MPn-derived methane production by epiphytic bacteria on pelagic Sargassum seaweed from 2017-2019 (Cyanobacteria Hydrocarbons project)

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Project
» Redox Cycling of Phosphorus in the Western North Atlantic Ocean (Phosphorus Redox Cycling)
» Collaborative Research: Do Cyanobacteria Drive Marine Hydrocarbon Biogeochemistry? (Cyanobacteria Hydrocarbons)
» Fall Semester Student Research in Oceanography and Marine Science at BIOS (Fall Student Research at BIOS)

Contributors	Affiliation	Role
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Abstract
The essential nutrient phosphorus is biologically scarce in the Sargasso Sea, yet the pelagic macroalgae Sargassum, for which this area of the North Atlantic Ocean is named, thrives. We tested the hypothesis that Sargassum holobionts utilize methylphosphonate (MPn) as an alternative source of phosphorus via bottle incubations, finding lysis liberated phosphonate-derived methane. The resulting data of methane production relative to various control conditions in the bottle system over time was used as an analog for MPn utilization, in line with previous studies. We noticed the methane production rates were ~linear for the first 2-3 days in each trial, after which production rates began to decrease, likely due to bottle effects. As such, the linear portion of methane production over time was used to calculate the anticipated MPn utilization rate in situ, where confounding variables induced by the experimental design would not impact the microbial activity. These MPn utilization rates are also reported, allowing for more appropriate comparison across trials and use in environmental impact estimates. The observed activity occurred at concentrations as low as 35 nM MPn and was inhibited by antibiotics, implicating microbial members of the holobiont capable of MPn lysis at realistic environmental concentrations. A survey of macroalgal species inhabiting the Sargasso Sea found a ubiquitous capacity for MPn lysis; such capacity was absent in species inhabiting phosphorus-replete waters of the California Current, pointing to phosphorus limitation as a selective pressure. These results suggest algal holobionts may conditionally acquire phosphorus from phosphonates while simultaneously serving as a source of atmospheric methane. Incubations of macroalgal holobionts were collected from surface waters (Sargassum spp) and shallow reefs (all other 2019) offshore 4km NE of Bermuda, while all 2017 macroalgal species were collected by scientific divers in shallow reefs offshore Santa Barbara, CA.

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Coverage

Temporal Extent: 2017-10-06 - 2019-10-09

Dataset Description

All samples reported collected via various small boat operations and scientific divers from UCSB (2017 trials) or BIOS (2018-2019 trials).

Methods & Sampling

Experiment Conditions and Details of Resulting Data

Pelagic Sargassum incubations were conducted at the Bermuda Institute of Ocean Sciences (BIOS) during the summers of 2018 and 2019. Samples were collected from 4 km northeast of Bermuda to ensure samples reflected offshore conditions. Following established protocols (Hanson, 1977; Lapointe, 1986; Schofield et al., 1998; Smith et al., 1973), Sargassum patches were collected via dip nets and placed in a cooler for transport back to land. The Sargassum samples were transferred to outdoor aquaria in full sunlight with flowing seawater taken from the adjacent bay to maintain ambient surface seawater temperatures until ready for use in incubations. These samples remained in the aquaria for no more than one full day before use and were only selected if in visibly good health.

For experiments, 1-2 g (wet weight) Sargassum samples (stipe, blades, and pneumatocysts) were clipped, rinsed to remove large macrofauna (Hanson, 1977; Lapointe, 1995), and placed into 150 mL borosilicate glass serum bottles. Serum bottles were then filled with 75 mL (in 2018) or 125 mL (in 2019) autoclaved seawater from the bay adjacent to BIOS, leaving sufficient headspace for subsampling. Note that this design eliminates the contribution of the pelagic bacterial community associated with the macroalgae. The components for specific treatments (including MPn, ammonium nitrate, sodium phosphate, and an antibiotic mixture containing 50 µg/mL ampicillin, 50 µg/mL kanamycin, 30 µg/mL nalidixic acid and 100 µg/mL streptomycin) were added from stock solutions, then the bottles were sealed with N-butyl rubber stoppers and crimp caps.

