Microbial Cellular Abundance Epifluorescent Microscopy from Neuse River Estuary, North Carolina USA from 2021-2022

Website: https://www.bco-dmo.org/dataset/925050
Data Type: experimental
Version: 1
Version Date: 2024-06-11

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
Gifford, Scott M.	University of North Carolina at Chapel Hill (UNC-Chapel Hill)	Principal Investigator
Cohn, Melanie R.	University of North Carolina at Chapel Hill (UNC-Chapel Hill)	Scientist
Parker, Sierra	University of North Carolina at Chapel Hill (UNC-Chapel Hill)	Student
Merchant, Lynne M.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Microbial cellular abundance was enumerated for two microcosm incubation experiments to track the growth response of the microbial community. This dataset targets bacterial and phytoplankton abundance through epiflorescent microscopy. Sample water originated from the West Bay of the Neuse River Estuary, North Carolina USA in 2021 and 2022. The microcosms were 60-L, conducted in biological duplicates under three light treatment incubations: 12 h light-dark cycle of photosynthetically active radiation (PAR), 12 h light-dark cycle of UV-B radiation, or darkness. Unfiltered sample water was stained with SYBR Green I (Fall 2021 experiment) or DAPI (Spring 2022 experiment) for enumeration at 60x for bacterial and cyanobacterial abundance. Autofluorescence from excitation at 620 nm and 60x was used to enumerate small phytoplankton and cyanobacteria. This dataset highlights patterns in microbial growth across treatments over the course of the incubation and is used to generate cell-specific, normalized rates for associated datasets in the project.

Table of Contents

- Coverage
- Dataset Description
  - Methods & Sampling
  - Data Processing Description
  - BCO-DMO Processing Description
- Related Publications
- Parameters
- Instruments
- Project Information
- Funding

Coverage

Location: West Bay of the Neuse River Estuary in North Carolina, USA. Surface water.
Spatial Extent: **Lat**:34.92567222 **Lon**:-76.36506944
Temporal Extent: 2021-09-02 - 2022-05-28

Methods & Sampling

Sample Collection. Sample water was collected from the microcosms every couple of days during the experiment incubation for enumeration of the microbial community via epiflorescent microscopy. The water was sampled from the microcosms using a 25-mL serological pipette (VWR) with the tip snapped off to widen the opening. The serological pipette was rinsed three times with Milli-Q water before drawing sample water from the center of the microcosm near the mixer. A new serological pipette was used for each treatment. To

a 50-mL falcon tube, 25 mL of sample was collected.

Staining Procedure. For Ecocosm I (Fall 2021) 10 uL of 100x SYBR Green I was added to duplicate eppendorf tubes with 1 mL of sample water for a final stain concentration of 10x and vortexed gently for 5 seconds. The sample was incubated in the dark at room temperature for 45 minutes. For Ecocosm II, DAPI stain was used to allow for cell-sizing analysis in addition to enumeration; 50 uL of 50x DAPI solution was added to 1 mL of sample as above and incubated in the dark for 10 minutes prior to slide preparation.

Slide Preparation. Samples were filtered down onto black, 25-mm filters (GTBP, Millipore Sigma, 0.22 um) with a backing filter (PVDF, Millipore Sigma, 25-mm, 0.45 um) on a 25-mm multifilter manifold (Millipore). Each filter well was filled with c.a. 5 mL of Milli-Q water then 1 mL of stained sample was added to a filtering well and filtered down under gentle (5-10 in Hg) vacuum pressure. The GTBP filters were mounted to slides with microscopy oil and a cover slip.

Imaging. The slides were imaged on a Nikon Ti2 inverted microscope using the NIS Elements software. SYBR-stained slides were imaged at 60x magnification at 470 nm and 620 nm excitation to capture autofluorescence. DAPI-stained slides were similarly counted at 60x under 395 nm and 620 nm excitation. Manual counts calculated from 10 fields of view are reported in this dataset. Further details on imaging techniques, analysis, and automated image analysis are found in Parker (2022) in the publications section.

List of equipment used

25-mL serological pipette for sampling (VWR, sterile)

50-mL Falcon tube (Corning)

1.7-mL eppendorf tubes (Eppendorf)

SYBR Green I in DMSO stain, 10,000x (Invitrogen): diluted to 100x (flow cytometry) with Milli-Q water, 0.2-um filtered, and frozen in 0.2 mL aliquots to prevent freeze-thaw cycles.

