Toxin content of Alexandrium catenella in response of nitrogen sources, algal alarm cues, and grazer exposure

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Project
» Chemical Defenses in a Toxic Dinoflagellate: Mechanisms and Constraints (Chemical Defenses)

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Abstract
Data include Paralytic Shellfish Toxin production of the marine dinoflagellate Alexandrium catenella. PST production of A. catenella was measured as a function of varying concentrations of added nitrogen sources (ammonium and urea), alarm cues from lysed conspecific (A. catenella Group I strains) and interspecific (the diatom, Thalassiosira weissflogii, and the green flagellate, Tetraselmis sp.) algae, and the presence of a grazer (the copepod Acartia hudsonica). Independent variable: treatment type such as nitrogen sources (mol per liter), algal alarm cues (carbon content per cell), and grazer exposure in either the F/2 or FSW assays (control) Dependent variables: cell density (cells per liter), cell toxin content (mol per cell), cell diameter (µm per cell) Data were published in: Griffin, J. E., Park, G., & Dam, H. G. (2019). Relative importance of nitrogen sources, algal alarm cues and grazer exposure to toxin production of the marine dinoflagellate Alexandrium catenella. Harmful algae, 84, 181-187. https://doi.org/10.1016/j.hal.2019.04.006

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Coverage

Spatial Extent: **Lat**:41.320717 **Lon**:-72.06196
Temporal Extent: 2011-09 - 2018-04

Methods & Sampling

Refer to the Methods section of Griffin, et al. (2019).

Culture and culturing condition:
All assays were run with the dinoflagellate *Alexandrium catenella* strain BF-5, a highly toxigenic strain isolated from the Bay of Fundy, Canada. Cells were cultured in F/2 medium (Guillard, 1975) without silicate. Batch cultures were kept under exponential-phase growth at a temperature of 18 °C, with a photoperiod of 12 h of light and 12 h of darkness. Fluorescent lights were used at an intensity of ~50 µM m^{-2} s^{-1}.

Nitrogen sources:
These assays simulated potential bias due to N-sources (ammonium and urea) excreted by the copepod grazer *Acartia hudsonica*. Treatments consisted of daily additions of either 0.083 µmol L^{-1} ammonium, 50 µmol L^{-1} ammonium, or 50 µmol L^{-1} urea over a 3 day period. The lower ammonium concentration (0.083 µmol L^{-1}) is an estimate of the nitrogen excreted per day from the 15 copepods in a bottle. The higher concentration of ammonium (50 µmol L^{-1}) represents the ammonium concentration in K-medium (Keller et al., 1987). The F/2 medium in nutrient-replete conditions contains nitrate as its N-source. Urea was used at a concentration of 50 µmol L^{-1}, which is far higher than could result from copepod excretion. The controls consisted of cells grown in F/2 medium (880 µmol L^{-1} nitrate and 36.3 µmol L^{-1} phosphate) or 0.22 µm filtered seawater (FSW) collected from Long Island Sound (~3 µmol L^{-1} nitrate and ~1 µmol L^{-1} phosphate).

Alarm cues:
Crushed cells of the highly toxigenic *Alexandrium catenella* (strain BF-5), the low toxigenic *A. catenella* (strain GTCN-16), *Tetraselmis* sp., and *Thalassisosira weissflogii* were added to separate experimental bottles. Added cells were crushed via sonic dismembrator on ice. Complete lysis of cells was confirmed by microscopic examination. 140 µgC of crushed cells for each species tested were added daily to bottles during the three-day period incubations. Cell-to-carbon conversion factors used were 2.7 x 10^{-3} µgC per *Alexandrium* cell, 8.0 x 10^{-5} µgC per *T. weissflogii* cell, and 5.1 x 10^{-5} µgC per *Tetraselmis* sp. cell desired quantity of carbon.

Grazer exposure:
Grazer assays measured the combined effects of copepod kairomone and other feeding-related cues on cell toxin production. Treatments consisted of additions of 15 adult female copepods (*Acartia hudsonica)* collected from Maine, USA, which have a history of exposure to *A. catenella* blooms. Copepods had been kept in culture conditions similar to the experimental algae, and grown on a mixed diet of nontoxigenic phytoplankton. At the conclusion of the grazing assays, contents of the bottles were first passed through a 63 µm mesh to separate grazers, eggs and fecal pellets from the *Alexandrium catenella* cells. The filtrate was then passed through a 10 µm mesh, which collected the washed *A. catenella* cells, which were then checked to ensure the absence of cells and resuspended in 0.22 µm-FSW in 50 mL centrifuge tubes.

Cell concentration and toxin analysis:
In the nitrogen source and alarm cue assays, which did not involve use of copepods, there was no need to pass cells through the 63 µm mesh before cells were collected on the 10 µm mesh. A fraction of the contents of each centrifuge tube was preserved using Lugol's solution, and two subsamples were counted under an inverted microscope for cell abundance. In the remaining fraction, cells were centrifuged at 4000 × g for 20 minutes. The supernatant was carefully discarded and the cell pellet was resuspended in 0.1 M acetic acid. Cells were then lysed using a sonic dismembrator on ice. The solution was centrifuged and the extract was filtered through a 0.45 µm ultracentrifuge cartridge to remove the cell particles. The supernatant was stored at -80°C to prevent chemical reactions from occurring during the period before processing. After toxin extraction, toxin analysis by High Performance Liquid Chromatography (HPLC) with fluorescent detection was used to determine the total toxin content.