To determine how much MPn to add to treatments, we estimated the amount of MPn in the region (further details related to how MPn concentrations were determined can be found at the end of this Methods & Sampling section of this metadata page). Lomas et al., (2009) determined that 80% of total dissolved phosphorus (TDP) is in the form of dissolved organic phosphorus (DOP). The majority of natural MPn is found associated with high molecular weight dissolved organic matter and ~77% of the DOP in the Sargasso Sea exists as high molecular weight DOP (HMWDOP) (Sosa et al., 2020). MPn specifically accounts for 6.6-16.4% of this HMWDOP pool (Repeta et al., 2016; Sosa et al., 2020). Additionally, the North Atlantic has a C:P ratio of ~290-390:1 (Kolowith et al., 2001; Sosa et al., 2020). Assuming these relationships are temporally and regionally persistent, reported data for this area would imply a mean concentration of 13.7 nM MPn for surface water in the region. Sosa et al. (2020) saw ~10.7 nM MPn in the northwest Sargasso Sea (~66 nM HMWDOP * 0.165 nM MPn per nM HMWDOP), which provides confidence to our estimates. To ensure the signal was detectable using our methods, an MPn amendment concentration of 1,420 nM (~100 times greater) was used to connect all trials. Amendments of N and P macronutrients were added in ratios relative to the MPn amendment concentration (0.5:1, 1:1, 2:1, etc.).

An additional 30 mL of ambient lab air was injected into each bottle to minimize the generation of a vacuum from time series headspace sampling. Each bottle was analyzed for the initial methane concentration in the headspace. Headspace sampling was performed in small batches to minimize disruption of incubations and methane concentrations were measured via Shimadzu GC-FID 8A with N2 as the carrier gas (Kinnaman et al., 2007). A 3 mL headspace volume was taken from each bottle by syringe and injected into the GC-FID's gas injection manifold. The syringe was purged three times with N2 gas before headspace sample collection and between each following measurement. Data was recorded with a Shimadzu C-R68A, and the instrument was calibrated with a 3-point calibration curve at the beginning of each sampling period. Air for headspace replenishment at the end of each sampling point either came from a metal flask pressurized with air of known methane content or ambient lab air that was analyzed on the GC-FID prior to injection.

Each condition was comprised of 3-5 replicates depending on the trial. All replicate bottles per condition were placed in a clear plastic bag and incubated in the outdoor aquaria, exposed to ambient sunlight and temperature. Control conditions accompanied each experiment and included: i) bottles containing only autoclaved seawater, ii) bottles containing autoclaved seawater and 1420 nM MPn, and iii) bottles containing autoclaved seawater and ~2 g wet weight Sargassum to ensure the measured methane was due only to MPn interaction with algal holobiont. These were compared to bottles containing autoclaved seawater, ~2 g wet weight Sargassum, and a specified concentration of MPn and/or other macronutrients. Some experiments included a dark (bottles wrapped in aluminum foil containing autoclaved seawater, ~2 g wet weight Sargassum, and 1420 nM MPn) or an antibiotic (containing autoclaved seawater, 1420 nM MPn, ~2 g wet weight Sargassum, and the antibiotic mixture) treatment. Additional details about each condition are provided in the dataset.

Experiments were also conducted with nine other species of macroalgae, five of which were collected from Bermuda and treated as described above. The other four species were collected by divers from the Pacific Ocean off the coast of Santa Barbara, California, in December 2017. Pacific incubations were conducted at the University of California, Santa Barbara in indoor aquaria equipped with UV grow lamps on a 12-hr timer that provided approximately full range of solar irradiance and were connected to flowing seawater piped in from nearshore. The experimental design followed that of the Sargassum incubations. However, the morphological features of the Pacific macroalgae required that a ½" diameter circular core was taken from the leaves of each species and placed in 150 mL borosilicate serum bottles. These headspace samples were quantified on a Picarro G2132-i Cavity Ring-Down Spectrometer (CRDS) with a slow, steady stream of carrier gas from a compressed cylinder of Breathing Air through the input port of the CRDS with an aluminum tube fitted with an injection port. Methane concentration data was collected for the carrier gas for at least 1 hour prior to each trial to establish a baseline methane concentration reading. This baseline value was adjusted accordingly for each sampling time point by taking the mean methane concentration value of the breathing air for at least 2 minutes at the beginning and ending of the sampling session. Subsamples of the bottle headspace were then injected in line with the breathing gas, allowing the methane concentration peak to fully return to baseline before the next bottle sample was injected. The times from the start and end of each bottle sample peak were recorded, and the full sampling session data file was downloaded at the end. The individual bottle peaks were then manually separated from the full file. The peak area was calculated by summing the mean methane concentration between each recorded measurement subtracted from the baseline methane concentration. Peak areas were then converted into moles of methane in the headspace by three-point calibration with methane standards.

