HPLC data from field sampling sites in the Neuse River Estuary, Pamlico Sound, and Onslow Bay in the coastal North Atlantic, offshore from North Carolina, USA, in 2021 and 2022

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Project
» Collaborative Research: Vitamin B1 Limitation and Advantageous Use of B1-related Compounds by Marine Bacterioplankton (VBLAM)

Contributors	Affiliation	Role
Paerl, Ryan	North Carolina State University (NCSU)	Principal Investigator
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Abstract
This dataset contains high performance liquid chromatography (HPLC) data from field sampling sites in the coastal North Atlantic, offshore from North Carolina, USA, during 2021 and 2022. Study description: This study collected physical, chemical, and biological parameters measured from field sampling locations within the Neuse River Estuary the Neuse River Estuary (NRE180), Pamlico Sound (PS), and Onslow Bay (OB1). Measurements were made from near-surface waters (0.5m). See "Related Datasets" section for data from coordinated sampling efforts. Vitamin B1 amendment bottle experiments were conducted using the sample surface water, as well as collections for metagenomes and transcriptomes. Water was collected by N. Curtis and A. Zhou with the assistance of the UNC-IMS MODMON staff.

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Coverage

Location: Coastal N. Atlantic offshore from North Carolina, USA (Morehead City, Pamlico Sound, and Neuse River Estuary). Near surface waters (0.5m)
Spatial Extent: **N**:35.1225 **E**:-76.2006 **S**:34.609368 **W**:-76.6771
Temporal Extent: 2021-09-27 - 2022-08-29

Methods & Sampling

Sampling summary: Discrete samples were all obtained from ~0.2m using a diaphragm pump and weighted, marked hose. All containers were kept in dark coolers at ambient temperature during transport to the laboratory. All filtration was done within a few hours of collection and when conditions permitted, on board the research vessel.

Diagnostic phytoplankton photopigments were identified, separated and quantified by high performance liquid

chromatography (HLPC) coupled to an in-line photodiode array spectrophotometer (Jeffrey et al. 1997): Known volumes of water sample (500-1000 milliliters, enough to obtain color on the filter) were vacuum filtered (less than 25 kiloPascals) through 25 or 47 millimeter Whatman glass microfibre filters (GF/F) under reduced light conditions. The filters were blotted dry, folded in half, wrapped in foil and then immediately frozen at -20 degrees Celsius until analysis. The filters were placed in 15 milliliter centrifuge tubes containing 1.5-3.0 milliliters of 100% acetone (HPLC Grade), sonicated for 30-60 seconds using a Fisher Sonic Dismembrator 300 with microtip and extracted at -20 degrees Celsius for 12-24 hours. After extraction the samples were centrifuged at 4500 rpm and the supernatant (i.e.- the combined extracted pigments) collected & filtered into amber glass autosampler vials using Millipex Millipore 0.45 micometer PTFE. Two hundred microliters of extractant from each vial was injected into the HPLC system using a Spectra Physics (now Thermo Separations Products) AS3000 autosampler and SP8800 pump, running a non-linear, 55 minute, 2-solvent gradient adapted from Van Heukelem et.al. 1994 or 1995?. The nonlinear, variable flow, binary gradient consisted of solvent A [80% methanol : 20% ammonium acetate (0.5 M adjusted to pH 7.2)] and B (80% methanol : 20% acetone). The extractant was separated into individual pigments using a series of C18 reverse-phase columns to optimize photopigment separations: The column order was a Rainin Microsorb guard column (0.46 x 1.5 centimeters, 3 micrometer packing) followed by a single monomeric reverse-phase C18 column (Rainin Microsorb-MV, 0.46 x 10 cm, 3 µm packing) followed by two polymeric reverse-phase C18 columns (Vydac 201TP5, 0.46 x 25 cm, 5 µm packing). The columns were kept at a constant 52 degrees Celsius in an Alltech 330 column heater. The separated pigments were then passed through an in line Shimadzu SPD-M10AV photodiode array detector which measured the absorbance of the sample/extractant, scanning the range of 350-800 nanometers every 2 seconds. The data was collected and analyzed using Shimadzu's EZChrom software (Agilent Technologies, Inc (n.d.)). Individual pigments are identified using a combination of peak retention time and absorbance spectrum shape. Retention times and absorbance spectra are identified for each pigment by analyzing known pigments (either as pure standards or pigments or isolated from algal cultures). Pigments are quantified from their peak areas, calculated at 440nm. A calibration curve is generated by injecting various volumes of a mixed standard composed of known quantities of seven pure pigment standards (fucoxanthin, zeaxanthin, bacteriochlorophyll a, canthaxathin, chlorophyll b, chlorophyll a, echinenone and ß-carotene) and calculating the peak areas of those pigments The peak areas are regressed against the known quantities of each pigment to calculate the slope (Response Factor) for that pigment. Response factors for pigments we do not have reference standards for are calculated using the ratio of absorbance coefficients of each pigment to its closest structurally related reference pigment, multiplying the known pigment's response factor by that ratio. Pigments extracted from the samples are then quantified by multiplying the peak areas of a chromatogram at 440nm by the response factors. Pigment values listed as below detection were below the software threshold for peak detection or had spectra below a similarity of 0.9 compared to library spectra. Technician expert judgment was used in difficult cases.

