Domoic acid assimilation in copepods by consuming organic polymers and Pseudo-nitzschia from experiments conducted using water samples collected in northern Gulf of Mexico in 2017 and 2018.

Website: https://www.bco-dmo.org/dataset/808413
Data Type: experimental, Other Field Results
Version: 1
Version Date: 2020-06-24

Project
» The biotic and abiotic controls on the Silicon cycle in the northern Gulf of Mexico (CLASiC)

Contributors	Affiliation	Role
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Abstract
Domoic acid assimilation in copepods by consuming organic polymers and Pseudo-nitzschia. Results from experiments designed to investigate the contribution of organic polymers and Pseudo-nitzschia to domoic acid trophic transfer. Water samples were collected in the northern Gulf of Mexico in 2017 and 2018.

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Coverage

Spatial Extent: **N**:30.278166 **E**:-87.554261 **S**:30.234973 **W**:-87.809526
Temporal Extent: 2017-07-12 - 2018-05-15

Dataset Description

Domoic acid assimilation in copepods by consuming organic polymers and Pseudo-nitzschia. Results from experiments designed to investigate the contribution of organic polymers and Pseudo-nitzschia to domoic acid trophic transfer. Water samples were collected in the northern Gulf of Mexico in 2017 and 2018. Related datasets: Organic polymers and domoic acid https://www.bco-dmo.org/dataset/808280 Domoic acid assimilation in copepods https://www.bco-dmo.org/dataset/808402

Methods & Sampling

Location

Water collection sites in the northern Gulf of Mexico, particularly at the mouth of Mobile Bay and Little Lagoon, AL.

Water Collection

Briefly, water was collected from the field using a 5-gallon bucket, pre-screened with a 200 µm nitex mesh, and gently poured into 10-20 L carboys and kept in the dark until returning to the laboratory for same-day processing.

Terminology

dDA – dissolved Domoic Acid
pDAa – particulate Domoic Acid (algal fraction)
pDAOP – particulate Domoic Acid (bound to organic polymers)
cDA – Domoic Acid in copepods
POC – Particulate Organic Carbon

Field-simulation experiments

Field water used for grazing experiments was collected during spring and summer (2017, 2018) from designated monitoring sites at Little Lagoon (Gulf Shores, Alabama, USA). The water was prefiltered with a 200 µm mesh, gently poured into carboys, and kept in the dark until the start of the laboratory experiment. Laboratory-reared adult Acartia tonsa, with no prior exposure to DA, were provided by the University of Southern Mississippi Gulf Coast Research Laboratory's Thad Cochran Marine Aquaculture Center and starved for 24 hours prior to experiment initiation.

Initial samples for Pseudo-nitzschia abundance and DA were collected. For cell abundance, 50 mL of seawater was preserved with 2 mL of Bouin's solution and stored at 4°C. A Sedgewick rafter slide was used to count cells in a 1 mL subsample. DA was measured in two forms, dDA and pDA. Seawater was filtered using a 25 mm glass fiber filter and 30 mL of filtrate was collected in a polypropylene conical tube and stored at -20°C for later analysis of dDA. pDA was sampled by filtering 100 mL of seawater under low vacuum through a 25 mm glass fiber filter and stored in a cryovial at -20°C.

The experimental design consisted of four treatments, each containing triplicate 1-L polycarbonate bottles. Two treatments contained seawater filtered through a 0.2 µm polycap filter (as described above); these treatments tested whether copepods could assimilate dDA through the proposed organic polymer-bound pathway. The remaining treatments contained seawater with a natural phytoplankton community that was concentrated by a factor of three, using a 20 µm mesh. After the bottles were filled with the appropriate water, 30 copepods were added to the necessary treatments and the experiments started. After 24 hours the copepods were collected on a 200 µm screen, gently rinsed with filtered seawater, placed in fresh filtered artificial seawater, and allowed to evacuate their guts for ~1 hour. Afterwards, copepods were once again screened and rinsed three times, and then stored in a cryovial at -20°C until analysis.