All methane data was normalized to initial algal wet weight and headspace volume for comparison across bottles. To eliminate inclusion of extraneous methane sources in the MPn consumption signal, methane production is reported in molar quantities in excess of the mean value of control conditions containing only sterile seawater for each sampling time. All methane production rates were calculated using data collected during the first 3 days of incubation only.

References for MPn Concentration Justifications

These outlined assumptions allow the estimated natural concentration of MPn in surface Sea Water for the Atlantic Ocean, which then informs the amount of MPn in surface seawater supplied to bottles in the experiment.

Assumptions Used to Estimate Surface Methylphosphonate Concentration (nM) in the Sargasso Sea

- A (Repeta et al., 2016; Sosa et al., 2020): HMWDOP * (0.066 to 0.164) = MPn
- B (Sosa et al., 2020): DOP * 0.77 = HMWDOP
- C (Lomas et al., 2009): TDP * 0.8 = DOP
- D (Wu et al., 2000): DOP = 0.94-0.99 * TDP
- E (Kolowith et al., 2001; Sosa et al., 2020): 290-390 C : 1 P

Note: Assumption D is used for the reported values from that citation only. Preference was given to Assumption C as it was more recently determined and thus more indicative of current conditions in the Sargasso Sea.

Reference, Nutrient Concentrations, Applied Assumptions, and Estimated Surface MPn Concentration (nM) in the Sargasso Sea

McCarthy, Hedges, & Benner, 1996

- Nutrient Concentration: 16 mM HMWDOC
- Applied Assumptions: A, E
- Estimated Surface MPn Concentration (nM): 3.6-9.1

Canellas et al., 2000

- Nutrient Concentration: 0-0.19 µM DOP
- Applied Assumptions: A, B
- Estimated Surface MPn Concentration (nM): 0.0-24.0

Wu et al., 2000

- Nutrient Concentration: 75±42 µM TDP
- Applied Assumptions: A, B, D
- Estimated Surface MPn Concentration (nM): 1.6-14.8

Cavender-Bares et al., 2001

- Nutrient Concentration: 0.1-0.5 µM DOP
- Applied Assumptions: A, B
- Estimated Surface MPn Concentration (nM): 5.1-63.1

Kolowith et al., 2001

- Nutrient Concentration: 42 nM HMWDOP
- Applied Assumptions: A
- Estimated Surface MPn Concentration (nM): 2.8-6.8

Mahaffey et al., 2004

- Nutrient Concentration: 0.07-0.43 µM DOP
- Applied Assumptions: A, B
- Estimated Surface MPn Concentration (nM): 3.6-54.3

Mather et al., 2008

- Nutrient Concentration: 0.03-0.31 µM DOP
- Applied Assumptions: A, B
- Estimated Surface MPn Concentration (nM): 1.5-39.2

Lomas et al., 2009

- Nutrient Concentration: 0.04-0.07 µM DOP
- Applied Assumptions: A, B
- Estimated Surface MPn Concentration (nM): 2-8.8

McLaughlin et al., 2013

- Nutrient Concentration: 0.1-0.17 µM TDP
- Applied Assumptions: A, B, C
- Estimated Surface MPn Concentration (nM): 4.1-17.2

Sosa et al., 2020

- Nutrient Concentration: 66 nM HMWDOP
- Applied Assumptions: A
- Estimated Surface MPn Concentration (nM): 4.3-10.7

Data Processing Description

Data Issues Noted by the Data Authors:

Problematic rows are listed below. Data values in these rows have concerningly high variability between replicates, and thus it is recommended by the authors that these values are not heavily trusted. They are retained in the data for transparency and because there is a chance that variability could be indicative of natural variation or due to the result of human or technical error.