See the "Related Datasets" section for in-situ hydrography methods and data collected as part of the same study at the same field sampling locations.

Data Processing Description

Formatting and organization of data was completed in Excel (Microsoft).

BCO-DMO Processing Description

BCO-DMO Data Manager Processing Notes:
* Data from source file BLAMM_METADATA_HPLC_7_3_24_RWP.xlsx Sheet "HPLC ugL" were imported into the BCO-DMO data system with "-7777" (meaning no data) and "-9999" (meaning below detection limit) values in numeric columns imported as blank (null) values.
* Additional *_flag columns added to provide information about what type of missing data each blank value corresponds to. For example Cantha_flag. Indicates if the blank value in the Cantha column is due to below detection limit (BDL) or not analyzed/measured/recorded (NA).

** Missing data in the BCO-DMO system displays as a blank (null values) by default and will vary depending upon the file format downloaded (blank in csv files, NaN in .mat matlab files, etc).

* Parameters (column names) renamed to comply with BCO-DMO naming conventions. See https://www.bco-dmo.org/page/bco-dmo-data-processing-conventions

* Site name (geolocation), lat, lon were added to the data table from an additional sheet provided in related dataset excel file (hydrography) BLAMM_METADATA_7_3_24_RWP.xlsx

* Decimals rounded to 5 decimal places.

* DateTime (UTC) column added in ISO 8601 format yyyy-mm-ddTHH:MM:SSZ using local date and time provided (EST/EDT). Date and time format changed to ISO 8601 format.

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Related Publications

Agilent Technologies, Inc. (n.d.). Chromatography Data Systems: OpenLab EZChrom [Computer Software]. https://www.agilent.com/en/product/software-informatics/analytical-software-suite/chromatography-data-systems/openlab-ezchrom
Software

Arar, E. J., Budde, W. L., & Behymer, T. D. (1997). Methods for the determination of chemical substances in marine and environmental matrices. National Exposure Research Laboratory, US Environmental Protection Agency, Cincinnati, OH.
Methods

Jeffrey, S. W., Mantoura, R. F. C., Wright, S. W., International Council of Scientific Unions., & Unesco. (1997). Phytoplankton pigments in oceanography: Guidelines to modern methods. Paris: UNESCO Pub.
Methods

Welschmeyer, N. A. (1994). Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. Limnology and Oceanography, 39(8), 1985–1992. doi:10.4319/lo.1994.39.8.1985
Methods

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Related Datasets

IsRelatedTo

Paerl, R., Curtis, N. (2024) **Hydrologic data from field sampling sites in the Neuse River Estuary, Pamlico Sound, and Onslow Bay in the coastal North Atlantic, offshore from North Carolina, USA, in 2021 and 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-08-19 http://lod.bco-dmo.org/id/dataset/935794 [view at BCO-DMO]
Relationship Description: Data from the same study and field sampling locations within the Neuse River Estuary the Neuse River Estuary (NRE180), Pamlico Sound (PS), and Onslow Bay (OB1).