Liquid chromatography-mass spectrometry method for domoic acid quantification

LC-MS sample preparation followed was modified from Wang et al. (2012) for the determination of dDA, pDA, pDAOP and cDA. The samples for DA determination were cleaned and concentrated using Bond Elut LRC - C18, 200 mg, solid-phase extraction (SPE) columns from Agilent Technologies. For dDA, 30 mL seawater samples were filtered using a 47 mm glass fiber filter; the filtrate was collected and acidified with formic acid to yield a 0.2% final solution. SPE columns were conditioned with one column volume of HPLC-grade methanol followed by one column volume of HPLC-grade water. Samples were then loaded on the SPE column and filtered at ~1 mL min-1 using a vacuum manifold, followed by 10 mL of 0.2% formic acid as a rinse for the sample tube and SPE column. The SPE column was then allowed to go dry and was eluted with 1.5 mL of 20 mM ammonium acetate in 50% methanol (pH 8) and collected in a glass tube. The tubes were centrifuged for 5 minutes at ~1300 x g, supernatant was transferred into an LC vial with a Pasteur pipette, and stored at 4°C until further analysis. For pDAa 100 mL of seawater were filtered through a 5 µm polycarbonate filter and stored in a 50 mL polypropylene tube at -20°C. Similarly, for pDAOP 150 mL of seawater was filtered through a pre-combusted 25 mm glass-fiber filter and stored at -20°C. Prior to concentration and clean-up for pDA, pDAOP, and cDA, the filters were submerged in 2 mL of 80% methanol and sonicated to ensure cells and copepods were lysed. Sonication pulses were done for a total of 45 seconds (5 seconds on/off) on a Sonics Materials Ultrasonic Processor (model - VCX 130) at 75% power. Subsequent clean-up using the SPE column is the same as for the dDA samples.

An ultra-performance liquid chromatography (UPLC) – tandem mass spectrometry (MS) system was used for

the quantification of DA.The LC-MS system consisted of Acquity UPLC system (Waters, Milford, MA) coupled to a 5500 QTRAP triple quadrupole / linear ion trap mass spectrometer equipped with a TurboIonSpray interface (Sciex, Foster City, CA, USA). The analytes were separated on a Luna C18 (2), 2.0 x 100 mm column (Phenomenex, Torrance, CA, USA) with column temperature held at 40ºC. The mobile phase was water (A) and 95% aqueous acetonitrile (B) with 0.1% formic acid additive and the flow rate was 0.4 ml/min. Gradient program was: 5% B for 3 min, linear gradient to 60% B at 10 min, 95% B at 10.1 min, hold at 95% B for 2 min. MS was operated in positive ion mode. Ion spray voltage was 5 kV and declustering potential was 80 V. Gas parameter settings were: nebulizer gas, 50 psi; turbo gas, 50 psi at 500ºC; curtain gas, 20 psi; and collision gas, medium setting. The collision energy applied was 25eV. The transitions used for selected reaction monitoring were m/z 312→266, 193, 220. The transition m/z 312→266 was used for quantitation.

For organic polymer formation and sorption of DA results and methodology see https://www.bco-dmo.org/dataset/808280.

Data Processing Description

BCO-DMO Data Manager Processing Notes:
* Data from sheet "Field_DA-P.nitz" from originally submitted Excel file "DATASET_DA-org polymers-cop grazing_BCO-DMO_params_IAM.xlsx" extracted to csv.
* Column names modified to meet BCO-DMO naming conventions designed for interoperability (only letter, numbers, and underscores, no spaces).
* Data loaded into the bco-dmo data system with -999 as a missing data identifier.
* Year in ISO timestamp changed from 2071 to 2017 in rows 17-31 of originally submitted Excel sheet.

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Data Files

File
field-da-pnitz.csv (Comma Separated Values (.csv), 7.16 KB) MD5:3a8ad36cd70c0621179341a9d1f45871
Primary data file for dataset ID 808413

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Related Publications

Marquez, Israel A., Abraham, Ann, Krause, Jeffrey W. (in review) Marine snow consumption facilitates domoic acid entry into the marine food web without direct ingestion of Pseudo-nitzschia. Harmful Algae.
Results

Wang, Z., Maucher-Fuquay, J., Fire, S. E., Mikulski, C. M., Haynes, B., Doucette, G. J., & Ramsdell, J. S. (2012). Optimization of solid-phase extraction and liquid chromatography–tandem mass spectrometry for the determination of domoic acid in seawater, phytoplankton, and mammalian fluids and tissues. Analytica Chimica Acta, 715, 71–79. doi:10.1016/j.aca.2011.12.013
Methods

