symbiosis, in part because it has historically been difficult to maintain DDAs in culture. Here, we isolated the DDA Hemiaulus-Richelia from the Sargasso Sea and developed a protocol to maintain it in laboratory culture for long time periods (years). Experiments were conducted to determine how Hemiaulus-Richelia responds to different nitrogen sources with measurements of growth and nitrogen fixation.

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Coverage

Spatial Extent: **Lat**:32.42 **Lon**:-63.48
Temporal Extent: 2018-05 - 2022-05

Methods & Sampling

Samples were collected from culture flasks of a *Hemiaulus-Richelia* diatom diazotroph association (DDA) over the course of 2.5 weeks. Sargasso Seawater collected on the R/V Atlantic Explorer AE1812 cruise in May 2018 (at 32.42°N, 63.48°W) was used for media and filtered via peristaltic pump over 0.2 micrometers (µm) 47-millimeter (mm) filters (Polyethersulfone filters Millipore Express PLUS #GPWP04700). All flasks were grown in f/5 media without nitrogen, one set of flasks had no added nitrogen (Only N2), one set 10 µM added ammonium treatment (+NH4), and one set 10 µM added nitrate treatment (+NO3). Flasks were grown at 22 degrees Celsius (°C), ~130 micromoles per square meter per second (µmol m-2 s-1) in a 12:12 light:dark

cycle. Two time points were used, an initial (T=0) and a final (T=15,17,18,19, depending on flask growth). Samples from the culture innoculum (Innoc1, Innoc2, Innoc3) were collected before all three innoculum flasks were thoroughly mixed and added to the experiment flasks.

Total chlorophyll a fluorescence was sampled by filtering over GF/F (~0.7 µm Whatmanfilters) and immediately extracted using 90% acetone over 24 hours (Strickland & Parsons, 1968). Total chlorophyll a, phaeophytin, and relative fluorescence were measured daily using a 10 AU fluorometer (Turner). FRR (Fv_Fm and sigma) was measured using a FIRe Fluorometer System (Satlantic) (Kolber et al., 1998) with settings of 100 microseconds (µs) Single Turnover Flash (STF), 80 µs STRI, 20 µs MTF, 40 µs MTRP, 100 µs MTRI, and with gain adjusted based on the fluorescence yield. Samples were preserved for cell counts during the experiment by preserving with 0.125% gluteraldehyde, flash freezing, and storing at -80°C. Samples were then thawed and aliquoted onto a Sedgewick Rafter (PYSER-SGI) and counted. Cells were counted on an Eclipse E800 (Nikon) light microscope, with phycoerythrin emission (565 nanometers (nm) ± 40 nm) and excitation (530 nm ± 30 nm) wavelength filters to count the diazotroph symbionts. Cell size was averaged using at least 30 pictures each of the host and symbiont per flask, and measured using ImageJ and an image of a stage micrometer (OMAX A36CALM1 0.01 mm) at the same magnification of cell pictures. Cell volume and surface area were calculated using 29-H (*Hemiaulus hauckii*) and 1-H shaped cells (*Richelia euintracellularis*) equations from Sun and Liu (2003). Dissolved and particulate nutrients were sampled from the same ~100 milliliters (mL) aliqout (Part_vol_mL). Particulate nutrients were filtered out using pre-combusted GF/F (~0.7 µm) Whatman filters. Dissolved nutrients (NO3_uM, NO2_uM, NH4_uM, SiOH4_uM, and PO4_uM) were immediately frozen before measuring on a Seal Analytical AA3 nutrient autoanalyzer and total reduced nitrogen (TDN_uM) was analyzed by oxidizing all nitrogen to nitrate following a persulfate odixation method (Knapp et al., 2005). Particulate nutrients (PC and PN) were immediately frozen until processing, during which samples were dried at 60 °C for 24 hours, packed in 9x10mm tin capsules (Costech) and sent for analysis on the Carlo Erba NC 2500 Elemental Analyzer (with a Costech zero-blank autosampler) at the Central Appalachians Stable Isotope Facility (CASIF) at the University of Maryland.