- Row 181: Order #180, Year 2018, Trial 3 Condition SW+P_MPn_S. natans, Bottle 3 (all time points)
- Row 185: Order #181, Year 2018, Trial 3, Condition SW+N_MPn+S. natans, Bottle 1 (all time points)
- O205-Q207: Order #205-207, Year 2018, Trial 4, Condition SW+S. fluitans, Bottles 1-3 (timepoint 1.5 days)
- Row 293: Order #329, Year 2019, Trial 2, Condition SW+140nM MPn+S. natans, Bottle 5 (all time points)

BCO-DMO Processing Description

Spaces removed from column names and replaced with underscores ("_").

Units and special characters removed from column names.

T1-T6 ID prefixes added to column headers.

Special character representation of 1/2 replaced in values with plain text representation of 1/2.

Merged Year, Month, and Day fields into one Date column.

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Data Files

File	
911212_v1_epiphytic_bacteria_methane_production.csv	(Comma Separated Values (.csv), 115.75 KB) MD5:55ba5292bdf44f66a2263ca888f71e0d
Primary data file for dataset ID 911212, version 1	

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Related Publications

Cavender-Bares, K. K., Karl, D. M., & Chisholm, S. W. (2001). Nutrient gradients in the western North Atlantic Ocean: Relationship to microbial community structure and comparison to patterns in the Pacific Ocean. Deep Sea Research Part I: Oceanographic Research Papers, 48(11), 2373–2395. doi:10.1016/s0967-0637(01)00027-9 https://doi.org/10.1016/S0967-0637(01)00027-9
Methods

Cañellas, M., Agustí, S., & Duarte, C. (2000). Latitudinal variability in phosphate uptake in the Central Atlantic. Marine Ecology Progress Series, 194, 283–294. https://doi.org/10.3354/meps194283
Methods

Cox, D. D., Parsons, R. J., Van Mooy, B. A, S., & Valentine, D. L., (Accepted). Methylphosphonate is Utilized by Commensal Microbiota of Macroalgae in the Oligotrophic Sargasso Sea. Journal of Geophysical Research - Oceans. DOI: 10.1029/2023JC020315
Results

Kolowith, L. C., Ingall, E. D., & Benner, R. (2001). Composition and cycling of marine organic phosphorus. Limnology and Oceanography, 46(2), 309–320. Portico. https://doi.org/10.4319/lo.2001.46.2.0309
Methods

Lomas, M. W., Burke, A. L., Lomas, D. A., Bell, D. W., Shen, C., Dyhrman, S. T., & Ammerman, J. W. (2009).

Sargasso Sea phosphorus biogeochemistry: an important role for dissolved organic phosphorus (DOP). https://doi.org/10.5194/bgd-6-10137-2009
Methods

Mahaffey, C., Williams, R. G., Wolff, G. A., & Anderson, W. T. (2004). Physical supply of nitrogen to phytoplankton in the Atlantic Ocean. Global Biogeochemical Cycles, 18(1). Portico. https://doi.org/10.1029/2003gb002129 https://doi.org/10.1029/2003GB002129
Methods

Mather, R. L., Reynolds, S. E., Wolff, G. A., Williams, R. G., Torres-Valdes, S., Woodward, E. M. S., Landolfi, A., Pan, X., Sanders, R., & Achterberg, E. P. (2008). Phosphorus cycling in the North and South Atlantic Ocean subtropical gyres. Nature Geoscience, 1(7), 439–443. https://doi.org/10.1038/ngeo232
Methods

