Reactive oxygen species are linked to the toxicity of the dinoflagellate Alexandrium spp. to protists

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

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Abstract
Data include the survival response of the ciliate, Tiarina fusus, and the heterotrophic dinoflagellate, Polykrikos kofoidii, to three strains in the Alexandrium tamarense species complex. Independent variable: protists (Tiarina fusus and Polykrikos kofoidii), 3 strains in the Alexandrium with a different paralytic shellfish toxin (PST) content (High, Low, No PST), and cell densities of each dinoflagellate isolate (cells per milliliter) Dependent variables: survived protist (number per well) and protist survival (%) Data were published in: Flores, H. S., Wikfors, G. H., & Dam, H. G. (2012). Reactive oxygen species are linked to the toxicity of the dinoflagellate Alexandrium spp. to protists. Aquatic microbial ecology, 66(2), 199-209. https://doi.org/10.3354/ame01570

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Coverage

Spatial Extent: **Lat**:41.320717 **Lon**:-72.06196
Temporal Extent: 2008-09 - 2010-05

Methods & Sampling

Refer to the Methods section of Flores et al. (2012).

Culture and culturing conditions:
Three strains in the *Alexandrium tamarense* species complex (hereafter referred to as *Alexandrium* spp.) and one strain each of the dinoflagellates *Lingulodinium polyedra* and *Scrippsiella trochoidea* were maintained in f/2 medium without silicate (Guillard 1975) at 18°C on a 12:12 h light-dark cycle. Cultures were transferred biweekly to fresh medium and were in exponential growth for all experiments. The ciliate, *Tiarina fusus*, was isolated from Long Island Sound off Avery Point, Connecticut in June 2008. Ciliate cultures were maintained in 25-cm² polystyrene tissue-culture flasks containing 20 ml of f/2 medium, to which the dinoflagellate, *Lingulodinium polyedra*, was added as a food source. The heterotrophic dinoflagellate, *Polykrikos kofoidii*, was isolated from Northport Bay, located on the north shore of Long Island, NY, during a bloom of *Alexandrium*

spp. in May 2009. *P. kofoidii* cultures were maintained in 6-well, polystyrene tissue-culture plates and were fed a mixture of *L. polyedra* and *Scrippsiella trochoidea*. All heterotrophic protist cultures were incubated at 18°C with a 12:12h light-dark cycle and were transferred weekly or biweekly into fresh medium containing prey.

Interactions between *Alexandrium* spp. and heterotrophic protists:
Observational experiments were conducted to examine qualitatively the effects of each *Alexandrium* spp. strain upon *Tiarina fusus* and *Polykrikos kofoidii*. Groups of 25 *T. fusus* or *P. kofoidii* cells were transferred by micropipette into individual wells of 12-well, polystyrene tissue-culture plates containing 2 ml of 0.2-µm filtered seawater (FSW). Both heterotrophic protist species were starved for 24 h prior to experimentation to ensure digestion of any recently-ingested *Lingulodinium polyedra* or *Scrippsiella trochoidea* cells from the stock cultures. Following starvation, aliquots of each *Alexandrium* spp. culture were added to the wells containing *T. fusus* or *P. kofoidii*. For each *Alexandrium* spp. strain, cell densities of 200 and 2,000 cells ml^{-1} were tested. Controls consisted of FSW and *L. polyedra* (200 or 2,000 cells ml^{-1}). The behavior of individual *T. fusus* and *P. kofoidii* cells was observed under a stereomicroscope at 15-min intervals for 2 h.

Culture filtrates and extracts:
Experiments were conducted with *Tiarina fusus* to examine the effects of cell-free *Alexandrium* spp. culture filtrates and extracts upon ciliate survival. *Alexandrium* spp. cultures were diluted with f/2 medium to a density of 1,000 cells ml^{-1}. An aliquot (20 ml) of each *Alexandrium* spp. culture was filtered gently through a 0.2-µm syringe filter, resulting in a filtrate free of both *Alexandrium* spp. cells and bacteria. To examine the possible effects of bacteria present in the *Alexandrium* spp. cultures on *T. fusus* survival, additional aliquots (20 ml) from each *Alexandrium* spp. culture were filtered through 5-µm syringe filters, allowing bacteria, but not *Alexandrium* spp. cells, to pass through the filter. Cell extracts from *Alexandrium* spp. cultures were prepared by sonicating *Alexandrium* spp. culture aliquots (20 ml), on ice, with a Fisher model 100 sonic dismembrator until cells were completely disrupted (as confirmed by microscopy). Following sonication, the extracted samples were filtered through a 0.2-µm syringe filter to remove cell debris. Filtrates and extracts were added (5 ml; in triplicate) to individual wells of a 12-well, polystyrene tissue-culture plate. Controls consisted of intact *Alexandrium* spp. cultures and FSW. Fifteen ciliates were added to each experimental well, and treatments were incubated and enumerated as described above.

