Nitrogen fixation incubation data from cruise TN368 in July 2019 for SPIROPA project

Website: https://www.bco-dmo.org/dataset/928568
Data Type: Cruise Results, experimental
Version: 1
Version Date: 2024-05-23

Project
» Collaborative Research: Shelfbreak Frontal Dynamics: Mechanisms of Upwelling, Net Community Production, and Ecological Implications (SPIROPA)

Contributors	Affiliation	Role
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Abstract
Continental shelves contribute a large fraction of the ocean's new nitrogen (N) via N2 fixation; yet, we know little about how physical processes at the ocean's margins shape diazotroph biogeography and activity. This dataset includes incubation data and nitrogen fixation rates using water collected at the Mid-Atlantic Bight shelfbreak front in July 2019. Rates were measured using the 15N2 bubble release method on repeat cross-frontal transects. This study was coordinated with the SPIROPA ('Shelfbreak Productivity Interdisciplinary Research Operation at the Pioneer Array') Project (https://www.bco-dmo.org/project/748894). See Selden et al. (2024) JGR-Oceans or contact Dr. Corday Selden (crselden@marine.rutgers.edu) for further information.

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Coverage

Location: Mid-Atlantic Bight shelfbreak front to the south of Massachusetts, USA. Multiple transects in the area of 70-71 degrees W longitude and 39-40.5 degrees N latitude. Sampling was restricted to waters above the 0.1% light level.
Spatial Extent: **N**:40.5 **E**:-70 **S**:39 **W**:-71
Temporal Extent: 2019-07-05 - 2019-07-18

Dataset Description

This nitrogen fixation incubation data is a supplement to BCO-DMO dataset https://www.bco-dmo.org/dataset/927927, which is hydrographic, nutrient, and mean nitrogen fixation rate data from the SPIROPA project's third cruise. Bottle data from the TN368 cruise (Chief Sci McGillicuddy) can be viewed here: https://www.bco-dmo.org/dataset/849340.

Glossary of terms:
A = Atom-% enrichment
APN = Atom-% enrichment of the particulate nitrogen pool
BDL = Below detection limit
DNQ = Detectable but not quantifiable
LOD = Limit of detection
LOQ = Limit of quantification
N2 = Nitrogen gas
PETG = Polyethylene Terephthalate Glycol
PN = Particulate nitrogen
NFR = Nitrogen fixation rate
SUR = Specific uptake rate
t0 = time initial
tf = time final

Methods & Sampling

Nitrogen fixation rates and hydrographic data were measured in July 2019 in conjunction with the SPIROPA (Shelfbreak Productivity Interdisciplinary Research Operation at the Pioneer Array) project cruises (Chief Scientist Dr. Dennis McGillicuddy). This case study was conducted at the Mid-Atlantic Bight shelfbreak front to the south of Massachusetts, USA to examine whether significant N2 fixation occurs in the continental shelf region. On July 5th, 2019, the vessel R/V Thomas G. Thompson (cruise TN368) departed Woods Hole, MA and performed multiple transects in the area of 70 to 71 degrees West longitude and 39 to 40.5 degrees North latitude. Sampling was restricted to waters above the 0.1% light level.

Water was collected in 10L Niskin bottles on a 24-bottle rosette. Vertical conductivity, temperature, and depth profiles were measured concurrently using a Sea-Bird 911+ CTD profiler. Samples to determine nitrate, phosphate, and silicate concentrations were filtered (0.4 um, polycarbonate) directly from the Niskin bottles into acid-washed polyethylene bottles and stored frozen until analysis via standard colorimetric methods at the WHOI Nutrient Analytical Facility using a SEAL Analytical AA3 HR discrete chemistry analyzer. Detection limits for nitrate, phosphate, and silicate methods were 0.040, 0.030 and 0.009 µM, respectively.