McCarthy, M., Hedges, J., & Benner, R. (1996). Major biochemical composition of dissolved high molecular weight organic matter in seawater. Marine Chemistry, 55(3–4), 281–297. https://doi.org/10.1016/s0304-4203(96)00041-2 https://doi.org/10.1016/S0304-4203(96)00041-2
Methods

McLaughlin, K., Sohm, J. A., Cutter, G. A., Lomas, M. W., & Paytan, A. (2013). Phosphorus cycling in the Sargasso Sea: Investigation using the oxygen isotopic composition of phosphate, enzyme-labeled fluorescence, and turnover times. Global Biogeochemical Cycles, 27(2), 375–387. Portico. https://doi.org/10.1002/gbc.20037
Methods

Sosa, O. A., Burrell, T. J., Wilson, S. T., Foreman, R. K., Karl, D. M., & Repeta, D. J. (2020). Phosphonate cycling supports methane and ethylene supersaturation in the phosphate-depleted western North Atlantic Ocean. Limnology and Oceanography, 65(10), 2443–2459. Portico. https://doi.org/10.1002/lno.11463
Methods

Sosa, O. A., Repeta, D. J., DeLong, E. F., Ashkezari, M. D., & Karl, D. M. (2019). Phosphate-limited ocean regions select for bacterial populations enriched in the carbon–phosphorus lyase pathway for phosphonate degradation. Environmental Microbiology, 21(7), 2402–2414. Portico. https://doi.org/10.1111/1462-2920.14628
Methods

Wu, J., Sunda, W., Boyle, E. A., & Karl, D. M. (2000). Phosphate Depletion in the Western North Atlantic Ocean. Science, 289(5480), 759–762. https://doi.org/10.1126/science.289.5480.759
Methods

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Parameters

Parameter	Description	Units
Order	Identifier for each sample	unitless
Date	Date the experiment was conducted	unitless
Trial	Identifier in conjunction with Year for samples run concurrently	unitless
Condition	Description of amendments, if any, to the bottle. SW = seawater; MPn = methylphosphonate; M. pyrifera = Macrocystis pyrifera (macroalgae Sargassum)	unitless
Number_of_Replicates	Number of technical measurement replicates performed for each bottle.	count
Initial_MPn	The amount of methylphosphonic acid added to the bottle at the start of incubation.	nM
Additional_Amendments	The nutrients added to the bottle at the start of incubation, with symbol identifiers relating to concentration; the key for these symbols is at the top left of the file.	unitless
Bottle	Identifier for each bottle in a condition.	unitless