DAPI, 1 mL (1 mg/mL) 4',5-diamidina-2-phenylindole (Fisher Scientific)

25-mm PVDF filter (Millipore Sigma)

25-mm, 0.22 um GTBP filter (Millipore Sigma)

Filter manifold (Millipore Sigma 1225: 12, 25-mm wells)

1mm glass microscope slide (Vista-Vision)

Glass cover slips No. 1.5 (Slip-Rite)

Microscopy immersion oil

Nikon Ti2 inverted microscope with excitation filters for: DAPI (395/25 nm), SYBR (470/40 nm), and autofloresence (620/60 nm)

Nikon Elements Acquisition Software

Data Processing Description

The number of cells counted across 10 fields of view at 60x magnification for each sample was averaged, multiplied by the dilution factor, and corrected for the percent of filter area counted. The results in this datafile include the 60x SYBR, DAPI, and autofluorescent manual microscopy counts reported in cells/L with and standard deviation for each microcosm. Additionally, the ratio of stained to autofluorecent cells is reported.

For Ecocosm I, microscopy counts were not taken on experiment days 3, 4, and 14: some associated data sets under this project were sampled on these days. In order to provide cell counts on these additional sampling days, the abundances were interpolated for the missing days using a model 1 linear regression of the surrounding timepoints to calculate the "missing" days. These dates are denoted by "micoscopy_interpolated" under the "Method" parameter.

BCO-DMO Processing Description

In the submitted file Results_BacAbundance_microscopy2.csv, the file was modified using the BCO-DMO laminar tool.

The second line was ignored which contained the following notes line:
Note: -7777=below detection, -8888=NA, -9999=missing

For the header "Size.Fraction.µm", removed the characters µm because the units will be noted in the parameters section of the dataset page.

Converted submitted location information to decimal degrees and added the values as parameter columns lat and lon in the dataset file.

Used 8 decimal point precision of lat and lon which represents precision of about 1.11 mm

Latitude: 34°55'32.42" N -> 34.92567222
Longitude: 76°21'54.25" W -> -76.36506944

Removed the last column which is empty and has no header name.

Renamed headers. Replace periods and colons with underscores to match BCO-DMO naming scheme.

Remove units from Excitation and Size Fraction headers because it will be noted in the Osprey parameters section. Replaced / with _per_.

In the columns cells_per_L_avg and cells_per_L_sd, replaced the values (9,999) with -9999, replace the values (7,777) with -7777 and replace the values (7,777,000) with -7777 to match the flag format provided.

Removed commas from the columns cells_per_L_avg and cells_per_L_sd in order to convert the column into a numeric format.

Converted the date format from %Y%m%d into an ISO format %Y-%m-%d.

Reordered the columns so that the lat, lon, and Date columns are at the front of the columns after Experiment_Name.

[table of contents | back to top]

Related Publications

Parker, Sierra. (2022). Visualizing The Invisible: Using Microscopy to Observe Microbial Communities Over Time. *The University of North Carolina at Chapel Hill University Libraries*. https://doi.org/10.17615/M47W-DQ06
https://doi.org/10.17615/m47w-dq06
Results

[table of contents | back to top]

Parameters

Parameter	Description	Units
Experiment_Name	Name of microcosm incubation experiment. Eco1 was initiated September 2, 2021 and Eco2 was initiated April 4, 2022	unitless
lat	Sampling location latitude, south is negative	decimal degrees
lon	Sampling location longitude, west is negative	decimal degrees
Date	Respiration assay start date	unitless
Incubation_Day	Days elapsed since microcosm 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
Method	Method of acquisition	unitless
Stain	Type of fluorecent dye used to stain cells. The flag -8888 represents no stain applied.	unitless
Excitation	The excitation wavelength/ filter cassette used on a Nikon Ti2 microscope for cell enumeration	nanometers (nm)
Magnification	The magnification of the objective lense used for cell enumeration	unitless
Size_Fraction	The filter fraction used for slide preperation.	micrometers (um)
cells_per_L_avg	The average cellular abundance from 10 fields of view. The flag -7777 denotes below detection and the flag -9999 denotes a missing value.	cells L-1
cells_per_L_sd	The standard deviation of cellular abundance from 10 fields of view. The flag -7777 denotes below detection and the flag -9999 denotes a missing value.	cells L-1
620_tot	The ratio of cells that autofluoreced under 620 nm excitation over the total number of SYBR- or DAPI-stained cells enumerated. The flag -9999 denotes a missing value.	unitless
ratio_err	The error propagation of the 620:tot ratio. The flag -9999 denotes a missing value.	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Nikon Ti2 inverted microscope
Generic Instrument Name	Inverted Microscope
Dataset-specific Description	Nikon Ti2 inverted microscope with excitation filters for: DAPI (395/25 nm), SYBR (470/40 nm), and autofloresence (620/60 nm).
Generic Instrument Description	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

[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]