Coastal water biogeochemistry collected aboard the R/V Endeavor along the North Atlantic coast from 2017-08-20 to 2017-08-28

Website: https://www.bco-dmo.org/dataset/765327
Data Type: Cruise Results
Version: 1
Version Date: 2019-04-24

Project
» Collaborative Research: Defining the Role of Biologically Produced Reactive Oxygen Species in Dark Mercury Cycling (ROS in Hg Cycling)

Contributors	Affiliation	Role
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Abstract
Samples were collected from four water column sites along the Northeast Coast of the United States in August 2017 aboard the R/V Endeavor. Samples were collected using a 12-bottle trace metal clean CTD (Conductivity, Temperature and Depth) rosette, and were kept clean by using acid washed tubing to collect water directly into acid-washed 1 L PTFE bottles. Sample depths were chosen based on the water column profile obtained from a separate 24-bottle CTD rosette system equipped with Seabird software, which also provided the temperature, dissolved oxygen, salinity, PAR, beam transmission and fluorescence profiles reported here.

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Coverage

Spatial Extent: **N**:41.18026 **E**:-67.92692 **S**:37.77692 **W**:-74.56461
Temporal Extent: 2017-08-20 - 2017-08-28

Dataset Description

Sample Collection
Samples were collected from four water column sites along the Northeast Coast of the United States in August 2017 aboard the R/V Endeavor. Samples were collected using a 12-bottle trace metal clean CTD (Conductivity, Temperature and Depth) rosette, and were kept clean by using acid washed tubing to collect water directly into acid-washed 1 L PTFE bottles. Sample depths were chosen based on the water column profile obtained from a separate 24-bottle CTD rosette system equipped with Seabird software, which also provided the temperature, dissolved oxygen, salinity, PAR, beam transmission and fluorescence profiles reported here.

Methods & Sampling

Chlorophyll
In the dark, 250 mL of seawater was filtered onto 25 mm GF/F filters. Samples were stored in the dark at -20C until analyzed according to protocols adapted from Strickland and Parsons (1972). Briefly, samples were extracted in 90% acetone in the dark (4C, 9 hr) and measured using an 10AU fluorometer (Turner). Sample signals were calibrated using a chlorophyll-a standard (Sigma C6144) and were corrected for pheopigments by accounting for the fluorescence of extracts before and after acidification to 0.003 M HCl.

Nitrogen Species
Concentrations of nitrate + nitrite were measured by chemiluminescence after reduction in a hot acidic vanadyl sulfate solution on a NOx analyzer (Braman and Hendrix, 1989). Concentrations of nitrite were quantified by using the Griess–Ilosvay method followed by measuring absorption 540 nm (Grasshoff et al., 1999), and nitrate was quantified by difference. Concentrations of ammonium were measured by fluorescence using the OPA method (Holmes et al., 1999).

Microbial Abundance
Seawater samples were preserved for flow cytometry with 0.5% glutaraldehyde (final concentration), flash frozen in liquid nitrogen and stored at -80°C until analysis. Bacteria and group-specific phytoplankton counts were conducted on a Guava EasyCyte HT flow cytometer (Millipore). Instrument-specific beads were used to calibrate the cytometer. Samples were analyzed at a low flow rate (0.24 µL s-1) for 3 min. To enumerate bacteria, samples were diluted (1:100) with filtered seawater (0.01 µm). Samples and filtered seawater blanks were stained with SYBR Green I (Invitrogen) according to the manufacturer's instructions and incubated in a 96-well plate in the dark at room temperature for 1 hr. Bacterial cells were counted based on diagnostic forward scatter vs. green fluorescence signals. Major phytoplankton groups were distinguished based on plots of forward scatter vs. orange (phycoerythrin-containing Synechococcus sp.), and forward scatter vs. red (eukaryotes). Size classes of eukaryotic phytoplankton were further distinguished based on forward scatter (pico-, nano- and large eukaryotes).

Dissolved organic carbon
Filtered water samples for total dissolved organic carbon were pipetted into acid-washed combusted glass vials, acidified to pH = 2 with 12 M hydrochloric acid, and stored at 4 °C until analysis on a Shimadzu TOC-5050A total organic carbon analyzer. The coefficient of variability between replicate injections was less than 1%.