T1_Timepoint	Elapsed time at timepoint 1 since incubation start.	days
T1_Timepoint_mean_CH4_production	Mean methane production relative to the SW condition since start of incubation for all bottles in the same condition per trial.	nmol g^-1
T1_Timepoint_CH4_no_sig_fig_rounding	Methane production relative to the SW condition since start of incubation (mean of 3 technical replicates).	nmol g^-1
T2_Timepoint	Elapsed time at timepoint 2 since incubation start, if any.	days
T2_Timepoint_mean_CH4_production	Mean methane production relative to the SW condition since start of incubation for 3 technical replicates (GC-FID Peaks), rounded to the nearest tenth.	nmol g^-1
T2_Timepoint_CH4_no_sig_fig_rounding	Mean methane production relative to the SW condition since start of incubation for 3 technical replicates (GC-FID Peaks).	nmol g^-1
T3_Timepoint	Elapsed time at timepoint 3 since incubation start, if any.	days
T3_Timepoint_CH4_production	Mean methane production relative to the SW condition since start of incubation for 3 technical replicates (GC-FID Peaks), rounded to the nearest tenth.	nmol g^-1
T3_Timepoint_CH4_no_sig_fig_rounding	Mean methane production relative to the SW condition since start of incubation for 3 technical replicates (GC-FID Peaks).	nmol g^-1
T4_Timepoint	Elapsed time at timepoint 4 since incubation start, if any.	days
T4_Timepoint_CH4_production	Mean methane production relative to the SW condition since start of incubation for 3 technical replicates (GC-FID Peaks), rounded to the nearest tenth.	nmol g^-1
T4_Timepoint_CH4_no_sig_fig_rounding	Mean methane production relative to the SW condition since start of incubation for 3 technical replicates (GC-FID Peaks).	nmol g^-1
T5_Timepoint	Elapsed time at timepoint 5 since incubation start, if any.	days
T5_Timepoint_CH4_production	Mean methane production relative to the SW condition since start of incubation for 3 technical replicates (GC-FID Peaks), rounded to the nearest tenth.	nmol g^-1
T5_Timepoint_CH4_no_sig_fig_rounding	Mean methane production relative to the SW condition since start of incubation for 3 technical replicates (GC-FID Peaks).	nmol g^-1
T6_Timepoint	Elapsed time at timepoint 6 since incubation start, if any.	days
T6_Timepoint_CH4_production	Mean methane production relative to the SW condition since start of incubation for 3 technical replicates (GC-FID Peaks), rounded to the nearest tenth.	nmol g^-1
T6_Timepoint_CH4_no_sig_fig_rounding	Mean methane production relative to the SW condition since start of incubation for 3 technical replicates (GC-FID Peaks).	nmol g^-1
TFinal_Trial_Duration	Elapsed time at final timepoint since incubation start.	days
TFinal_Final_CH4	Mean methane production relative to the SW condition since start of incubation for 3 technical replicates (GC-FID Peaks), rounded to the nearest tenth.	nmol g^-1
TFinal_Final_CH4_no_sig_fig_rounding	Mean methane production relative to the SW condition since start of incubation for 3 technical replicates (GC-FID Peaks).	nmol g^-1
TFinal_Percentage_MPn_Addition_Utilized	Ratio of final methane production to initial MPn amendment, reported in %.	unitless

Best_Fit_Rate_by_Bottle_m	Slope of best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for each bottle.	unitless
Best_Fit_Rate_by_Bottle_b	Y-intercept of best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for each bottle.	unitless
Best_Fit_Rate_by_Bottle_R	R value of best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for each bottle.	unitless
Best_Fit_Rate_by_Bottle_R_squared	R^2 value of best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for each bottle.	unitless
Best_Fit_Rate_by_Bottle_N	Number of observations used to make the best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for each bottle.	unitless
Best_Fit_Rate_by_Bottle_P	Probability of the true dataset coming from the same population as the best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for each bottle.	unitless
Mode	Mode for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	nmol g^-1
Skewness_Score	Skewness score of dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless
Skewness_Interpretation	Interpretation of skewness score for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless
Kurtosis_Score	Kurtosis score for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless
Kurtosis_Interpretation	Interpretation of kurtosis score for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless
JB_test_Statistic	Jarque–Bera goodness-of-fit test statistic for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless
P_value	Probability that the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition has a skewness and kurtosis similar to a normal distribution.	unitless

Mean	Mean value for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	nmol g^-1
Median	Median value for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	nmol g^-1
Standard_Deviation	Standard deviation from the mean for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	nmol g^-1
Coefficient_of_Variation	Coefficient of variation from the mean for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless
Standard_Error	Stadard error from the mean for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless
Percent_Error	Ratio of standard error for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition to the mean for the same dataset, reported in %.	unitless
Range	Difference between the highest and lowest values for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	nmol g^-1
Interquartile_Range	Difference between the 75th and 25th percentiles for the dataset used to make best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	nmol g^-1
Best_Fit_Rate_by_Condition_m	Slope of best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless
Best_Fit_Rate_by_Condition_b	Y-intercept of best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless
Best_Fit_Rate_by_Condition_R	R value of best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless
Best_Fit_Rate_by_Condition_R_squared	R^2 value of best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless

Best_Fit_Rate_by_Condition_N	Number of observations used to make the best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless
Best_Fit_Rate_by_Condition_P	Probability of the true dataset coming from the same population as the best-fit correlation between elapsed time (TX_Timepoint) on x-axis and methane production (TX_Timepoint_CH4_production) on y-axis for all bottles of the same condition.	unitless