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Parameters

Parameter	Description	Units
Station	Sampled station	unitless
Trip_Code	Individual trip code	unitless
Site	Site name (geolocation)	unitless

Lat	Site latitude	decimal degrees
Lon	Site longitude	decimal degrees
Date	Date when the in situ measurements were made. When the collection was split over two days, a single date was used based on the upstream or majority stations. (local time zone EST/EDT)	unitless
Chlide_a	Chlorophyllide a concentration by HPLC analysis.	micrograms per liter (ug/L)
Chl_c1c2	Chlorophyll c1 and c2 concentration by HPLC analysis.	micrograms per liter (ug/L)
Perid_corr	Peridinin concentration by HPLC analysis.	micrograms per liter (ug/L)
Perid_corr_flag	Perid_corr flag. Indicates if the blank value in the Perid_corr column is due to below detection limit (BDL) or not analyzed/measured/recorded (NA).	unitless
But_fuco	19'-Butanoyloxyfucoxanthin concentration by HPLC analysis.	micrograms per liter (ug/L)
But_fuco_flag	But_fuco flag. Indicates if the blank value in the But_fuco column is due to below detection limit (BDL) or not analyzed/measured/recorded (NA).	unitless
Fuco_corr	Peridinin concentration by HPLC analysis.	micrograms per liter (ug/L)
Hex_fuco	19'-Hexanoyloxyfucoxanthin concentration by HPLC analysis (micrograms per liter).	micrograms per liter (ug/L)
Hex_fuco_flag	Hex_fuco flag. Indicates if the blank value in the Hex_fuco column is due to below detection limit (BDL) or not analyzed/measured/recorded (NA).	unitless
Neo	9'-cis Neoxanthin concentration by HPLC analysis.	micrograms per liter (ug/L)
Viola	Violaxanthin concentration by HPLC analysis.	micrograms per liter (ug/L)
Diadino	Diadinoxanthin concentration by HPLC analysis.	micrograms per liter (ug/L)
Anth	Antheraxanthin concentration by HPLC analysis.	micrograms per liter (ug/L)

Myxo	Myxoxanthophyll concentration by HPLC analysis.	micrograms per liter (ug/L)
Myxo_flag	Myxo flag. Indicates if the blank value in the Myxo column is due to below detection limit (BDL) or not analyzed/measured/recorded (NA).	unitless
Allo_corr	Alloxanthin concentration by HPLC analysis.	micrograms per liter (ug/L)
Diato	Diatoxanthin concentration by HPLC analysis.	micrograms per liter (ug/L)
Diato_flag	Diato flag. Indicates if the blank value in the Diato column is due to below detection limit (BDL) or not analyzed/measured/recorded (NA).	unitless
Monado	Monadoxanthin concentration by HPLC analysis.	micrograms per liter (ug/L)
Monado_flag	Monado flag. Indicates if the blank value in the Monado column is due to below detection limit (BDL) or not analyzed/measured/recorded (NA).	unitless
Lut	Lutein concentration by HPLC analysis.	micrograms per liter (ug/L)
Zea_corr	Zeaxanthin concentration by HPLC analysis.	micrograms per liter (ug/L)
Gyro	Gyroxanthin concentration by HPLC analysis.	micrograms per liter (ug/L)
Gyro_flag	Gyro flag. Indicates if the blank value in the Gyro column is due to below detection limit (BDL) or not analyzed/measured/recorded (NA).	unitless
Cantha	Canthaxanthin concentration by HPLC analysis.	micrograms per liter (ug/L)
Cantha_flag	Cantha flag. Indicates if the blank value in the Cantha column is due to below detection limit (BDL) or not analyzed/measured/recorded (NA).	unitless
Chl_b_corr	Chlorophyll b concentration by HPLC analysis.	micrograms per liter (ug/L)
Chl_a_corr	Chlorophyll a concentration by HPLC analysis.	micrograms per liter (ug/L)
Echin	Echinenone concentration by HPLC analysis.	micrograms per liter (ug/L)

Echin_flag	Echin flag. Indicates if the blank value in the Echin column is due to below detection limit (BDL) or not analyzed/measured/recorded (NA).	unitless
B_car	ß-Carotene (beta-carotene) concentration by HPLC analysis.	micrograms per liter (ug/L)
B_car_flag	B_car flag. Indicates if the blank value in the B_car column is due to below detection limit (BDL) or not analyzed/measured/recorded (NA).	unitless
TotalChla	Total chlorophyll a concentration derived from the sum of Chlorophytes, Cryptophytes, Cyanobacteria, Diatoms, and Dinoflagellates (all in µg L-1). This concentration should equal total chlorophyll a generated from HPLC analysis.	micrograms per liter (ug/L)

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	High performance liquid chromatography (HLPC) coupled to an in-line photodiode array spectrophotometer (Jeffrey et al. 1997). Study also used a Shimadzu SPD-M10AV photodiode array detector.
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	
Generic Instrument Name	Laboratory Autosampler
Dataset-specific Description	Spectra Physics (now Thermo Separations Products) AS3000 autosampler and SP8800 pump
Generic Instrument Description	Laboratory apparatus that automatically introduces one or more samples with a predetermined volume or mass into an analytical instrument.