Reactive oxygen species
Water samples were collected directly from a trace metal clean rosette into acid-washed, opaque bottles and stored in a shipboard flow-through seawater incubator. A subset of each water sample was filtered (0.2 um), amended with 50 µM diethylene-triaminepentaacetic acid (DTPA, Sigma), and aged overnight in the shipboard seawater flow through incubator (termed AFSW). Additionally, particle associated superoxide signals were determined by filtering (0.2 um) a subset of each water sample and measuring the chemiluminescent signal within 25-30 minutes after filtering (termed FFSW). Superoxide signals were measured by pumping unfiltered water (UFSW), FFSW, or AFSW from dark bottles using a high accuracy peristaltic pump directly into a flow-through FeLume Mini system (Waterville Analytical, Waterville ME) within the ship laboratory. Superoxide detection was based on the reaction between superoxide and a chemiluminescent probe, a methyl cypridina luciferin analog (MCLA, Santa Cruz Biotechnology) (Rose et al., 2008) as before (Roe et al., 2016). The travel time of the water samples in the opaque FeLume tubing was approximately 20 sec. Data was collected for several minutes (~2-4 min) once a steady-state signal was achieved. At least 12 hours following filtering, the superoxide signals within the AFSW for each depth was measured to establish the baseline. At the end of each run, 800 U L-1 superoxide dismutase (SOD, Sigma) was added to seawater samples. The total dark superoxide signal produced in the seawater was defined as the difference between the UFSW signal (subtracted by the SOD baseline) and AFSW signal (subtracted by the SOD baseline) (similar to (Roe et al., 2016)). The particle associated signal was defined as the UFSW signal (subtracted by the SOD baseline) and FFSW signal (subtracted by the SOD baseline) (similar to (Roe et al., 2016)). While the SOD baseline has an autoxidation artifact, this artifact is canceled by taking the difference between two signals. The chemiluminescent signals were converted to superoxide concentration by conducted calibrations in the same aged-filtered seawater used for the baseline at each depth. Calibrations were conducted using potassium dioxide (Sigma) as detailed previously (Zhang et al., 2016).

Designations are:
Total superoxide = [(UFSW) - (UFSW-SOD)] - [(AFSW)- (AFSW-SOD)]
Particle superoxide = [(UFSW) - (UFSW-SOD)] - [(FFSW)- (FFSW-SOD)]

Hydrogen peroxide concentrations. Hydrogen peroxide concentration was quantified based on the oxidation of

colorless AmplifluTM Red (AR, Sigma) to pink-colored resorufin by hydrogen peroxide. For hydrogen peroxide concentration analysis, pre-mixed AR and horse radish peroxidase (HRP) stock solution was added at a final concentration of 50 µmol L-1 AR and 1 kU L-1 HRP to filtered (0.2 um) and unfiltered seawater samples in a clear 96-well microplate (Zhang et al., 2016). Light absorbance was measured at 570 nm (Abs570, maximum absorbance of resorufin) and 700 nm (Abs700, to account for background absorbance) on a SpectraMax® M3 multi-mode microplate reader. The difference between Abs570 and Abs700 (i.e., Abs570-700) was used for calculating hydrogen peroxide concentrations in seawater samples based on a calibration. The calibration factor was determined by standard addition of hydrogen peroxide into 0.2-µm filtered seawater from each station and depth as described previously (Zhang et al., 2016). To account for autoxidation of AR, 200 kU L-1 catalase (Sigma) was added to the blanks prior to the addition of AR and HRP. The hydrogen peroxide concentrations in seawater samples were determined by applying the calibration factor to the blank-corrected Abs570-700values. Net and gross hydrogen peroxide concentrations were defined as the levels measured in unfiltered and filtered seawater samples, respectively.

Manganese
Seawater samples (1 L) were filtered through 0.2 µm membrane filters (Millipore) within one hour of collection using acid-washed Savillex vacuum-filtration rigs. The filtrate was poured into new 15 mL falcon tubes and the filter was immediately amended with a leuco-based dye for Mn oxide concentration in a separate 15 mL falcon tube.

