Microbial respiration from microcosm experiments conducted under three light treatments using water originating from West Bay of the Neuse River Estuary, North Carolina USA in 2021 and 2022

Website: https://www.bco-dmo.org/dataset/908626
Data Type: experimental
Version: 1
Version Date: 2023-10-09

Project
» Bacteria as Biosensors of Carbon and Energy Flow in Marine Ecosystems: Quantitative Links Between Substrates, Transcripts, and Metabolism (Bacterial DOC Sensor)

Contributors	Affiliation	Role
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Abstract
Microbial respiration assays were conducted for two microcosm incubation experiments. Sample water originated from West Bay of the Neuse River Estuary, North Carolina USA in 2021 and 2022. The microcosms were 60-liters, conducted in biological duplicates under three light treatment incubations: 12-hour light-dark cycle of photosynthetically active radiation (PAR), 12-hour light-dark cycle of UV-B radiation, or darkness. Respiration assays of the unfiltered and 5-micron filtered community were initiated every few days using foil-membrane optodes to examine light effects on community respiration.

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Coverage

Spatial Extent: **Lat**:34.925672 **Lon**:-76.365069
Temporal Extent: 2021-09-03 - 2022-05-23

Methods & Sampling

Surface water samples for these experiments were collected from West Bay of the Neuse River Estuary, North Carolina USA (34°55'32.42" N, 76°21'54.25" W). The microcosms were 60 liters. The experiments were conducted in biological duplicates under three light treatment incubations: 12-hour light-dark cycle of photosynthetically active radiation (PAR), 12-hour light-dark cycle of UV-B radiation, or darkness. Samples were collected from the microcosms in duplicate every few days for over one month to examine how light and

the resulting microbial activity altered the DOC pool over time.

For each 60-liter (L) microcosm, water was sampled daily to weekly at the University of North Carolina over the month-long (September 2021) experiment or two-month-long experiment (April 2022). Water was sampled from the microcosms using a 50-milliliter (mL) serological pipette and auto pipettor. The pointed end of the pipette was snapped off to provide a wide, sterile pipette mouth. The pipette was twice rinsed with Milli-Q water prior to sampling to limit potential carbon contamination and attached to MasterFlex tubing. A vacuum pump with a trap was used under gentle pressure to fill 500 mL of sample water into a 1-L borosilicate bottle.

To size fractionate the sample water, a filter tower (47-millimeter (mm), 5-micrometer (um) polycarbonate filter) was twice rinsed with c.a. 50 mL of sample water and discarded. Then, 250 mL of sample was gravity filtered through the tower to generate a less 5 um fraction. For the respiration incubation assay, biological oxygen demand bottles (BODs) were rinsed three times with c.a. 20 mL of sample water (unfiltered or less 5 um), then filled to the brim. Air bubbles were removed from the BOD by taping the sides of the bottle with the stopper for 1 minute, then quickly inserting the glass stopper. The filtering and filling process was done in a temperature-controlled room to reduce changes in sample and bottle temperature during handling. All bottles, filter towers, and tubing in contact with sample water were acid washed with 10% HCl and triple rinsed with Milli-Q water before each sampling event and rinsed with Milli-Q, then sample water between treatment replicates.

The BODs have a PreSens foil-membrane optode mounted on the inside of the bottle and were fitted with a holder to align an external fiber optic cable with the sensor spot (detailed in Cohn, et al. 2023). The BODs and one abiotic control BOD of Milli-Q were then placed in a water bath in the temperature-controlled room and covered to prevent light from entering the bath. The BODs were incubated for an average of two days, recording an oxygen concentration every 30 seconds, then exported from the PreSens Measurement 2 Studio for analysis (note, the incubation time can be greatly shortened, however drawdown rates were linear and the long incubation was allowed due to logistical constraints).

Instruments and Materials:

Hydrochloric acid (36% w/w, ThermoFisher) was diluted to 10% (v/v) with Milli-Q water for acid washing procedures.