Physical separation from live *Alexandrium* spp. culture:
In order to determine if the observed mortality of *Tiarina fusus* exposed to *Alexandrium* spp. is a result of physical contact with and/or ingestion of the dinoflagellate. Groups of 15 *T. fusus* cells were placed into individual wells of 12-well, polystyrene tissue-culture plates containing FSW (2.5 ml). A culture plate insert with a10-µm nylon mesh bottom was added to each experimental well. Aliquots (1.5 ml) of each *Alexandrium* spp. culture were added to each culture insert, resulting in a final concentration of dissolved compounds in the treatment equivalent to a ~1,000 cells ml^{-1} *Alexandrium* spp. culture. The 10-µm mesh separating the *Alexandrium* spp. culture from *T. fusus* cells prevented physical contact between the species while permitting exchange of dissolved compounds. Controls consisted of *Alexandrium* spp. cultures (1,000 cells ml^{-1}) in direct contact with *T.fusus,* and FSW. All experimental treatments and controls were conducted in triplicate and were incubated and enumerated as described in the above experiments.

Mitigation of toxicity:
Experiment was conducted to examine the effects of scavengers of reactive oxygen species on *Tiarina fusus* and *Polykrikos kofoidii* survival when exposed to *Alexandrium* spp. The antioxidant enzymes peroxidase (MP Biomedicals, #191370), catalase (MP Biomedicals, #100429), and superoxide dismutase (MP Biomedicals, # 190117) were prepared as aqueous solutions according to manufacturer specifications. All solutions were used within 1 h of preparation or were frozen immediately (-20°C) and thawed just before use. An additional treatment testing the protease, trypsin, was included to examine the possibility that protein or protein-like compounds are responsible for the toxicity of *Alexandrium* spp. to protists. *Alexandrium* spp. cultures were diluted with f/2 medium to a density of 1,000 cells ml^{-1}. Each *Alexandrium* spp. culture was subdivided, and peroxidase (1.25 µg ml^{-1}), catalase (2 U ml^{-1}), superoxide dismutase (5 U ml^{-1}), or trypsin (500 µg ml^{-1}) was added. Aliquots (5 ml, in triplicate) of each culture were added to individual wells of 12-well, polystyrene tissue-culture plates. A group of 15 *T. fusus* or *P. kofoidii* cells was added to each experimental well, and treatments were incubated and enumerated as described above. Controls consisted of ciliates and heterotrophic dinoflagellates exposed to *Alexandrium* spp. cultures without the addition of the enzymes and also FSW with the addition of each enzyme.

Data Processing Description

Data processing:

Differences between treatments were assessed by one-way or two-way ANOVA. Post hoc comparisons employed the Tukey-Kramer method. In all cases, significance levels were set at $p < 0.05$.

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Data Files

File
flores_2012.csv (Comma Separated Values (.csv), 14.33 KB) MD5:438600fef13d5a56eb62f460db431533 Primary data file for dataset ID 853804

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Related Publications

Flores, H., Wikfors, G., & Dam, H. (2012). Reactive oxygen species are linked to the toxicity of the dinoflagellate Alexandrium spp. to protists. Aquatic Microbial Ecology, 66(2), 199–209. doi:10.3354/ame01570
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

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Parameters

Parameter	Description	Units
Alexandrium	Three Alexandrium strains: High PST (NB-05), Low PST (CB-307), and No PST (CCMP115) and Controls: 0.2-um filtered seawater (FSW) and L. polyedra	unitless
Densitiy	Cell concentration, ranging from 63 to 1,000 cells ml-1	cells per milliliter (cells ml-1)
Protist	Ciliate, Tiarina fusus, and heterotrophic dinoflagellate, Polykrikos kofoidii.	unitless
Filtrate	0.2 um and 5.0 um filtrates or sonicated cell extracts from Alexandrium spp.	unitless
Separation	Separated from living Alexandrium spp. cultures by a 10 um mesh (+ mesh) and Controls: exposed to live Alexandrium spp. without separation (-mesh) and FSW	unitless
Mitigation	The addition of the enzymes peroxidase, catalase, superoxide dismutase (SOD), or trypsin and Controls: exposed to live Alexandrium spp. cultures without the addition of enzymes	unitless
Survived_protist	Survived protists (numbers out of 15 individuals)	number of individuals per well (Ind. well-1)
Survival	Protist survival (%)	Percentage
Figure	Figure number in Flores et al. (2012)	unitless

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Instruments

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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