The leucoberbelin blue (LBB) assay for Mn oxides (denoted MnOx hereafter) was previously adapted from Altman (1972) to examine coastal water column sites (Oldham et al., 2015; 2017a, 2017b, 2017c). In this assay, the filter is amended with 3 mL of 20 µM LBB dye solution (LBB, Sigma-Aldrich). The dye color forms upon oxidation of the LBB molecule by Mn oxides and can be measured spectrophotometrically. The LBB stock solution was prepared by dissolving the powder in Milli-Q water to a concentration of 4 % and adding 40 µL of 10 M sodium hydroxide (NaOH) per 10 mL of stock solution. Working solutions are subsequently prepared by diluting the stock solution into 1 % acetic acid, to 0.4 % LBB. A calibration curve was generated using KMnO4, for which equivalent absorbance for Mn(IV) is calculated based on 2.5 more Mn(IV) being required relative to Mn(VII) to oxidize the LBB. In our set-up, a 100 cm pathlength cell (Liquid Waveguide Capillary Cell) was coupled to a Flame UV-Vis (Ocean Optics), set up with SpectraSuite software. Using a 100 cm pathlength cell allows for a detection limit of 0.2 nM but also requires our re-filtration of samples (0.2 µm luer-lok syringe filter, Millipore) prior to injection into the cell to avoid particulate interference and clogging of the capillary cell. Samples reacted with the LBB dye overnight in the dark prior to analysis, then absorbance at 623 nm was recorded. If sample absorbance was too high, samples were diluted 10-20 times in Milli-Q water.

For soluble Mn speciation analysis, an established spectrophotometric porphyrin addition method was employed (Madison et al., 2011; Oldham et al., 2017a), which uses the ligand T(4-CP)P (or α, β, γ, δ-tetrakis(4-carboxyphenyl)porphine, to 2.33 x 10-7 M final sample cocentration). In this method, cadmium chloride ($CdCl_2$; to 2.4 x 10-7 M) is added to form a complex with the porphyrin, in the presence of an imidazole tetraborate buffer (pH = 8.2). The sample is added to the mixture (diluted 10-fold with Milli-Q water to avoid chloride interference) and any Mn(II) in the sample undergoes a metal substitution reaction with the Cd over the course of a 1 hour reaction in a 90°C water bath. The solution is cooled, then analyzed using the 100-cm UV-Vis spectrophotometric set-up described above. Total dissolved Mn is analyzed in the same way, but after the addition of 1.4 µM hydroxylamine hydrochloride to the sample (reacted overnight in a refrigerator). The difference between the total dissolved Mn and the Mn(II) gives the Mn(III)-L in the sample. We note that during the heated reaction with no reducing agent, it is likely that some Mn(III)-L complexes undergo a ligand substitution reaction with the added porphyrin, and thus our method likely underestimates Mn(III)-L, particularly for weaker complexes. For all samples, assays were run in triplicate for both Mn(II) and Mn total, and peak height for all assays was determined using a baseline subtraction performed using ECD-Soft peak correction software.

Data Processing Description

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- added ISO Date fromate generated from date and time values
- concatenated the two sheets "CTD data" and "Full Data for discrete depths".
- added a column to identify the sheet the data came from

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Data Files

File
combined.csv (Comma Separated Values (.csv), 507.14 KB) MD5:42bafa2b7c76280fb80425004a49269b Primary data file for dataset ID 765327

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Related Publications

Braman, R. S., & Hendrix, S. A. (1989). Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium(III) reduction with chemiluminescence detection. Analytical Chemistry, 61(24), 2715–2718. doi:10.1021/ac00199a007
Methods

Holmes, R. M., Aminot, A., Kerouel, R., Hooker, B. A., & Peterson, B. J. (1999). A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences, 56(10), 1801-1808. doi:10.1139/f99-128 https://doi.org/10.1139/cjfas-56-10-1801
Methods

Roe, K. L., Schneider, R. J., Hansel, C. M., & Voelker, B. M. (2016). Measurement of dark, particle-generated superoxide and hydrogen peroxide production and decay in the subtropical and temperate North Pacific Ocean. Deep Sea Research Part I: Oceanographic Research Papers, 107, 59–69. doi:10.1016/j.dsr.2015.10.012
Methods

Rose, A. L., Moffett, J. W., & Waite, T. D. (2008). Determination of Superoxide in Seawater Using 2-Methyl-6-(4-methoxyphenyl)-3,7- dihydroimidazo[1,2-a]pyrazin-3(7H)-one Chemiluminescence. Analytical Chemistry, 80(4), 1215–1227. doi:10.1021/ac7018975
Methods