This dataset presents the full incubation result with all replicates. The associated environmental (hydrographic) data is presented in a separate dataset along with the mean uptake and fixation rates. Please refer to BCO-DMO dataset https://www.bco-dmo.org/dataset/927927

Nitrogen fixation rate measurements (NFR) were quantified on repeat cross-frontal transects using the $^{15}N_2$ bubble release technique (Chang et al., 2019; Klawonn et al., 2015), which is a variant of the $^{15}N_2$ tracer method (Montoya et al., 1996) which accounts for the slow dissolution time of N_2 gas (White et al., 2020). The particular approach used for this study have been described in Selden et al. (2019), Selden, Chappell, et al. (2021), and Selden, Mulholland, et al. (2021). To measure $^{15}N_2$ uptake, triplicate PETG bottles (0.5-2 L) were rinsed with whole water then filled, ensuring that no bubbles remained. Using a gas tight syringe (VICI Precision Sampling), $^{15}N_2$ gas (~99%, Cambridge Isotope Laboratories) was added through a flexible silicon septa. Samples were gently rocked for 15 min as described by Selden et al. (2019). Any remaining bubble was subsequently removed to ensure constant isotopic enrichment over the incubation period, and bottles were incubated on-deck for approximately 24 hours. As the vessel moved across the front, a large temperature gradient was encountered (from 16°C to 26°C). To avoid temperature-shocking the samples during transit, we maintained three on-deck incubators that recirculated water through aquaria chillers (MC-1/10HP, AquaEuroUSA) housed in custom casings (built from King Starboard plastic sheet) for protection and maintained approximate temperature conditions for shelf, frontal, and offshore waters, respectively.

At the end of the incubation period, a 6 milliliter sample aliquot was collected from each bottle and transferred to a helium-purged Exetainer™ containing 50 µl zinc chloride (50% w/v). These samples were stored at room temperature and upside-down in 15 ml Falcon™ tubes, submerged in ultrapure water, until analysis at the UC Davis Stable Isotope Facility. The remaining incubation volume was then filtered for initial particulate nitrogen (PN) samples. Initial and final PN isotopic composition and concentration were analyzed at Old Dominion University on a Europa 20/20 isotope ratio mass spectrometer with an automated N and carbon preparation module. Low mass samples (<10 µg N) were excluded from downstream analysis as IRMS response can become non-linear at low mass (White et al., 2020).

Specific uptake rates were calculated following Montoya et al. (1996), with equations detailed below.

Data Processing Description

Specific N_2 uptake rates (SUR) were calculated following Montoya et al. (1996):

Equation 1: SUR = (APN_tf - APN_t0) / (AN2 - APN_t0)] x (1/time)

where A represents the atom-% enrichment of the initial (t=0) or final (t=f) particulate nitrogen (PN) pool, or the nitrogen (N_2) pool. Across all experiments, AN2 averaged 3.89 ± 2.0%. Experiments with <1% enrichment were excluded from downstream analysis. The specific uptake rate represents the relative contribution of diazotroph-derived N to PN turnover within a given sample. Absolute N_2 fixation rates (NFR) were calculated as:

Equation 2: NFR = SUR x [PN]

where [PN] indicates the particulate nitrogen concentration. Limits of detection and quantification were calculated per incubation by propagating the minimum detectable difference between initial and final ^{15}N atom-% enrichment of the PN pool (3σ and 10σ), three sigma and ten sigma respectively, n≥7, 12.5 µg N standards measured daily through Eqn. 2 (Ripp, 1996; White et al. 2020). A rate was only considered detectable if two of three replicate incubations yielded detectable rates.

BCO-DMO Processing Description

- Imported data from source file "Selden_JGR24_Nfix_rates_per_bottle.csv" into the BCO-DMO data system.
- Changed date format from m/d/yyyy to yyyy-mm-dd (ISO Date 8601 format) and converted datetimes from EST to UTC.
- Added latitude and longitude columns based on datetimes and depths.
- Added official cruise ID corresponding to the the provided deployment and cruise name
- Rounded values according to the PI's/submitter's indicated precision
- Modified parameter (column) names to conform with BCO-DMO naming conventions. The only allowed characters are A-Z,a-z,0-9, and underscores. No spaces, hyphens, commas, parentheses, or Greek letters.

