Influence of predator-prey evolutionary history, chemical alarm-cues and feeding selection on induction of toxin production in a marine dinoflagellate

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

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Abstract
Data include induced toxin production in the marine dinoflagellate Alexandrium catenella (formerly known as A. fundyense). The hypothesis that history of co-occurrence between predator and prey affects the ability of prey to recognize and respond to predators with increased toxin production was tested for this copepod-alga interaction. Independent variable: direct and indirect induction of toxin production, algal alarm-cue, and feeding selectivity Dependent variables: toxicity (pgSTXeq per cell) and ingestion rate (cells per copepod per hour) Data were published in: Senft-Batoh, C. D., Dam, H. G., Shumway, S. E., Wikfors, G. H., & Schlichting, C. D. (2015). Influence of predator–prey evolutionary history, chemical alarm-cues, and feeding selection on induction of toxin production in a marine dinoflagellate. Limnol. Oceanogr., 60(1), 318-328. https://doi.org/10.1002/lno.10027

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Coverage

Spatial Extent: **Lat**:41.320717 **Lon**:-72.06196
Temporal Extent: 2010-09 - 2012-01

Acquisition Description

Refer to the Methods section of Senft-Batoh, et al. (2015).

Phytoplankton Culturing:
The dinoflagellates, *Alexandrium fundyense* (toxic strain BF-5, isolated from Bay of Fundy, Canada) and *Alexandrium tamarense* (nontoxic, isolated from Mumford Cove, Groton, Connecticut), were grown in semi-continuous culture in F/2 medium without silicate. Cultures were maintained in, and all experiments were conducted in, an environmental chamber with fluorescent lighting set to a 12 h:12 h light:dark photoperiod (100 μmol m^{-2} s^{-1} photosynthetically active radiation) and 18°C. The *Alexandrium fundyense* strain was used in grazer-enhanced toxin production, feeding-selection, and algal alarm-cue assays, while *Alexandrium tamarense* was used in the feeding-selection and alarm-cue assays. Aside from production of paralytic shellfish toxin, *A. tamarense* and *A. fundyense* are nearly identical in shape, size, and carbon and nitrogen content per cell.

Copepod Collection and culturing:
The calanoid copepod *Acartia hudsonica* was collected from Casco Bay, Maine (43°39'N, 74°47'W; historically co-occurring with toxic *Alexandrium*, and Little Egg Harbor, Tuckerton, New Jersey (39°63'N, 74°33'W). Triplicate cultures for each copepod population were maintained with a mixed phytoplankton food medium replenished thrice weekly. Copepods were cultured for at least three generations (~3 months) prior to experiments to eliminate maternal and environmental effects. Animals (eggs to adults) in cultures were transferred monthly to new containers. Prior to assays, adult, female copepods were acclimatized to experimental conditions for 24 h and starved during that period to ensure complete gut evacuation.

Direct and indirect induction of toxin production by copepods:
Direct and indirect mechanisms of toxin induction were tested simultaneously using experimental cages. 1 L polycarbonate beakers with bottoms made of 10 μm mesh were nested within another 1 L beaker containing 500 mL of toxic *Alexandrium fundyense* (300 cells mL^{-1}). The mesh isolated these cells from materials within the cage. Adult female *Acartia hudsonica* (15 individuals) from either Maine or New Jersey were added to each cage and offered a diet of toxic *A. fundyense* (300 cells mL^{-1}) or were starved (no addition of algal food). Triplicate treatments (n=3) of the combinations of copepods and algal food within the cages were: 1) Maine copepods fed toxic algae; 2) Maine copepods starved; 3) New Jersey copepods fed toxic algae; 4) New Jersey copepods starved. Control cages (n=3) contained 300 cells mL^{-1} of toxic algae and no copepods. Assays were run for 72 h, long enough to ensure induction of toxin production, and incubation conditions were identical to those of the algal cultures. Cages were lifted every 12 hours to ensure exchange of cues between compartments. At the termination of the assay, cells of *Alexandrium fundyense* within cages (where applicable) and below cages were collected from treatments and controls for toxin analysis (*see* below). Cells within cages (treatments and controls) were enumerated microscopically, before and after incubation, to calculate copepod ingestion rates (Frost 1972). Differences in ingestion rate between the populations were assessed by a t-test.

Induction of toxin production by algal alarm cue:
To determine if alarm cues released by *Alexandrium fundyense*, and a congener (but non-toxic) species, *Alexandrium tamarense,* could induce toxin production in a culture of *A. fundyense*, extracts of sonicated, conspecific cells or cells of *A. tamarense* (equivalent to 50,000 cells; complete lysis confirmed microscopically), were added daily, over a 3 day period, to triplicate treatments (n=3 for *A. fundyense* and *A. tamarense* extracts) of 500 mL, nutrient-replete (F/2) cultures of *A. fundyense* (300 cells mL^{-1}). The daily re-inoculation of cue-receiving cultures with sonicated extracts ensured that potentially labile alarm cues were maintained throughout the duration of the experiment. Extracts were not added to control cultures (n=3).