Carbon and nitrogen fixation measurements were taken using ^{13}C and ^{15}N stable isotope incubations (Hama et al. 1983, Montoya et al. 1996) during the last 24 hours of the experiment (T=Final), following methods from White et al. (2020) and Klawonn et al. (2015). Sample aliqouts (~550 mL) were added to 630 mL polycarbonate bottles (Nalgene), with 214 mL of 0.05 grams per milliliter (g/mL) of $H_{13}CO_3$ (Sodium Bicarbonate, 13C 99% Cambridge Isotopes) for a final concentration of 200 µM and 63 mL $^{15}N_2$ solution using Cambridge Isotopes $^{15}N_2$, 98%+ (Lot No. I-24583/AR0483820) for a final $^{15}N_2$ dilution of 10% v/v. The $^{15}N_2$ (Cambridge Isotopes 15N2 98%+, Cat # NLM-363-1-LB, Lot # 1-24583/AR0483820) solution added to each flask was made using the dissolution method (Mohr et al. 2010; Klawonn et al. 2015) and letting serum bottles with dissolved gas sit for ~12 hours before the start of the incubation to allow more $^{15}N_2$ to move into solution. After 24 hours, 10 mL of incubation sample was collected through the bottle septum caps, added to helium-flushed 20 mL vials with 20 mm rubber butyl septa crimp caps (Sigma-Aldrich) for % $^{15}N_2$ dissolved gas analysis. A 50% solution of $ZnCl_2$ was added to each 20 mL vial for sample preservation and vials were stored upside down, submerged in DI water before sending for sample analysis at the UC Davis Stable Isotope Facility for analysis on the GasBench-Precon-IRMS for $^{15}N_2/N_2$ atom % measurements. After dissolved gas samples were collected from incubation bottles, 225-275 mL isotope incubation volume was filtered over pre-combusted GF/F (~0.7 µm) filters (Whatman), which were then immediately frozen and processed in the same way as particulate samples before sending to the CASIF at the University of Maryland (UMD) for isotope analysis ($d^{15}N$ and $d^{13}C$) on a Thermo Fisher Delta V+ isotope ratio mass spectrometer interfaced with the Carlo Erba NC 2500 Elemental Analyzer. One sample (NegCon) was incubated with a diatom (*Thalassiosira pseudonana* - f/2 media) with no nitrogen fixers to test for contamination ($^{15}NO_3$ or $^{15}NH_4$) in the $^{15}N_2$ gas stock, from which there was no indication of contamination.

The culture used in this experiment was isolated in 2018; these data were collected in 2022.

Data Processing Description

R v4.3.0 was used for all isotope incubation calculations for carbon (pp_rate_ugCperLperday) and nitrogen fixation (NFR_nmolNperLperday).

BCO-DMO Curation Notes

- Imported original file "Meta-DDA_NitAdd_2022.csv" into the BCO-DMO system.

- Marked "NA" as a missing data value; note that missing data are empty/blank in the final CSV file.
- Saved the final file as "921924_v1_dda_nutrient_acquisition.csv".

Problem Description

Any data not collected were originally indicated by "NA" in the data file. In the final CSV file available here, "NA" has been removed, so blanks/empty values indicate "no data".

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Data Files

File	
921924_v1_dda_nutrient_acquisition.csv	(Comma Separated Values (.csv), 8.55 KB) MD5:bb81bddfc288c5b64ca0b93642679982
Primary data file for dataset ID 921924, version 1	

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Related Publications

Hama, T., Miyazaki, T., Ogawa, Y., Iwakuma, T., Takahashi, M., Otsuki, A., & Ichimura, S. (1983). Measurement of photosynthetic production of a marine phytoplankton population using a stable 13C isotope. Marine Biology, 73(1), 31–36. https://doi.org/10.1007/bf00396282 https://doi.org/10.1007/BF00396282
Methods

Klawonn, I., Lavik, G., Böning, P., Marchant, H. K., Dekaezemacker, J., Mohr, W., & Ploug, H. (2015). Simple approach for the preparation of 15−15N2-enriched water for nitrogen fixation assessments: evaluation, application and recommendations. Frontiers in Microbiology, 6. https://doi.org/10.3389/fmicb.2015.00769
Methods

Knapp, A. N., Sigman, D. M., & Lipschultz, F. (2005). N isotopic composition of dissolved organic nitrogen and nitrate at the Bermuda Atlantic Time-series Study site. Global Biogeochemical Cycles, 19(1). doi:10.1029/2004gb002320
Methods

Kolber, Z. S., Prášil, O., & Falkowski, P. G. (1998). Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1367(1-3), 88–106. doi:10.1016/s0005-2728(98)00135-2
Methods

Mohr, W., Großkopf, T., Wallace, D. W. R., & LaRoche, J. (2010). Methodological Underestimation of Oceanic Nitrogen Fixation Rates. PLoS ONE, 5(9), e12583. https://doi.org/10.1371/journal.pone.0012583
Methods

