Cell-growth gene expression reveals a direct fitness cost of grazer-induced toxin production in red tide dinoflagellate prey

Website: https://www.bco-dmo.org/dataset/853900
Data Type: experimental, Other Field Results
Version: 1
Version Date: 2021-06-16

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

Contributors	Affiliation	Role
Dam, Hans G.	University of Connecticut (UConn)	Principal Investigator, Contact
Park, Gihong	University of Connecticut (UConn)	Contact
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Data include Paralytic Shellfish Toxins (STX and its derivatives) of the marine dinoflagellate Alexandrium catenella, cell growth rates, and relative gene expression (RGE) level of the toxin gene compared to the reference gene as values of the independent and dependent variables. Independent variable: exposure time (per day) of cells either without grazers (control) or with grazers (treatment) Dependent variables: cell density (cells per liter), cell toxin content (mol per cell), cell growth rate (per day), and RGE (unitless) Data were published in: Park, G., & Dam, H. G. (2021). Cell-growth gene expression reveals a direct fitness cost of grazer-induced toxin production in red tide dinoflagellate prey. Proceedings of the Royal Society B, 288(1944), 20202480. https://doi.org/10.1098/rspb.2020.2480

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Coverage

Spatial Extent: **Lat**:41.320717 **Lon**:-72.06196
Temporal Extent: 2011-09 - 2018-05

Methods & Sampling

Refer to the Methods section of Park & Dam (2021).

Sample collection and culture:
The toxigenic dinoflagellate, *Alexandrium catenella* (strain BF-5, isolated from the Bay of Fundy, Canada) was grown in F/2 medium without silicate. Cultures were maintained in the exponential growth phase and all experiments were conducted in an environmental chamber kept at 18°C and illuminated with fluorescent lighting (~100 µM m^{-2} s^{-1}) set to a 12 h:12 h light:dark photoperiod. The calanoid copepod *Acartia hudsonica,* historically co-occurring with toxic *A. catenella,* was collected from Casco Bay, Maine, U.S.A. (43°39′N, 74°47′W), a location in which blooms of *A. catenella* are common. Triplicate copepod cultures were maintained with a mixed diet of *Rhodomonas* sp., *Tetraselmis* sp., and *Thalassiosira weissflogii*.

Grazer-induced toxin production assay:
Alexandrium catenella cells were placed in 500 ml bottles (300 cells ml^{-1}) either without copepods (controls: constitutive toxin production) or with 20 adult female *A. hudsonica* (treatments) for a period of 96 h. Experiments were done in quadruplicate sets for both control and treatment, and carried out at 18 °C in a walk-in environmental chamber as described above. For the time series analysis, *A. catenella* cells were harvested at the conclusion of each exposure time (0, 4, 8, 24, 48, 72, and 96 h) after which cells were gently separated by wet-sieving onto a 63 µm mesh to remove copepods, nauplii, eggs, and fecal pellets. Two aliquots from each bottle were filtered onto 5 µm pore size polycarbonate membranes; one for toxin analysis and another for RNA extraction.

Toxin and gene expression analysis:
PST concentrations were determined by reverse-phase ion-pairing high performance liquid chromatography (HPLC) using the post-column oxidative fluorescence method. Gene expression analyses were conducted using reverse transcription quantitative PCR (RT-qPCR). From previous studies several pairs of primers were designed and tested for specificity and PCR efficiency by RT-qPCR. All quantitative PCRs (qPCRs) were performed on a StepOnePlus™ real-time PCR system (Applied Biosystems) and run in 10 µL reactions with Fast SYBR® Green Master Mix (Applied Biosystems).

Data Processing Description

Data Processing:
A Kruskal-Wallis one-way ANOVA on ranks was used and all pairwise comparisons among controls/treatments were assessed with the Student-Newman-Keul's (SNK) post-hoc procedure. The effects of day, presence of grazer, and their interaction on cell concentration, toxin content, toxin profile, growth rate, and relative gene expression (four independent replicates per test and two samples for PST analysis) were tested by two-way ANOVA. Time-dependent changes in these variables were also tested separately by regression analysis for the control and treatments. Ingestion rates were tested with a Mann-Whitney U Test to compare the Frost equation to our modified equation. All statistical analyses were performed using SigmaPlot version 11.0 and SPSS version 26 software.

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Data Files

File
park_dam_2021.csv (Comma Separated Values (.csv), 4.73 KB) MD5:268dae7956bb150a223dd17a6d71bc06 Primary data file for dataset ID 853900

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Related Publications

Park, G., & Dam, H. G. (2021). Cell-growth gene expression reveals a direct fitness cost of grazer-induced toxin production in red tide dinoflagellate prey. Proceedings of the Royal Society B: Biological Sciences, 288(1944), 20202480. doi:10.1098/rspb.2020.2480
Results

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Parameters

Parameter	Description	Units
Name	Alexandrium catenella cells, either without grazers (control) or with grazers (treatment)	unitless
Day	Experimental time for a period of 4 days	days
Hour	Harvested time at the conclusion of each exposure hr (0, 4, 8, 24, 48, 72, and 96 hr)	hours
Cell_Number	Cell concentration	cells per milliliter (cells ml-1)
Growth_Rate	Net growth rate	per day (d-1)
Toxin_Content	Total cellular toxin content	femtomoles per cell (fmol cell-1)
utox	Toxin production rate	per day (d-1)
Rtox	Net toxin production rate	femtomoles per cell per day (fmol cell-1 d-1)
GTX3	gonyautoxin 3	femtomoles per cell (fmol cell-1)
GTX4	gonyautoxin 4	femtomoles per cell (fmol cell-1)
NEO	neosaxitoxin	femtomoles per cell (fmol cell-1)
STX	saxitoxin	femtomoles per cell (fmol cell-1)
C2	C-2 toxin	femtomoles per cell (fmol cell-1)
RGE_stxA4	Relative gene expression level of the stxA4 gene compared to the reference gene (lbpn)	unitless
RGE_stxG	Relative gene expression level of the stxG gene compared to the reference gene (lbpn)	unitless
RGE_cyc	Relative gene expression level of the cyc gene compared to the reference gene (lbpn)	unitless
g	Grazing rate	per day (d-1)
g_modified	Modified grazing rate (this study)	per day (d-1)
Cm	Average cell concentration	cells per milliliter (cells ml-1)
Cm_modified	Modified average cell concentration (this study)	cells per milliliter (cells ml-1)
I	Ingestion rate	cells per copepod per day (cells cop-1 d-1)
I_modified	Modified ingestion rate (this study)	cells per copepod per day (cells cop-1 d-1)

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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	Fastprep-24 Tissue and Cell Homogenizer (MP Biomedicals, Solon, OH)
Generic Instrument Name	Homogenizer
Dataset-specific Description	The FastPrep of benchtop instrument utilizes bead-beating technology to lyse, homogenize and grind routine and difficult samples in 40 seconds or less. A completely self-contained system, FastPrep instrument eliminates the risk of cross-contamination and time-consuming clean up. The use of interchangeable adapters offers exceptional versatility in sample size and temperature with ambient, cryogenic and all-metal options.
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	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	StepOnePlus™ real-time PCR system (Applied BiosystemsTM)
Generic Instrument Name	qPCR Thermal Cycler
Dataset-specific Description	The system can be setup in a variety of configurations and comes ready to use, out of the box, with intuitive data analysis and instrument control software. Utilizing robust LED based 4-color optical recording, the StepOnePlus™ Real-Time PCR System is designed to deliver precise, quantitative Real-Time PCR results for a variety of genomic research applications.
Generic Instrument Description	An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).

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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.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1130284

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