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Related Publications

Chang, B. X., Jayakumar, A., Widner, B., Bernhardt, P., Mordy, C. W., Mulholland, M. R., & Ward, B. B. (2019). Low rates of dinitrogen fixation in the eastern tropical South Pacific. Limnology and Oceanography, 64(5), 1913–1923. Portico. https://doi.org/10.1002/lno.11159
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

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

Ripp, J. (1996). Analytical detection limit guidance and laboratory guide for determining method detection limits. Wisconsin Department of Natural Resources, Laboratory Certification Program. https://www.iatl.com/content/file/LOD%20Guidance%20Document.pdf
Methods

Selden, C. R., Chappell, P. D., Clayton, S., Macías-Tapia, A., Bernhardt, P. W., & Mulholland, M. R. (2021). A coastal N2 fixation hotspot at the Cape Hatteras front: Elucidating spatial heterogeneity in diazotroph activity

via supervised machine learning. Limnology and Oceanography, 66(5), 1832–1849. Portico. https://doi.org/10.1002/lno.11727
Results

Selden, C. R., Mulholland, M. R., Bernhardt, P. W., Widner, B., Macías-Tapia, A., Ji, Q., & Jayakumar, A. (2019). Dinitrogen Fixation Across Physico-Chemical Gradients of the Eastern Tropical North Pacific Oxygen Deficient Zone. Global Biogeochemical Cycles, 33(9), 1187–1202. doi:10.1029/2019gb006242 https://doi.org/10.1029/2019GB006242
Methods

Selden, C. R., Mulholland, M. R., Crider, K. E., Clayton, S., Macías-Tapia, A., Bernhardt, P., McGillicuddy, D. J., Zhang, W. G., & Chappell, P. D. (2024). Nitrogen Fixation at the Mid-Atlantic Bight Shelfbreak and Transport of Newly Fixed Nitrogen to the Slope Sea. Journal of Geophysical Research: Oceans, 129(4). American Geophysical Union (AGU). https://doi.org/10.1029/2023jc020651 https://doi.org/10.1029/2023JC020651
Results
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Methods

Selden, C. R., Mulholland, M. R., Widner, B., Bernhardt, P., & Jayakumar, A. (2021). Toward resolving disparate accounts of the extent and magnitude of nitrogen fixation in the Eastern Tropical South Pacific oxygen deficient zone. Limnology and Oceanography, 66(5), 1950–1960. Portico. https://doi.org/10.1002/lno.11735
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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Related Datasets

IsRelatedTo

McGillicuddy, D. J., Sosik, H. M., Zhang, W. G., Smith, W. O., Stanley, R., Turner, J., Petitpas, C. (2022) **Bottle sample data from CTD casts from the third cruise of SPIROPA project, R/V Thomas G. Thompson cruise TN368, to the New England Shelfbreak in July of 2019.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 2) Version Date 2022-06-08 doi:10.26008/1912/bco-dmo.849340.2 [view at BCO-DMO]

IsSupplementTo

Mulholland, M., Chappell, P. Dreux, Selden, C. (2024) **Hydrography, nutrients, and nitrogen uptake rates from SPIROPA cruise TN368 in July 2019.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-05-20 http://lod.bco-dmo.org/id/dataset/927927 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
spl_id	Unique identifier for individual N2 uptake incubation bottles; meant for internal lab use (may be of value to anyone who wishes to discuss specific samples with corresponding authors)	dimensionless