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Instruments

Dataset-specific Instrument Name	Shimadzu GC-FID 8A
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Headspace sampling was performed in small batches to minimize disruption of incubations and methane concentrations were measured via Shimadzu GC-FID 8A with N2 as the carrier gas (Kinnaman et al., 2007). A 3 mL headspace volume was taken from each bottle by syringe and injected into the GC-FID's gas injection manifold. The syringe was purged three times with N2 gas before headspace sample collection and between each following measurement. Data was recorded with a Shimadzu C-R68A, and the instrument was calibrated with a 3-point calibration curve at the beginning of each sampling period.
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	Picarro G2132-1 Cavity Ring-Down Spectrometer (CRDS) with a compressed cylinder of breathing air as carrier gas
Generic Instrument Name	Spectrometer
Dataset-specific Description	The morphological features of the Pacific macroalgae required that a ½" diameter circular core was taken from the leaves of each species and placed in 150 mL borosilicate serum bottles. These headspace samples were quantified on a Picarro G2132-i Cavity Ring-Down Spectrometer (CRDS) with a slow, steady stream of carrier gas from a compressed cylinder of Breathing Air through the input port of the CRDS with an aluminum tube fitted with an injection port.
Generic Instrument Description	A spectrometer is an optical instrument used to measure properties of light over a specific portion of the electromagnetic spectrum.

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Project Information

Redox Cycling of Phosphorus in the Western North Atlantic Ocean (Phosphorus Redox Cycling)

Coverage: western north Atlantic

NSF Award Abstract:

Redox Cycling of Phosphorus in the Western North Atlantic Ocean
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Understanding controls on the growth of plankton in the upper ocean, which plays an essential role in the sequestration of carbon dioxide, is an important endeavor for chemical oceanography. Phosphorus is an essential element for marine plankton, and has been a research focus of chemical oceanography for nearly a century. Yet, phosphorus redox cycling rates are almost completely unknown throughout the ocean, and the specific molecular identities of the phosphonates, a form of phosphate, in seawater have defied elucidation. This project will explore and refine entirely new pathways for the biological cycling of phosphorus. This project will support teaching and learning by funding the PhD research of a graduate student, and through the continuation of conducting K-12 classroom laboratory modules and hosting 6-8th grade science fair participants in the investigator's lab.

Phosphorus has never been viewed by oceanographers as an element that actively undergoes chemical redox reactions in the water column, and it was believed to occur only in the +5 valence state, in compounds such as phosphate. However, over the last 17 years, numerous lines of geochemical and genomic information have emerged to show that phosphorus in the +3 valence state (P(+3)), particularly dissolved phosphonate compounds, may play a very important role within open ocean planktonic communities. This is particularly true in oligotrophic gyres such as the Sargasso Sea, where growth of phytoplankton can be limited by the scarcity of phosphate. To better understand these new data, the investigators will design and execute a research program that spans at-sea chemical oceanographic experimentation, state-of-the-art chromatography and mass spectrometry, and novel organic synthesis of 33P-labeled P(+3) compounds. Specifically, they will answer questions about rates of production and consumption of low molecular weight P(+3) compounds, the impact of phosphate availability on the production and consumption of P(+3) compounds, and the groups of phytoplankton that utilize low molecular weight P(+3) compounds. Results of this project have the potential to contribute to the transformation of our understanding of the marine phosphorus cycle.

Collaborative Research: Do Cyanobacteria Drive Marine Hydrocarbon Biogeochemistry? (Cyanobacteria Hydrocarbons)

Coverage: North Atlantic Sub-tropical Gyre

NSF Award Abstract:
While the release of petroleum hydrocarbons into the ocean is recognized as an environmental and human hazard, a recent study has estimated that on an annual basis, the release of natural hydrocarbons by a single phytoplankton group (cyanobacteria) contributes at least ten times more total hydrocarbon to the surface ocean. This project will be the first in-depth study of the latent biogeochemical cycling of this huge pool of biogenic hydrocarbons. Using field studies, laboratory incubations of cyanobacteria, and state-of-the art chemical analysis, the researchers will examine the molecular structures, rates and mechanisms of production and removal, and the environmental conditions that control the cycling of this major pool of oceanic hydrocarbons. The results of this study will reveal significant new knowledge for improved understanding of a major carbon cycle in the ocean. Additionally, data could indicate a role for cyanobacterial hydrocarbons in preparing natural marine bacteria to respond to, and degrade petroleum spills, as well as a possible atmospheric impact (e.g. cloud formation) resulting from air-sea exchange of certain components of the hydrocarbon pool.