Dataset-specific Instrument Name	
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	High performance liquid chromatography (HLPC) coupled to an in-line photodiode array spectrophotometer (Jeffrey et al. 1997).
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

Dataset-specific Instrument Name	
Generic Instrument Name	Turner Designs Trilogy fluorometer
Generic Instrument Description	The Trilogy Laboratory Fluorometer is a compact laboratory instrument for making fluorescence, absorbance, and turbidity measurements using the appropriate snap-in application module. Fluorescence modules are available for discrete sample measurements of various fluorescent materials including chlorophyll (in vivo and extracted), rhodamine, fluorescein, cyanobacteria pigments, ammonium, CDOM, optical brighteners, and other fluorescent compounds.

Dataset-specific Instrument Name	Shimadzu UV-160U spectrophotometer
Generic Instrument Name	UV Spectrophotometer-Shimadzu
Generic Instrument Description	The Shimadzu UV Spectrophotometer is manufactured by Shimadzu Scientific Instruments (ssi.shimadzu.com). Shimadzu manufacturers several models of spectrophotometer; refer to dataset for make/model information.

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Project Information

Collaborative Research: Vitamin B1 Limitation and Advantageous Use of B1-related Compounds by Marine Bacterioplankton (VBLAM)

Coverage: Pamlico Albemarle Sound System, NC and Coastal N. Atlantic Ocean.

NSF Award Abstract:
Planktonic marine bacteria significantly impact global elemental cycling, productivity, and water quality. Recent evidence shows that abundant and diverse marine bacterioplankton require external vitamin B1 or precursors (B1 herein) to survive, and addition of these nutrients stimulates bacterial production. This suggests that it is favorable for most marine bacteria to rely on supplied B1, making B1 dynamics an environmentally relevant test case to study nutrient exchanges within the planktonic microbiome. Notably though, links between extracellular B1 availability and the composition, function, and fitness of marine bacteria are poorly understood. This project sheds light on 1) which activities and interactions are modulated by B1 availability, and 2) the benefits of exogenous B1 use by ubiquitous marine bacterioplankton. Experiments are being conducted to

address uncertainty regarding B1 limitation of natural bacterioplankton, help predict plankton responses to natural or anthropogenic increases in B1 and reveal more on the rules governing nutrient exchange between plankton. Greater knowledge of the advantages of bacterial B1 use will benefit fields beyond oceanography, such as synthetic biology which focuses on streamlined microbial product generation. B1-deficiency in animals is a current concern in marine ecosystems. Greater knowledge of costs and quotas at the microbial level will best position the larger community to ask deficiency questions at other trophic levels. The teaching and training components of this project include support for graduate students and a post-doctoral scholar. The major outreach component is a newly conceived Mobile Aquatic Microbial Laboratory (MAML) that seeks to improve public awareness of aquatic microbes and their ecosystem impact, as well as convey concepts of nutrient limitation and why cells need vitamins. Pre- and post-assessment and social distancing measures are integrated into MAML, as are incentives for participants to share via social media images and contribute to the program.

This project investigates the impact of vitamin B1 and B1 congeners on bacterioplankton in marine ecosystems where vitamins have garnered little recent consideration as influential nutrients. The work is conducted in the Neuse River estuary, the second largest estuary in the lower USA, and in the offshore coastal ocean. Nutrient amendment experiments are being conducted to test whether B1/congeners modulate bacterioplankton growth, composition, and gene transcription (reflecting putative function). Complementary lab-based experiments with wildtype and genetically engineered bacterioplankton are being conducted to test whether use of exogenous B1/congener significantly improves cell fitness and to address why reliance upon exogenous B1/congener is so prevalent among wild bacterioplankton. Additional lab experiments with isolates are being conducted to examine the protein-related cost of B1 *de novo* biosynthesis versus exogenous B1/congener use as well as cell quotas of B1 and precursors using mass spectrometry techniques. The work reaches beyond recent genome-based extrapolations to address how exogenous B1/congener availability shapes bacterioplankton metabolism, community structure, microbial interactions, and fitness.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2049388

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