Strickland, J. D. H. and Parsons, T. R. (1972). A Practical Hand Book of Seawater Analysis. Fisheries Research Board of Canada Bulletin 157, 2nd Edition, 310 p.
Methods

Zhang, T., Hansel, C. M., Voelker, B. M., & Lamborg, C. H. (2016). Extensive Dark Biological Production of Reactive Oxygen Species in Brackish and Freshwater Ponds. Environmental Science & Technology, 50(6), 2983–2993. doi:10.1021/acs.est.5b03906
Methods

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Parameters

Parameter	Description	Units
Station	station identifier	unitless
lat	station latitude with north positive	decimal degrees
long	station longitude with east positive	decimal degrees
Year	year of collection in yyyy format	unitless

Month	month of collection in mm format	unitless
Day	day of collection in dd format	unitless
Time	time of collection in HH:MM format	unitless
ISO_datetime_UTC	date and time following ISO format	yyyy-MM-dd'T'HH:mm:ss'Z'
Depth	water depth	meters (m)
Temp	Water temperature from CTD	degrees Celsius (C)
Florescence	water flourescence from CTD	miligrams per meter cubed (mg/m3)
Chlorophyll	Chlorophyll a concentration	micrograms per liter (ug/L)
Chloro_sd	Chlorophyll a concentration standard deviation	micrograms per liter (ug/L)
PAR	Photosynthetically active radiation (CTD)	microeinteins per meter squared second (microE/m2s)
Beam_Transmission	Beam transmission (CTD)	percent
Salinity	Water salinity (CTD)	PSU
O2	Water oxygen concentration	mililiters per liter (mL/L)
NO2	Water nitrite concentration	micromole (uM)
NH4	Water ammonium concentration	micromole (uM)
NO3	Water nitrate concentration	micromole (uM)
Nanoeuks	Nanoeukaryote abundance	cells per mililiter (cells/mL)

Nanoeuks_sd	Nanoeukaryote abundance standard deviation	cells per mililiter (cells/mL)
Picoeuks	Picoeukayrote abundance	cells per mililiter (cells/mL)
Picoeuks_sd	Picoeukayrote abundance standard deviation	cells per mililiter (cells/mL)
Synechococcus	Synechococcus abundance	cells per mililiter (cells/mL)
Synechococcus_sd	Synechococcus abundance standard deviation	cells per mililiter (cells/mL)
Bacteria	Bacteria concentration	cells per mililiter (cells/mL)
Bacteria_sd	Bacteria concentration standard deviation	cells per mililiter (cells/mL)
DOC	Dissolved organic carbon	miligrams per liter (mg/L)
DOC_sd	Dissolved organic carbon standard deviation	miligrams per liter (mg/L)
Tot_O2	Total superoxide	picomole (pM)
Tot_O2_sd	Total superoxide standard deviation	picomole (pM)
Part_O2	Particle associated superoxide	picomole (pM)
Part_O2_sd	Particle associated superoxide standard deviation	picomole (pM)
Net_H2O2	Net hydrogen peroxide	nanomole (nM)
Net_H2O2_sd	Net hydrogen peroxide standard deviation	nanomole (nM)
Gross_H2O2	Gross hydrogen peroxide	nanomole (nM)

Gross_H2O2_sd	Gross hydrogen peroxide standard deviation	nanomole (nM)
Total_dHg	Total dissolved Hg	picomole (pM)
Total_dHg_sd	Total dissolved Hg standard deviation	picomole (pM)
Hg_0	Elemental Hg	picomole (pM)
Hg_0_sd	Elemental Hg standard deviation	picomole (pM)
dMn_II	Dissolved Mn(II)	nanomole (nM)
dMn_II_sd	Dissolved Mn(II) standard deviation	nanomole (nM)
dMn_T	Total dissolved Mn	nanomole (nM)
dMn_T_sd	Total dissolved Mn standard deviation	nanomole (nM)
dMn_III_L	Dissolved Mn(III)-L	nanomole (nM)
dMn_III_L_sd	Dissolved Mn(III)-L standard deviation	nanomole (nM)
MnOx	Mn oxides	nanomole
sheet	Name of the sheet from the original data file	unitless