collection_date_EST	Datetime of sample collection in eastern standard time (EST)	dimensionless
latitude	Latitude of sampling site	decimal degrees
longitude	Longitude of sampling site	decimal degrees
cruise	Cruise identification	dimensionless
cast	Cast identifier for cruise TN368 on R/V T.G. Thompson	dimensionless
z_m	Depth of sample collection	meters (m)
time_hr	Duration of incubation	hours
vol_L	Incubation volume	liters (L)
apn_tf	Atom% 15N enrichment at final time point (i.e., at time equals incubation duration)	atom percent
pn_uM_tf	Particulate nitrogen concentration at final time point	micromoles nitrogen per liter (umol N2/L)
pnpc_flag	Flag indicating whether an independent sample was available to determine particulate nitrogen concentration at the time of sample collection; "NO_SPL" indicates that no sample was available; "INSUFFICIENT_N_MASS" indicates that the sample collected for initial PN determination did not have sufficient mass (>10 ug N) to allow for robust isotopic analysis	dimensionless
apn_t0	Atom% 15N enrichment at initial time point	atom percent
pn_uM_t0	Particulate nitrogen concentration at initial time point	micromoles nitrogen per liter (umol N2/L)
an2_flag	Flag indicating whether a subsample to directly determine 15N enrichment of the N2 pool in the specific incubation bottle was analyzed; "NO_AN2_SPL_RUN" indicates that the sample was lost, damaged, or otherwise unable to be analyzed	dimensionless
an2	Atom% 15N enrichment of N2 pool in incubation bottle; subsampled at final time point	atom percent

mindetdiff	The minimum detectable difference between the atom% enrichment at the initial and final time points of the incubation; calculated as 3 times the standard deviation of 7 replicate 12.5 ug N standards (sulfanilimide)	atom percent
apn_t0_flag	Flag indicates where the atom% 15N enrichment of the N2 pool came from; "MEAN_APNT0_USED_IN_CALC" indicates that the mean atom% enrichment achieved across experiments was used to calculate a rate; no flag indicates that a direct measurement was available and used	dimensionless
mass_flag	Flag indicates where the particulate nitrogen concentration used to calculate a N2 fixation rate came from; "Tf_MASS_USED" indicates that no data was available for the initial time point to calculate an average across the incubation, and concentration was instead assumed constant across the incubation (and the final time point concentration value was used); no value indicates that the average across the incubation period was used	dimensionless
pn_uM_used	The actual value for particulate nitrogen concentration used to calculate N2 fixation rate; where concentrations were available at both initial and final time points, the average was used	micromoles nitrogen per liter (umol N2/L/day)
sur	Specific N2 uptake rate	per day
nfr	N2 fixation rate	nanomoles nitrogen per liter per day (nmol N2/L/day)
lod	Limit of detection for N2 fixation rate at given sampling location	nanomoles nitrogen per liter per day (nmol N2/L/day)
loq	Limit of quantification for N2 fixation rate for given incubation experiment	nanomoles nitrogen per liter per day (nmol N2/L/day)
lod_sur	Limit of detection for specific N2 uptake rate for given incubation experiment	per day
loq_sur	Limit of quantification for specific N2 uptake rate for given incubation experiment	per day
det_flag	flag indicates whether N2 uptake was detectable and/or quantifiable; "BDL" indicates that it was below the detection limit; "DNQ" indicates that it was detectable but not quantifiable	dimensionless

ISO_DateTime_UTC_if_EST	Datetime of sampling in UTC (if original datetimes were EST or GMT-5)	unitless
ISO_DateTime_UTC_if_EDT	Datetime of sampling in UTC (if original datetimes were actually EDT or GMT-4)	unitless

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Instruments

Dataset-specific Instrument Name	AquaEuroUSA Aquaria chillers (MC-1/10HP)
Generic Instrument Name	Aquarium chiller
Dataset-specific Description	To avoid temperature-shocking the samples, three on-deck incubators recirculated water through aquaria chillers (MC-1/10HP, AquaEuroUSA) housed in custom casings.
Generic Instrument Description	Immersible or in-line liquid cooling device, usually with temperature control.

Dataset-specific Instrument Name	incubator
Generic Instrument Name	Incubator
Dataset-specific Description	To avoid temperature-shocking the samples, three on-deck incubators recirculated water through aquaria chillers.
Generic Instrument Description	A device in which environmental conditions (light, photoperiod, temperature, humidity, etc.) can be controlled. Note: we have more specific terms for shipboard incubators (https://www.bco-dmo.org/instrument/629001) and in-situ incubators (https://www.bco-dmo.org/instrument/494).

Dataset-specific Instrument Name	Europa 20/20 isotope ratio mass spectrometer with automated nitrogen and carbon preparation module
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	Initial and final PN isotopic composition and concentration were analyzed on a Europa 20/20 isotope ratio mass spectrometer with an automated N and carbon preparation module.
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	Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset-specific Description	A CTD rosette equipped with 24 10L Niskin bottles was used to sample water in the Mid Atlantic Bight
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.