Montoya, J. P., Voss, M., Kahler, P., & Capone, D. G. (1996). A Simple, High-Precision, High-Sensitivity Tracer Assay for N(inf2) Fixation. Applied and Environmental Microbiology, 62(3), 986–993. https://doi.org/10.1128/aem.62.3.986-993.1996
Methods

Strickland, J.D.H and Parsons, T.R. (1968) A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada Bulletin 167, 71-75 [as seen in The Quarterly Review of Biology (1969) 44(3), 327–327. doi:10.1086/406210]
Methods

Sun, J., Liu, D. (2003). Geometric models for calculating cell biovolume and surface area for phytoplankton. Journal of Plankton Research, 25(11), 1331–1346. doi:10.1093/plankt/fbg096
Methods

White, A. E., Granger, J., Selden, C., Gradoville, M. R., Potts, L., Bourbonnais, A., Fulweiler, R. W., Knapp, A. N.,

Mohr, W., Moisander, P. H., Tobias, C. R., Caffin, M., Wilson, S. T., Benavides, M., Bonnet, S., Mulholland, M. R., & Chang, B. X. (2020). A critical review of the 15N2 tracer method to measure diazotrophic production in pelagic ecosystems. Limnology and Oceanography: Methods, 18(4), 129–147. Wiley. https://doi.org/10.1002/lom3.10353
Methods

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Parameters

Parameter	Description	Units
Flask	Either flask (F1-F16, Innoc1-3) or sample id (NegCon)	unitless
Time_gp	Time point group (either initial or final)	unitless
Timepoint	Day each sample was collected	days
Treatment	Type of nutrient addition treatment: either no added nitrogen (Only N2, control), 10 uM added ammonium (+NH4), or 10 uM added nitrate (+NO3). Innoculum cultures are indicated with Innoc, and the isotope incubation negative control is indicated with NegCon.	unitless
RFU_Turner	chlorophyll a relative fluorescence	relative fluorescence units
chla	total chlorophyll a concentration	micrograms per cell (ug/cell)
phaeophytin	phaeophytin concentration	micrograms per cell (ug/cell)
Hemiaulus_cellspermL	Cell counts of Hemiaulus hauckii	cells per milliliter (cells/mL)
Richelia_cellspermL	Cell counts of Richelia intracellularis	cells per milliliter (cells/mL)
Asymb_cellspermL	Cell counts of Hemiaulus hauckii host cells without symbionts	cells per milliliter (cells/mL)
Hemaiulus_mean_SA	mean surface area of Hemiaulus hauckii cells	square micrometers (um^2)
Hemaiulus_mean_vol	mean volume of Hemiaulus hauckii cells	cubic micrometers (um^3)
Richelia_mean_SA	mean surface area of Richelia intracellularis cells	square micrometers (um^2)

Richelia_mean_vol	mean volume of Richelia intracellularis cells	cubic micrometers (um^3)
NO3_uM	dissolved nitrate concentration	micromolar (uM)
NO2_uM	dissolved nitrite concentration	micromolar (uM)
NH4_uM	dissolved ammonium concentration	micromolar (uM)
SiOH4_uM	dissolved silicate concentration	micromolar (uM)
PO4_uM	dissolved phosphate concentration	micromolar (uM)
TDN_uM	total dissolved nitrogen concentration	micromolar (uM)
Part_vol_mL	volume of sample filtered for particulate nutrients	milliliters (mL)
PC	particulate carbon	micromolar (uM)
PN	particulate nitrogen	micromolar (uM)
Fv_Fm	FRR variable fluorescence (Fv/Fm) or PSII photosynthetic efficiency	unitless
sigma	FRR PSII Absorption cross-section	square Angstroms per quanta (Å^2 (quanta)^-1)
d15N	delta 15N	parts per thousand (ppt)
massN_mg	mass of particulate N for delta 15N samples	milligrams (mg)
d13C	delta 13C	parts per thousand (ppt)
massC_mg	mass of particulate C for delta 13C samples	milligrams (mg)
atperc_15N2_N2	atom % of 15N2/N2 dissolved gasses	percent
vol_filt_L	volume of isotope incubation filtered	liters (L)

inc_time_min	duration of the isotope incubation experiments	minutes
pp_rate_ugCperLperday	carbon fixation rate	micrograms C per liter per day (ug C L^-1 d^-1)
NFR_nmolNperLperday	nitrogen fixation rate	nanomoles N per liter per day (nM N L^-1 d^-1)