Serological pipette, 50 mL, sterile, cotton plug removed (VWR)

1/4 inch Masterflex L/S Platinum-Cured Silicone Tubing

GAST vacuum pump (Model: DOA-P704-AA) used at <-5 in Hg

1-L borosilicate media bottle (VWR)

500-mL amber bottle-top filter holder (Nalgene)

5 micron polycarbonate filter (Millipore Sigma, 47 mm)

60-mL borosilicate biological oxygen demand bottles with glass stopper (VWR)

PreSens oxygen optode sensor spot (SP-PSt3-NAU)

PreSens polymer optical fiber (POF)

PreSens temperature/pressure sensor (Pt-100)

PreSens sensor meter (Oxy-4 SMA [G2] for all sample bottles, Oxy-1 SMA for the abiotic control BOD)

water bath (60-L igloo cooler) filled with DI water, covered in black bags to block light, an aquarium pump for water circulation, all placed in an environmental controlled room at in situ temperature

Data Processing Description

PreSens Precision Measurement Studio 2 (v4.0.0.2323) was used with the PreSens sensors for data collection, acquiring one oxygen concentration measurement per BOD bottle every 30 seconds. The pressure and salinity were set manually. Initial temperature correction was performed by the PMS2 software using the Pt100 sensor. The data were exported as Excel books for further analysis.

R Studio (version 2022.11.0-daily+155) was used to plot the oxygen drawdown rate over time for each respiration assay. The first 2.5 hours of data were removed due to abiotic acclimation of the BOD bottle and sensor spot. The remaining data were trimmed if nonlinearity became apparent at the end of the incubation period. The change in oxygen concentration of the abiotic Milli-Q control bottle was subtracted from the sample oxygen values as an additional temperature correction measure. A rolling mean with a sliding window of 12.5 minutes was applied to the oxygen values, and then a model I linear regression was calculated. The slope of the linear regression represents the respiration rate as oxygen consumption over time. This rate is reported along with the residuals, residual standard error, and respiration rate converted to carbon units using a static respiratory quotient of 1.4 O2:CO2.

BCO-DMO Processing Description

- Imported original file named "Results_Respiration.xlsx" into the BCO-DMO system.
- Flagged '-9999' as a missing data value; missing data are blank/empty in the final CSV file.
- Renamed fields to comply with BCO-DMO naming conventions.
- Converted the 'Date' column to YYYY-mm-dd format.
- Added the 'ISO_DateTime_Local' column for the date-time in ISO 8601 format.
- Replaced non-standard Greek "mu" character with "u" in the 'Size_fraction_um' column.
- Saved the final file as "908626_v1_microbial_respiration.csv".

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Data Files

File	
908626_v1_microbial_respiration.csv	(Comma Separated Values (.csv), 16.41 KB) MD5:c0cc22c5bcef7cbc0f017195acacfbcf
Primary data file for dataset ID 908626, version 1.	

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Related Publications

Cohn, M. R., Stephens, B., Meyer, M. G., Sharpe, G., Niebergall, A. K., Graff, J. R., Cassar, N., Marchetti, A., Carlson, C. A., & Gifford, S. (2023). Microbial Respiration in Contrasting Ocean Provinces via High-Frequency Optical Assays. https://doi.org/10.1101/2023.07.20.549894
Methods

PreSens Measurement Studio 2. (n.d.). Retrieved March 18, 2021, from https://www.presens.de/products/detail/presens-measurement-studio-2
Software

RStudio Team (2022) RStudio: Integrated Development for R. Version 2022.11.0-daily+155. RStudio, Inc., Boston, MA. http://www.rstudio.com/
Software

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Parameters

Parameter	Description	Units
Experiment_Name	Name of incubation experiment; either "Eco1" or "Eco2". Eco1 was initiated September 2, 2021 and Eco2 was initiated April 4, 2022.	unitless
Date	Respiration assay start date	unitless
Time_EDT	Respiration assay start time (time zone = EST/EDT)	unitless
Incubation_day	Days elapsed since microcosm incubation initiation	days
Treatment	Light treatment applied to incubation: "L" = 12 h PAR/dark; "V" = 12 h UV-B/dark; "D" = dark; and "in situ" = at time of collection.	unitless
Tank_ID	Identifier for microcosm replicate (two tanks per light treatment)	unitless
Size_fraction_um	Filter fraction of microcosm sample water for respiration assay	micrometers (um)
respiration_uM_O2_d_1	The respiration rate as oxygen drawdown	micromoles O2 per day (uM O2 d-1)
respiration_uM_C_d_1	The respiration rate using a respiratory quotient of 1.4 O2:CO2	micromoles C per day (uM C d-1)
r2	The residual of the model I linear regression (respiration rate)	unitless
RSE	The residual standard error of the model I linear regression	unitless
No_pts	Number of assay points used in rate calculation	unitless
ISO_DateTime_Local	Date and time of respiration assay start (time zone = EST/EDT)	unitless