Data Processing Description

Data Processing:
A multiple comparison analysis of variance (ANOVA) with the Student–Newman–Keul's (SNK) post-hoc test was performed for each assay, to test for the treatment effect and differences within treatments, using the statistical program R version 3.3.1. The program R and SPSS version 26 were also used to create box and whisker plots to represent toxin production for each assay.

Data Files

File
griffin_2019.csv(Comma Separated Values (.csv), 1.44 KB) MD5:0e73aef79c782e9d80efe08e5aa40cac
Primary data file for dataset ID 853877

Related Publications

Griffin, J. E., Park, G., & Dam, H. G. (2019). Relative importance of nitrogen sources, algal alarm cues and grazer exposure to toxin production of the marine dinoflagellate Alexandrium catenella. Harmful Algae, 84, 181–187. doi:10.1016/j.hal.2019.04.006
Results

Guillard, R. R. L. (1975). Culture of Phytoplankton for Feeding Marine Invertebrates. Culture of Marine Invertebrate Animals, 29–60. doi:10.1007/978-1-4615-8714-9_3
Methods

Keller, M. D., Selvin, R. C., Claus, W., & Guillard, R. R. L. (2007). MEDIA FOR THE CULTURE OF OCEANIC ULTRAPHYTOPLANKTON1,2. Journal of Phycology, 23(4), 633–638. doi:10.1111/j.1529-8817.1987.tb04217.x
Methods

Parameters

Parameter	Description	Units
Media	F/2 medium: nutrient-replete condition; 0.22 um-filtered seawater (FSW): nutrient-limited condition	unitless
Treatment_Type	N-sources: Low ammonium (0.083umol_NH4); high ammonium (50umol_NH4); urea=50 umol Lu1 (in micromoles per liter (umol L-1)). Alarm cues: lysed high toxigenic A. catenella (High_tox); lysed low toxigenic A. catenella (Low_tox); lysed Thalassiosira weissflogii (TW); lysed Tetraselmis sp. (Tetra) (in micrograms Carbon per cell (ug C per cell)). Grazer exposure: exposed to copepod Acartia hudsonica (Grazer). Control: F/2 medium or FSW.	see description
Cell_Number	Cell concentration	cells per milliliter (cells ml-1)
Diameter	The mean size of Alexandrium catenella cells	micrometer (um)
Toxicity	Total cellular toxin content in saxitoxin equivalents	picograms saxitoxin equivalents per cell (pg STXeq cell-1)
Toxin_Content	Total cellular toxin content	femtomoles per cell (fmol cell-1)

Instruments

Dataset-specific Instrument Name	HPLC system (Waters, Milford, MA)
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	High Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). Scanning Fluorescence Detector (Waters 474, Waters, Milford, MA) was used for in-vitro diagnostic testing to analyze compounds of STX and its derivatives.
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	Olympus IX70 inverted system microscope
Generic Instrument Name	Inverted Microscope
Dataset-specific Description	The IX70 inverted tissue culture microscope is a research-level instrument capable of imaging specimens in a variety of illumination modes including brightfield, darkfield, phase contrast, Hoffman modulation contrast, fluorescence, and differential interference contrast.
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.

Dataset-specific Instrument Name	Sonic dismembrator (Model 50, Fisher Scientific)
Generic Instrument Name	ultrasonic cell disrupter (sonicator)
Dataset-specific Description	The Fisher Scientific™ Model 50 Sonic Dismembrator is compact, portable and extremely simple to operate. Weighing less than 4 lb., this model is the smallest unit on the market and is highly effective for cell disruption, sample preparation and many other small volume applications.
Generic Instrument Description	Instrument that applies sound energy to agitate particles in a sample.

Project Information

Chemical Defenses in a Toxic Dinoflagellate: Mechanisms and Constraints (Chemical Defenses)

Coverage: New England waters from Connecticut to Maine

Description from NSF award abstract:
Species of the dinoflagellate genus Alexandrium occur around the globe, and some species, because of their toxin production, have been hypothesized to be keystone species. Alexandrium produces chemical compounds that appear to target different consumers. Neurotoxins such as PST target metazoan grazers. In preliminary experiments in their laboratory, the investigators also verified the presence of reactive oxygen species that target, at a minimum, protistan grazers. Such compounds reduce grazer fitness, and, at least in the case of PST, have been shown to have profound evolutionary effects on grazers. Grazer adaptation, in turn, can affect Alexandrium population dynamics. A common assumption is that production of toxic compounds in phytoplankton represents an adaptive defense. However, unequivocal experimental evidence in support of this hypothesis is scarce. This project will be a rigorous experimental test of the chemical defense hypothesis. The project's investigators will investigate a series of experimentally falsifiable hypotheses with both metazoan and protistan grazers challenged with Alexandrium. This project will provide novel understanding of, and insight into, the factors that determine grazer-induced toxin production, the relationship between degree of chemical defense and susceptibility to grazing, and the costs and tradeoffs of the purported mechanisms of chemical defense in Alexandrium. Verification or refutation of the chemical defense hypothesis is essential to conceptual models of the formation, control and persistence of toxic algal blooms, and chemically-mediated predator-prey interactions.

Funding

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