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Instruments

Dataset-specific Instrument Name	Carlo Erba NC 2500 Elemental Analyzer
Generic Instrument Name	Carlo Erba NC 2500 elemental analyzer
Dataset-specific Description	Particulate nutrients were measured on a Carlo Erba NC 2500 Elemental Analyzer (with a Costech zero-blank autosampler).
Generic Instrument Description	A laboratory instrument that simultaneously determines total nitrogen, total hydrogen, and total carbon in a solid sample. The sample is completely and instantaneously oxidized 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 helium carrier gas. The gases are separated in the column and quantified or they can be introduced into another instrument, such as an isotope ratio mass spectrometer, for further analysis. The instrument was originally manufactured by CE instruments (formerly Carlo Erba) and has since been replaced by Thermo Scientific (part of Thermo Fisher Scientific). This model is no longer in production.

Dataset-specific Instrument Name	Thermo Fisher Delta V+ isotope ratio mass spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	Particulate isotope associated with isotope incubations were measured on a Thermo Fisher Delta V+ isotope ratio mass spectrometer interfaced with the Carlo Erba NC 2500 Elemental Analyzer. Both instruments are at the CASIF at the University of Maryland (UMD).
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	GasBench-Precon-IRMS
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	Samples for 15N2/N2 atom % were analyzed on the GasBench-Precon-IRMS at the UC Davis Stable Isotope Facility.
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	Costech zero-blank autosampler
Generic Instrument Name	Laboratory Autosampler
Dataset-specific Description	Particulate nutrients were measured on a Carlo Erba NC 2500 Elemental Analyzer (with a Costech zero-blank autosampler).
Generic Instrument Description	Laboratory apparatus that automatically introduces one or more samples with a predetermined volume or mass into an analytical instrument.

Dataset-specific Instrument Name	Eclipse E800 (Nikon) light microscope
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	A Sedgewick Rafter (PYSER-SGI), Eclipse E800 (Nikon) light microscope, and stage micrometer (OMAX A36CALM1 0.01 mm) were used for cell size and count measurements.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

Dataset-specific Instrument Name	FIRe Fluorometer System (Satlantic)
Generic Instrument Name	Satlantic Fluorescence Induction and Relaxation of Emission Spectrometer
Dataset-specific Description	The FIRe Fluorometer System (Satlantic) was used for FRR measurements.
Generic Instrument Description	The Satlantic FIRe (Fluorescence Induction and Relaxation) System is a bio-optical technology used to measure variable chlorophyll fluorescence in photosynthetic organisms. Based on the Fast Repetition Rate Fluorometry (FRRF) technique, the FIRe was developed in collaboration with Dr. Maxim Gorbunov and Dr. Paul Falkowski from Rutgers University. More information on FIRe (PDF).

Dataset-specific Instrument Name	Sedgewick Rafter (PYSER-SGI)
Generic Instrument Name	Sedgewick Rafter Counting Chamber
Dataset-specific Description	A Sedgewick Rafter (PYSER-SGI), Eclipse E800 (Nikon) light microscope, and stage micrometer (OMAX A36CALM1 0.01 mm) were used for cell size and count measurements.
Generic Instrument Description	Sedgewick Rafter Counting Chambers are transparent slides widely water analysis, culture inspection, and for other applications where particles per unit volume in fluid must be determined. The slide has a base that is ruled in one-thousand 1-millimeter squares. When a liquid is held in the cell by a coverglass, the grid subdivides 1 milliliter of liquid into 1 microliter volume.

Dataset-specific Instrument Name	10 AU fluorometer (Turner)
Generic Instrument Name	Turner Designs Fluorometer 10-AU
Dataset-specific Description	A 10 AU fluorometer (Turner) was used for fluorescence measurements.
Generic Instrument Description	The Turner Designs 10-AU Field Fluorometer is used to measure Chlorophyll fluorescence. The 10AU Fluorometer can be set up for continuous-flow monitoring or discrete sample analyses. A variety of compounds can be measured using application-specific optical filters available from the manufacturer (read more from Turner Designs, turnerdesigns.com, Sunnyvale, CA, USA).