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Instruments

Dataset-specific Instrument Name	preSens oxygen optode sensor spot (SP-PSt3-NAU)
Generic Instrument Name	Oxygen Sensor
Generic Instrument Description	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Dataset-specific Instrument Name	GAST vacuum pump
Generic Instrument Name	Pump
Dataset-specific Description	GAST vacuum pump (Model: DOA-P704-AA) used at
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

Dataset-specific Instrument Name	PreSens temperature/pressure sensor (Pt-100)
Generic Instrument Name	Water Temperature Sensor
Generic Instrument Description	General term for an instrument that measures the temperature of the water with which it is in contact (thermometer).

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Project Information

Bacteria as Biosensors of Carbon and Energy Flow in Marine Ecosystems: Quantitative Links Between Substrates, Transcripts, and Metabolism (Bacterial DOC Sensor)

Coverage: Estuaries and Coastal Ecosystems of North Carolina

NSF Award Abstract:
The formation and flux of organic material is the foundation of ocean ecosystems, which in turn, substantially influences the global carbon cycle. As such, a fundamental goal in the ocean sciences is increasing our ability to identify marine organic matter's sources, transformations, and sinks, as well as how these components may change due to anthropogenic activities. Understanding these components is especially important in estuarine and coastal systems given these ecosystems are critical zones of organic carbon transformations. However, the dissolved organic carbon (DOC) pool in these systems consists of numerous different compounds from a multitude of sources that can turn over at vastly different rates (minutes to millennia). This makes it difficult to identify which DOC compounds support microbial growth, limiting the incorporation of microbial metabolism into predictive ecosystem models. Novel approaches are therefore needed to identify the DOC substrates driving microbial metabolism in ocean ecosystems. This project is premised on the idea that the bacterial cellular system is the ultimate chemical sensor of the organic environment and that the information recorded in the cell's active gene pool (transcripts) can be leveraged to make insights into DOC composition when the relationships between organic substrate availability, gene activity, and metabolism are known. This project identifies substrate-transcript relationships for a model marine bacterium, as well as the growth and metabolic outcomes of substrate availability. These insights are used to identify the biologically active DOC substrates in coastal environments when the model organism is added directly to coastal samples, and to interpret both historical and current environmental RNA and DNA data sets. This work provides novel insights into the substrates driving the ocean's carbon cycle and how marine bacterial cellular systems are regulated. Bioassays are developed that can be applied in many different aquatic environment settings. The project trains graduate and undergraduate students directly involved in the research and minority undergraduates will be recruited to use research modules for hands-on study of cell cultivation, bioinformatics, and microbial metabolism. High school students will be engaged through a module developed for an aquatic microbiology field trip and subsequent sample and data analysis.

Bacterial processing of dissolved organic carbon (DOC) mediates the flux of gigatons of carbon in the ocean,

yet a significant hurdle to incorporating bacterial metabolism into ocean models is the inability to quantify the DOC substrates supporting bacterial metabolism and their transformation. Metatranscriptomics (sequencing of community mRNAs) has the potential to be a sensitive method for surveying bacterioplankton responses to the DOC pool and making insights into its composition but is currently limited by insufficient knowledge as to how transcriptional patterns relate to substrate availability. This project will identify carbon substrates supporting microbial metabolism and their transformation in estuarine-coastal ecosystems by elucidating the relationships between transcript abundances and carbon substrate availability. It aims to bridge the gap between model organism and environmental -omic studies by creating quantitative inventories of transcripts in response to defined substrates, and then using these calibrated transcriptional signals to interpret environmental DOC bioassays and metatranscriptomes. The first component of the project will establish genome-wide transcript-substrate relationships in a model marine bacterium in response to individual, environmentally-relevant carbon substrates. The second component will determine the extent to which transcription and metabolism are altered when the bacterium is exposed to complex mixtures of defined and undefined substrates, revealing the potential for transcription to identify individual substrates within a complex DOC pool and how metabolic processing may shape the DOC pools labile and refractory components. Finally, these calibrated transcriptional responses will be used to identify the DOC substrates driving bacterial metabolism in an estuarine-coastal system via DOC drawdown bioassays in which the model organism is added to natural seawater samples, as well as community wide bacterioplankton responses to the extant DOC pool via metatranscriptomics.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1850692

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