Dissolved inorganic nutrients from two microcosm incubation experiments conducted on sample water collected from West Bay of the Neuse River Estuary, North Carolina USA in 2021 and 2022

Website: https://www.bco-dmo.org/dataset/944277
Data Type: experimental
Version: 1
Version Date: 2024-12-02

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
Dissolved inorganic nutrients were collected 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. 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 concentrations of dissolved inorganic nutrients (total dissolved nitrate and nitrite or NOx, ammonium or NH4, and soluble reactive phosphorus or SRP) over time. Concentrations of these inorganic nutrients were quantified via colorimetric assays on a Genesys 10S UV-Vis spectrophotometer or Tecan Infinite 200Pro plate reader.

Table of Contents

Coverage

Spatial Extent: **Lat**:34.92567222 **Lon**:-76.36506944
Temporal Extent: 2021-09-02 - 2022-04-04

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) and distributed into six microcosms. 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. For each 60-liter 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 peristaltic pump (Masterflex) under gentle (75%) pressure through in-line 3 micron (um) and 0.2 micron, 47-millimeter (mm) polycarbonate filters (Millipore Sigma) which were flushed with 250 milliliters (mL) of Milli-Q water. All tubing, filter holders, and 60-milliter (mL) HDPE collection bottles (Fisher Scientific) were acid washed in 10% (v/v) HCl for six hours and triple rinsed with Milli-Q water. <0.2 um filtrate was collected from each tank for dissolved inorganic nutrient analysis and stored for one month at -20 degrees Celisus (°C). Acid-washed glassware was used for NOx and NH4 assays, and RBS phosphate-free detergent washed glassware was used for SRP assay.

NOx was quantified using 2% vanadium chloride in hydrochloric acid to reduce nitrate to nitrite, then using the Greiss reaction on 1 mL sample volumes following Garcia-Robledo et al. (2014). 10 standard solutions ranging from 0.1 to 10 micromolar (uM) were created in saltwater (sodium chloride in Milli-Q at in situ salinity).

NH4 was measured using a colorimetric alkalic nitroprusside reaction in 96-well plates as described in Bower and Holm-Hanson, 1980. 10 standard solutions of NH4 were created ranging from 0.5 to 25 uM using saltwater similarly to NOx standards. For summer samples, NOx and NH4 absorbance were measured with quartz cuvettes at 540 nanometers (nm) (NOx) and 630 nm (NH4). Spring samples were measured on a Tecan plate reader.

Soluble reactive phosphorus SRP was measured using a modified molybdenum blue assay from Murphy and Riley (1962). Seven standards were prepared ranging from 0.1 to 4 uM in saltwater similar to NOx and NH4. All measurements were quantified with quartz cuvettes at 885 nm.

Data Processing Description

For each assay, standard curves were created. The limit of detection (LOD) was calculated from this standard curve, where the LOD= 3.3 times the standard error of standard curve divided by the slope of the calibration curve.

BCO-DMO Processing Description

- Imported original file "Results_inorganics.xlsx" into the BCO-DMO system.
- Marked "-9999" as a missing data value (missing data are empty/blank in the final CSV file).
- Renamed fields to comply with BCO-DMO naming conventions.
- Filled in experiment start dates in Date_Start column.
- Rounded values in columns NOx, NH4, and SRP to 2 decimal places as advised by data provider.
- Saved the final file as "944277_v1_dissolved_inorganic_nutrients.csv".

Problem Description

Note the LOD for each nutrient is different between Eco1 and Eco2 experiments (with the exception of SRP). LOD's are as followed, Eco1 NOx= 0.5 uM, Eco1 NH4= 1 uM, Eco1 SRP= 0.1 uM, Eco2 NOx= 0.1 uM, Eco2 NH4= 0.5 uM, Eco2 SRP= 0.1 uM.

[table of contents | back to top]

Data Files

File
944277_v1_dissolved_inorganic_nutrients.csv (Comma Separated Values (.csv), 6.67 KB) MD5:006a46ed322375b7e9c2c03c578fceed Primary data file for dataset ID 944277, version 1

[table of contents | back to top]

Related Publications

Bower, C. E., & Holm-Hansen, T. (1980). A Salicylate–Hypochlorite Method for Determining Ammonia in Seawater. Canadian Journal of Fisheries and Aquatic Sciences, 37(5), 794–798. doi:10.1139/f80-106
Methods

García-Robledo, E., Corzo, A., & Papaspyrou, S. (2014). A fast and direct spectrophotometric method for the sequential determination of nitrate and nitrite at low concentrations in small volumes. Marine Chemistry, 162, 30–36. https://doi.org/10.1016/j.marchem.2014.03.002
Methods

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta, 27, 31–36. doi:10.1016/s0003-2670(00)88444-5
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
Experiment	Name of incubation experiment	unitless
Date_Start	Start date of experiment. Eco1 was initiated September 2, 2021 and Eco2 was initiated April 4, 2022.	unitless
Incubation_Day	Days elapsed since incubation initiation	days
Treatment	Light treatment applied to incubation: 12 h PAR/dark (L), 12 h UV-B/dark (V), dark (D), or in situ at time of collection (in situ)	unitless
Tank_ID	Identifier for microcosm replicate (two tanks per light treatment)	unitless
Replicate	Replicate from individual micocosm	unitless
NOx	Total nitrate and nitrite concentration	micromolar (uM)
NH4	Ammonium concentration	micromolar (uM)
SRP	Soluble reactive phosphorus concentration	micromolar (uM)

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Tecan Infinite 200Pro (i-control v2.0.10.0)
Generic Instrument Name	plate reader
Dataset-specific Description	used for NOx and NH4 measurements from spring samples
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

Dataset-specific Instrument Name	Genesys 10S UV-Vis Spectrophotometer (ThermoFisher)
Generic Instrument Name	Spectrometer
Dataset-specific Description	used in scanning mode, fast, at 1 nm intervals to measure the absorbance of the filtrate
Generic Instrument Description	A spectrometer is an optical instrument used to measure properties of light over a specific portion of the electromagnetic spectrum.

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

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

[table of contents | back to top]