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Project Information

META-DDA: METabolic Activities of Diatom-Diazotroph Associations (META-DDA)

Coverage: Oligotrophic and shelf waters

Diatom diazotroph associations (DDAs) have broad geographic distributions, provide bioavailable nitrogen to the biosphere via nitrogen fixation, affect ecosystem functioning and influence biogeochemical cycling. Despite the importance of these symbioses, little is known about their basic physiology and metabolism because DDAs are rarely brought into and kept in culture. Without cultured strains, it is difficult to study experimentally how the partners interact, share and potentially compete for resources. Here we evaluate and model key physiological characteristics of a DDA (Hemiaulus hauckii-Richelia intracellularis) isolated from the Sargasso Sea to investigate how their physiology is altered by nitrogen sources and temperature. The isolated strain is growing well, fixing nitrogen and can be manipulated in the laboratory, allowing for an unprecedented view into the physiology and metabolism of a biogeochemically important ocean symbiosis. Empirical data informs the development of quantitative cell flux model of DDAs (CFM-DDA) embedded into a simple ecosystem model to test how different nitrogen sources and temperatures shape the niche of DDAs. The physiology of DDAs is compared to asymbiotic diatoms to examine the conditions where diazotrophic symbionts benefit the host diatoms and allow them to expand their ecological niche. This research addresses fundamental knowledge gaps that will lead to an enhanced understanding of DDA distributions and activities both in today's ocean and in a future ocean with altered temperature and nutrient fields.

NSF Award Abstract:

Phytoplankton are photosynthetic microbes that inhabit the surface ocean, form the base of marine food webs, and drive the global cycling of elements like carbon and nitrogen. To survive in regions with limiting nutrients for growth, some phytoplankton have evolved symbiotic relationships. In many cases, it remains unknown how the symbioses influence the survival of each partner or their impacts on ecosystem function and cycling of nutrients. This project focuses on the symbiotic relationship between two phytoplankton -- a single-celled eukaryote diatom and a single-celled nitrogen fixing cyanobacteria called a diazotroph. These diatom-diazotroph associations (DDAs) have broad geographic distributions, provide bioavailable nitrogen to the biosphere via the fixation of nitrogen gas, affect marine food webs, and influence the cycling of carbon and nitrogen. Despite the importance of these symbioses, little is known about their basic physiology and metabolism because it has been difficult for researchers to grow DDAs in the laboratory. In this project, a team of investigators from the University of Rhode Island is applying a method they developed to grow DDAs in the laboratory and conducting experiments on the effects of temperature and nutrients on DDA cellular metabolism. The newly-generated laboratory data is informing the development of a computer model of DDA cellular functioning that embedded in a simple ecosystem model to test how different nitrogen sources and temperatures influence DDA ecology and ecosystem function. The project is addressing fundamental knowledge gaps, leading to an enhanced understanding of DDA geographic distributions and activities both in today's ocean and in a future ocean with altered temperature and nutrient fields. Broader impacts of this study include the provision of DDA cultures to the oceanographic community, graduate student training, computational model distribution, and outreach to the broader community. Graduate students supported by the project are being cross-trained in experimental and modeling approaches. Outreach to the broader community includes hosting a high school student intern in the lab each year and the development of educational videos for the general public and K-12 students. Collectively, these activities are designed to broaden the public understanding of DDAs, a globally significant symbiosis.

This project is examining the cellular metabolism and physiology of diatom-diazotroph associations (DDAs) and evaluating their ecosystem and biogeochemical impacts by addressing the following critical, longstanding questions: 1) What is the cellular response of the Hemiaulus DDA to different nitrogen sources? 2) How does the thermal niche of the DDA influence nitrogen fixation, nutrient stoichiometries, and geographic distribution? 3) How does DDA physiology and metabolism differ from asymbiotic diatoms, and what are the ecosystem-level impacts of the symbiosis? Due to a scarcity of culture data, major ecological models assume DDAs gain 100% of their nitrogen from N2, although there is intriguing experimental evidence suggesting otherwise. If DDA physiology is affected by different N sources, key assumptions in modern ecosystem models will be altered, refining our understanding of the role DDAs play in ecosystem and biogeochemical functioning. In addition to N sources, temperature is an important regulator of cellular metabolism and a key variable in ecosystem models. The team of researchers is examining the roles of each partner in the symbiosis in setting the DDA thermal niche and examining ecosystem-level impacts via modeling both in the present day and future oceans. Finally, the impacts of the endosymbiont on the host genome, transcriptome, and resulting physiology are practically unknown. Comparison of DDAs with asymbiotic diatoms is providing new insights into the metabolic modifications of the host and providing new understanding of DDAs as a symbiosis. Addressing these three questions advances fundamental understanding of the impact of this widely-distributed symbiosis.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2227425

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