Toxin analysis:
Cells from treatments and controls were collected on a 10 µm mesh and resuspended in filtered seawater in a 50 mL centrifuge tube. Replicate subsamples (1 mL) were taken from each tube, and cells were enumerated microscopically to determine the number of cells present in each extract (50,000-150,000 cells, depending on the assay). Cells were centrifuged at 4,000 x g for 20 minutes. The seawater supernatant was decanted, and the cell pellet was resuspended in 1 mL of 0.1 M acetic acid. Cells were lysed using a probe sonic dismembrator. Sonication was conducted with the tube immersed in ice to prevent heating of the samples. Samples were then centrifuged again at 4,000 x g for 20 minutes to remove cell debris. The acetic acid supernatant (extract) was filtered through a 0.45 µm ultracentrifuge filter cartridge to remove any remaining particles. Samples were stored at -80°C until analysis.

Concentrations of saxitoxin (STX), neosaxitoxin (NEO) and gonyautoxins 1 through 4 (GTX 1-4), as well as the sulfamate congeners, C1 and C2 were measured using high-performance liquid chromatography with fluorescence detection (Oshima 1995) after calibration with standards (Certified Reference Materials Program, NRC Institute for Marine Biosciences, Canada) and expressed as mass of STX equivalents per cell according to conversion factors of Oshima (1995).

Processing Description

Data Processing:
A one-way ANOVA coupled with post-hoc Holm-Sidak means analysis was used to determine significance of differences in cellular toxin content of *A. fundyense* for assays on direct and indirect induction of toxin production by copepods and algal alarm-cue. A 2x2 factorial ANOVA was also performed for indirect induction measurements to test significance of the effects of population, diet, or the interaction of these two variables upon cell-toxin content of *A. fundyense*, and the effects of the mechanism, grazer population and the interaction. Ingestion rates of copepods on both species of *Alexandrium* were determined by cell disappearance according to the methods of Frost (1972). Selection for cells of lower toxin content (positive selection) was indicated if more than 50% of the total ingested cells were nontoxic (Chesson's alpha index of selectivity, Chesson, 1983). Statistical analyses for this and all prior assays were performed using SigmaPlot version 11.0 software.

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Related Publications

Chesson, J. (1983). The Estimation and Analysis of Preference and Its Relatioship to Foraging Models. Ecology, 64(5), 1297–1304. doi:10.2307/1937838
Methods

Frost, B. W. (1972). Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod Calanus pacificus. Limnology and Oceanography, 17(6), 805–815. doi:10.4319/lo.1972.17.6.0805
Methods

Oshima, Y. (1995). Post-column derivatization HPLC methods for paralytic shellfish poisons. In: Manual on Harmful Marine Microalgae (eds. Hallegraeff, G. M., Anderson, D. M., Cembella, A. D. & Enevoldsen, H. O.). IOC manuals and guides No. 33, UNESCO, pp. 81-94.
Methods

Senft-Batoh, C. D., Dam, H. G., Shumway, S. E., Wikfors, G. H., & Schlichting, C. D. (2015). Influence of predator-prey evolutionary history, chemical alarm-cues, and feeding selection on induction of toxin production in a marine dinoflagellate. Limnology and Oceanography, 60(1), 318–328. doi:10.1002/lno.10027
Results

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Parameters

Parameter	Description	Units
Induction	Copepod-induced toxin production: direct induction (within cage), indirect induction (outside cage; cue-receiving cells), algal cue (receiving lysed cells), and control (no copepod)	unitless
Copepod	Acartia hudsonica collected from Maine (ME) and New Jersey (NJ, starved copepods or copepods grazing on toxic Alexandrium)	unitless
Alexandrium	Alexandrium strains: toxic strain (TOX) and non toxic strain (NONTOX)	unitless
Algal_cue	Algal cue induction: lysed cells of nontoxic Alexandrium (NONTOX_extract) and lysed cells of toxic Alexandrium (TOX_extract)	unitless
Toxicity	Total cellular toxin content in saxitoxin equivalents	picograms saxitoxin equivalents per cell (pg STXeq cell-1)
Ingestion	Ingestion rate of copepods from Maine and New Jersey	cells per copepod per hour (cells copepod-1 hour-1)
Figure	Figure number in Senft-Batoh et al. (2015)	unitless

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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	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.

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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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