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Parameters

Parameter	Description	Units
ISO_DateTime_UTC	Date/Time (UTC) in ISO 8601 format yyyy-mm-ddTHH:MMZ	unitless
Latitude_N	Latitude in decimal degrees	decimal degrees
Longitude_W	Longitude in decimal degrees	decimal degrees
Date	Local date water was collected in format yyyymmdd	unitless
Time	Local time water was collected in format hhmm (24 hr)	unitless
Experiment	Experiment name	unitless
Treatment	Treatment name	unitless
Replicate_bottle	Letters denote a unique bottle that was sampled for each measurement	unitless
dDA_conc	dissolved Domoic Acid in seawater	micrograms (µg) per liter (L)
pDAa_conc	particulate Domoic Acid particles (> 5 um) in seawater	nanograms (ng) per liter (L)
Cell_tox	Cell toxicity, domoic acid normalized per Pseudo-nitzschia cell	picograms (pg) per cell
Pnitz_density	Pseudo-nitzschia cell concentration	cells per liter
DA_cop_indiv	Total Domoic Acid in copepods normalized per individual	picograms (pg) per copepod

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Costech International Elemental Combustion System (ECS) 4010
Generic Instrument Description	The ECS 4010 Nitrogen / Protein Analyzer is an elemental combustion analyser for CHNSO elemental analysis and Nitrogen / Protein determination. The GC oven and separation column have a temperature range of 30-110 degC, with control of +/- 0.1 degC.

Dataset-specific Instrument Name	Sonics Materials Ultrasonic Processor (model - VCX 130)
Generic Instrument Name	Homogenizer
Generic Instrument Description	A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others.

Dataset-specific Instrument Name	Acquity UPLC system coupled to a 5500 QTRAP
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Acquity UPLC system (Waters, Milford, MA) coupled to a 5500 QTRAP triple quadrupole / linear ion trap mass spectrometer equipped with a TurboIonSpray interface (Sciex, Foster City, CA, USA).
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

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Project Information

The biotic and abiotic controls on the Silicon cycle in the northern Gulf of Mexico (CLASiC)

Coverage: Northern Gulf of Mexico, specifically the Louisiana Shelf region dominated by the discharge of the Mississippi River on the western side of the delta

NSF Award Abstract:
The Louisiana Shelf system in the northern Gulf of Mexico is fed by the Mississippi River and its many tributaries which contribute large quantities of nutrients from agricultural fertilizer to the region. Input of these nutrients, especially nitrogen, has led to eutrophication. Eutrophication is the process wherein a body of water such as the Louisiana Shelf becomes enriched in dissolved nutrients that increase phytoplankton growth which eventually leads to decreased oxygen levels in bottom waters. This has certainly been observed in this area, and diatoms, a phytoplankton which represents the base of the food chain, have shown variable silicon/nitrogen (Si/N) ratios. Because diatoms create their shells from silicon, their growth is controlled not only by nitrogen inputs but the availability of silicon. Lower Si/N ratios are showing that silicon may be playing an increasingly important role in regulating diatom production in the system. For this reason, a scientist from the University of South Alabama will determine the biogeochemical processes controlling changes in Si/N ratios in the Louisiana Shelf system. One graduate student on their way to a doctorate degree and three undergraduate students will be supported and trained as part of this project. Also, four scholarships for low-income, high school students from Title 1 schools will get to participate in a month-long summer Marine Science course at the Dauphin Island Sea Laboratory and be included in the research project. The study has significant societal benefits given this is an area where $2.4 trillion gross domestic product revenue is tied up in coastal resources. Since diatoms are at the base of the food chain that is the biotic control on said coastal resources, the growth of diatoms in response to eutrophication is important to study.

Eutrophication of the Mississippi River and its tributaries has the potential to alter the biological landscape of the Louisiana Shelf system in the northern Gulf of Mexico by influencing the Si/N ratios below those that are optimal for diatom growth. A scientist from the University of South Alabama believes the observed changes in the Si/N ratio may indicate silicon now plays an important role in regulating diatom production in the system. As such, understanding the biotic and abiotic processes controlling the silicon cycle is crucial because diatoms

dominate at the base of the food chain in this highly productive region. The study will focus on following issues: (1) the importance of recycled silicon sources on diatom production; (2) can heavily-silicified diatoms adapt to changing Si/N ratios more effectively than lightly-silicified diatoms; and (3) the role of reverse weathering in sequestering silicon thereby reducing diffusive pore-water transport. To attain these goals, a new analytical approach, the PDMPO method (compound 2-(4-pyridyl)-5-((4-(2-dimethylaminoethylamino-carbamoyl)methoxy)phenyl)oxazole) that quantitatively measures taxa-specific silica production would be used.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1558957
U.S. Food and Drug Administration (FDA)	5U19FD005923-04

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