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Deployments

TN368

Website	https://www.bco-dmo.org/deployment/848750
Platform	R/V Thomas G. Thompson
Start Date	2019-07-05
End Date	2019-07-18
Description	DOI: https://doi.org/10.7284/908710

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Project Information

Collaborative Research: Shelfbreak Frontal Dynamics: Mechanisms of Upwelling, Net Community Production, and Ecological Implications (SPIROPA)

Website: http://science.whoi.edu/users/olga/SPIROPA/SPIROPA.html

Coverage: Shelf break south of New England, OOI Pioneer Array

NSF award abstract:

The continental shelf break of the Middle Atlantic Bight supports a productive and diverse ecosystem. Current paradigms suggest that this productivity is driven by several upwelling mechanisms at the shelf break front. This upwelling supplies nutrients that stimulate primary production by phytoplankton, which in turn leads to enhanced production at higher trophic levels. Although local enhancement of phytoplankton biomass has been observed in some circumstances, such a feature is curiously absent from time-averaged measurements, both from satellites and shipboard sampling. Why would there not be a mean enhancement in phytoplankton biomass as a result of the upwelling? One hypothesis is that grazing by zooplankton prevents accumulation of biomass on seasonal and longer time scales, transferring the excess production to higher trophic levels and thereby contributing to the overall productivity of the ecosystem. However, another possibility is that the net impact of these highly intermittent processes is not adequately represented in long-term means of the observations, because of the relatively low resolution of the in-water measurements and the fact that the frontal enhancement can take place below the depth observable by satellite. The deployment of the Ocean

Observatories Initiative (OOI) Pioneer Array south of New England has provided a unique opportunity to test these hypotheses. The combination of moored instrumentation and autonomous underwater vehicles will facilitate observations of the frontal system with unprecedented spatial and temporal resolution. This will provide an ideal four-dimensional (space-time) context in which to conduct a detailed study of frontal dynamics and plankton communities needed to examine mechanisms controlling phytoplankton populations in this frontal system. This project will also: (1) promote teaching, training and learning via participation of graduate and undergraduate students in the research , (2) provide a broad dissemination of information by means of outreach in public forums, printed media, and a video documentary of the field work, and (3) contribute to improving societal well-being and increased economic competitiveness by providing the knowledge needed for science-based stewardship of coastal ecosystems, with particular emphasis on connecting with the fishing industry through the Commercial Fisheries Research Foundation.

The investigators will conduct a set of three cruises to obtain cross-shelf sections of physical, chemical, and biological properties within the Pioneer Array. Nutrient distributions will be assayed together with hydrography to detect the signature of frontal upwelling and associated nutrient supply. The investigators expect that enhanced nutrient supply will lead to changes in the phytoplankton assemblage, which will be quantified with conventional flow cytometry, imaging flow cytometry (Imaging FlowCytobot, IFCB), optical imaging (Video Plankton Recorder, VPR), traditional microscopic methods, and pigment analysis. Zooplankton will be measured in size classes ranging from micro- to mesozooplankton with the IFCB and VPR, respectively, and also with microscopic analysis. Biological responses to upwelling will be assessed by measuring rates of primary productivity, zooplankton grazing, and net community production. These observations will be synthesized in the context of a coupled physical-biological model to test the two hypotheses that can potentially explain prior observations: (1) grazer-mediated control and (2) undersampling. Hindcast simulations will also be used to diagnose the relative importance of the various mechanisms of upwelling. The intellectual merit of this effort stems from our interdisciplinary approach, advanced observational techniques, and integrated analysis in the context of a state-of-the-art coupled model. The project will address longstanding questions regarding hydrodynamics and productivity of an important ecosystem, leading to improved understanding of physical-biological interactions in a complex continental shelf regime. Given the importance of frontal systems in the global coastal ocean, it is expected that knowledge gained will have broad applicability beyond the specific region being studied.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1657803

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