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Instruments

Dataset-specific Instrument Name	NOx analyzer
Generic Instrument Name	Chemiluminescence NOx Analyzer
Dataset-specific Description	Concentrations of nitrate + nitrite were measured by chemiluminescence after reduction in a hot acidic vanadyl sulfate solution on a NOx analyzer (Braman and Hendrix, 1989)
Generic Instrument Description	The chemiluminescence method for gas analysis of oxides of nitrogen relies on the measurement of light produced by the gas-phase titration of nitric oxide and ozone. A chemiluminescence analyzer can measure the concentration of NO/NO2/NOX. One example is the Teledyne Model T200: https://www.teledyne-api.com/products/nitrogen-compound-instruments/t200

Dataset-specific Instrument Name	CTD Seabird
Generic Instrument Name	CTD Sea-Bird
Dataset-specific Description	Samples were collected using a 12-bottle trace metal clean CTD (Conductivity, Temperature and Depth) rosette
Generic Instrument Description	A Conductivity, Temperature, Depth (CTD) sensor package from SeaBird Electronics. This instrument designation is used when specific make and model are not known or when a more specific term is not available in the BCO-DMO vocabulary. Refer to the dataset-specific metadata for more information about the specific CTD used. More information from: http://www.seabird.com/

Dataset-specific Instrument Name	Guava EasyCyte HT flow cytometer (Millipore)
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Bacteria and group-specific phytoplankton counts were conducted on a Guava EasyCyte HT flow cytometer (Millipore).
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. Description from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm

Dataset-specific Instrument Name	10AU fluorometer (Turner)
Generic Instrument Name	Fluorometer
Dataset-specific Description	Briefly, samples were extracted in 90% acetone in the dark (4C, 9 hr) and measured using an 10AU fluorometer (Turner).
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	Shimadzu TOC-5050A total organic carbon analyzer
Generic Instrument Name	Total Organic Carbon Analyzer
Dataset-specific Description	Filtered water samples for total dissolved organic carbon were pipetted into acid-washed combusted glass vials, acidified to pH = 2 with 12 M hydrochloric acid, and stored at 4 °C until analysis on a Shimadzu TOC-5050A total organic carbon analyzer.
Generic Instrument Description	A unit that accurately determines the carbon concentrations of organic compounds typically by detecting and measuring its combustion product (CO2). See description document at: http://bcodata.whoi.edu/LaurentianGreatLakes_Chemistry/bs116.pdf

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Deployments

EN604

Website	https://www.bco-dmo.org/deployment/765654
Platform	R/V Endeavor
Start Date	2017-08-20
End Date	2017-08-28

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Project Information

Collaborative Research: Defining the Role of Biologically Produced Reactive Oxygen Species in Dark Mercury Cycling (ROS in Hg Cycling)

Coverage: Coastal North Atlantic

NSF Abstract:

Mercury (Hg) is a toxic trace element that bioaccumulates into marine food webs, imposing a health threat to humans through the consumption of seafood. However, controls on the cycling of Hg in the ocean are poorly understood. Most research to date has focused on sun-lit and/or Hg-laden environments, where light-induced chemical and mercury resistance reactions, respectively, have been identified as dominant pathways for Hg cycling. The paradigm that dark Hg reactions are irrelevant is fading and it is now apparent that dark redox reactions, both reduction and oxidation, are important in the cycling of Hg. In this study, researchers at the Woods Hole Oceanographic Institution and Colorado School of Mines will obtain a better understanding of the biogeochemical reactions responsible for dark redox transformations of mercury (Hg) in marine systems. The researchers will explore the relationship between microbial activity, reactive oxygen species, and Hg speciation in a series of laboratory- and field-based investigations to obtain a mechanistic understanding of dark Hg cycling. By identifying new controls on the redox cycling of Hg in the ocean, this research will help inform global and ecosystem models used to predict Hg bioavailability.

Broader Impacts: The proponents plan to educate high school teachers from Boston Green Academy in South Boston on mercury biogeochemistry and have one teacher participate in the summer research cruises, as well as develop science curricula to engage the underrepresented students at the school in science. One postdoc and one graduate student from Woods Hole Oceanographic Institution and one graduate student from the Colorado School of Mines would be supported and trained as part of this project. It is anticipated that undergraduate students would have the opportunity to participate in the study as summer interns.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1355720

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