This project will support undergraduate and graduate students, a postdoctoral investigator, and a new faculty member, and will engage participants from minority-serving institutions in California and North Carolina. Plans are also included to establish links with oil spill and biofuel researchers in order to evaluate additional practical applications for the data resulting from this study.

The annual production of 308,000,000 - 771,000,000 tons of hydrocarbons by cyanobacteria has recently been reported and is a factor of 10 larger than marine petroleum hydrocarbon input from spills and natural seeps. Consequently, these biogenic hydrocarbons almost certainly have significant implications for the carbon

cycle and the bacterial community composition in the ocean but have never been the subject of rigorous study. This project will investigate the distribution, partitioning, and cycling of biogenic hydrocarbons in the ocean, focusing on the abundance and molecular diversity of biogenic hydrocarbons in relation to cyanobacterial populations; the extent to which volatilization to the atmosphere acts as a sink for biogenic hydrocarbons; and the rate at which hydrocarbons are produced by cyanobacteria and consumed by hydrocarbon-degrading bacteria. Field studies across natural gradients in phytoplankton community structure and abundance will employ state of the art chemical analysis to evaluate the distribution of biogenic hydrocarbons, and together with incubation experiments will determine quantitative rates for biogenic hydrocarbons cycling in the surface ocean. Laboratory studies will augment field studies by assessing hydrocarbon production and loss mechanisms under carefully controlled laboratory conditions. Together, the project will obtain a quantitative understanding of this important component of the oceanic carbon cycle.

Fall Semester Student Research in Oceanography and Marine Science at BIOS (Fall Student Research at BIOS)

Website: https://www.nsf.gov/awardsearch/showAward?AWD_ID=1757475&HistoricalAwards=false

Research training for the next generation of marine scientists and oceanographers is an important focus for the Division of Ocean Sciences. The Research Experiences for Undergraduates (REU) program at the Bermuda Institute of Ocean Sciences (BIOS), which is incorporated in New York, will provide undergraduate students with experiential research training in the ocean sciences during the fall semester in Bermuda. The program will introduce eight undergraduate students per year to the techniques, skills and intellectual processes required to conduct research in oceanography and the marine sciences, including projects with long-term, ocean observation programs and near-shore projects based in ecology and molecular biology.

All REU students have an opportunity to join BIOS scientists for a research cruise aboard the R/V Atlantic Explorer. REU students will also meet visiting students from the USA, Canada and the UK and local scientists from the Bermuda Government and from industry. The BIOS REU Site will provide a total of twenty-four undergraduates with internships over the three-year period. Students will conduct independent research projects with the guidance of research mentors and will participate in a variety of professional development activities, including workshops on scientific writing and ethics, the graduate school experience and career options, as well as field trips.

The program conducts a national search for applicants and seeks to engage students who are from schools with limited research opportunities. Most of the funding provided supports student stipends, housing and travel to attend the program. This project supports the national goals of developing the next generation of scientists and the scientific workforce.The range of potential research projects for REU students at BIOS is highly varied. Students may work on deep-sea oceanography, near-shore marine ecology, molecular biology, and marine and atmospheric geochemistry. BIOS is uniquely situated in the middle of the Atlantic Ocean, allowing students the opportunity to study the open ocean in conjunction with established oceanographic time-series programs or the near-shore environment of one of the most northerly coral reef systems in the world.

Additional opportunities include studying the relationship between global change and the physiological function and geographic distribution of marine animal species; zooplankton ecology and population dynamics using traditional morphological and distributional studies paired with cutting-edge molecular tools; nitrogen cycling in the ocean; deployments of autonomous underwater vehicles, and the interaction between the marine boundary layer and the free troposphere.This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1536346
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