Predator Induced and Non-Induced Eastern Oyster Shell Thickness

Website: https://www.bco-dmo.org/dataset/939528
Version: 1
Version Date: 2024-10-04

Project
» Collaborative Research: Keystone chemicals: Identifying general and universal molecules of fear (Identifying molecules of fear)

Contributors	Affiliation	Role
Weissburg, Marc	Georgia Institute of Technology (GA Tech)	Principal Investigator
Dickinson, Gary H.	The College of New Jersey (TCNJ)	Co-Principal Investigator
Belgrad, Benjamin A.	Dauphin Island Sea Lab (DISL)	Scientist
Roney, Sarah Hope	Georgia Institute of Technology (GA Tech)	Student
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Table of Contents

- Dataset Description
  - Methods & Sampling
  - Data Processing Description
  - BCO-DMO Processing Description
- Parameters
- Instruments
- Project Information
- Funding

Methods & Sampling

Eastern oysters were spawned at the Auburn University Shellfish Laboratory in Dauphin Island, AL, USA and juveniles were raised in a nursery system at Dauphin Island Sea Lab, Dauphin Island, AL, USA. Data collection took place at The College of New Jersey, Ewing, NJ, USA.

Oyster Culturing:
The oyster spat used in this experiment were preserved from a previous study on shell strength of predator-induced spat, which confirmed that exposure to predator cues resulted in stronger shells, measured as the amount of force (N) required to break shells standardized to size (Belgrad et al. 2021). Oysters were spawned and cultured at Auburn University Shellfish Laboratory on Dauphin Island, AL, USA, in May 2019. Oyster larvae were settled onto sun-bleached oyster shell where they metamorphosized into spat-on-shell and were housed in four flow-through holding tanks (2.4 meters x 0.9 meters) filled to a water depth of 0.4 meters (m) and flowing at a rate of 36.9 liters per minute (L/min) with natural seawater from Mobile Bay, AL, USA. Oyster spat-on-shell were randomly placed in seven oyster aquaculture baskets (~140 adult shells per basket, 20,000 spat/tank) spaced evenly along the length of the tank and suspended within the tanks to avoid sediment smothering the spat (28 baskets, 80,000 spat total). Two of the holding tanks were kept with only oysters to serve as controls (non-induced), whereas the two treatment tanks also each held four caged live adult blue crabs (*Callinectes sapidus*, Rathbun) to add predator cues to the tanks (induced). These crabs were fed one adult oyster (~5.0 centimeters in length) daily and were replaced with healthy, fresh-caught crabs at least biweekly. Oyster cages were rotated daily around crab cages to reduce differences in growth due to proximity to cue sources or water intake. A subset of spat-on-shell were removed from each tank after four weeks and eight weeks of culturing under these conditions and stored in 70% ethanol until March 2023 when shell structure analysis began.

Sample Preparation:

Oyster spat-on-shell were removed from ethanol solution in March 2023. A total of 5 left (top) shell valves from each nursery tank per induction state and age group (n = 10 per induction state and age group; 40 valves total) were carefully removed from individual spat and any soft tissue was removed using forceps and scalpel. The separated shell valves sat in 100% ethanol overnight to assist with removal of tissue remains, then were rinsed with DI water, dried at room temperature for 2-3 hours and finished drying in a low-temperature vacuum oven at 45 degrees Celsius (°C) and 25 millimeters of mercury (Hg mm) for 2 hours. The shell valves were then mounted and polished following standard techniques (Prezant et al. 2022). Each valve was placed in a 32-millimeter (mm) mounting cup, with the ventral edge of the shell affixed to the base of the cup using a coil mounting clip that had been glued to the bottom of the cup. This was left to dry overnight, before mounting with Bisphenol A Epichlorohydrin epoxy and hardener mixed in a 10:3 ratio (Allied High Tech). After the epoxy hardened for 24 hours, the mounted valves were removed from their mounting cups and ground to a plane that was visually approximated near the center of the shell running along the longest axis from anterior to posterior end. The shell valves were then polished to 0.04 micrometers (µm) using polycrystalline diamond solution and colloidal silica suspension (Allied High Tech).

Panoramic images of each shell sample were taken under polarized light using a reflected light microscope (Zeiss Axioscope.A1 with a Zeiss, AxioCam 105 color camera), with the analyzer set to 10°. Panoramas were constructed using imaging software (Zeiss Zen 3.8). Four-week-old spat valves were imaged under a 5x objective, and eight-week-old spat valves were imaged under a 2.5x objective.

Determining Shell Thickness:
Measurements of the thickness of the foliated layer, prismatic layer, and total shell thickness took place in ImageJ - FIJI 1.54f (Schindelin et al. 2012) for both four-week and eight-week-old oysters of both induction states. Panoramic images had a grid placed over them (100 µm² for four-week-old, and 200 µm² for eight-week old) and images were divided approximately into thirds. All thickness measurements were taken from the middle third of the shell valve, with one measurement each for the foliated layer, prismatic layer, and total thickness within each grid square (n = 19-31 per four-week-old shell, and n = 16-22 measurements per eight-week-old shell, depending on overall spat size). Grid measurements were averaged to yield a single thickness value for each layer of each shell sample.

Data Processing Description

Images panoramas were created in Zeiss Zen 3.8 and shell thickness measurements were collected using Fiji Is Just ImageJ (FIJI) 1.54f. Data were recorded in Microsoft Excel.

BCO-DMO Processing Description

currently being processed

[table of contents | back to top]

Parameters

Parameters for this dataset have not yet been identified

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Zeiss AxioCam 105 color camera
Generic Instrument Name	Camera
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Zeiss Axioscope
Generic Instrument Name	Microscope - Optical
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

[table of contents | back to top]

Project Information

Collaborative Research: Keystone chemicals: Identifying general and universal molecules of fear (Identifying molecules of fear)

Coverage: Wassaw Sound, GA, US and Dauphin Island, AL

NSF Award Abstract:
Many prey species use chemicals released in predator urine to detect imminent danger and respond appropriately, but the identity of these ‘molecules of fear’ remains largely unknown. This proposal examines whether prey detect different estuarine predators using the same chemical or whether the identity of the chemical signals varies. Experiments focus on common and important estuarine prey, mud crabs and oysters, and their predators including fishes, crustaceans and marine snails. Bioactive molecules are being collected from predators and prey and characterized. The goal is to determine if there are predictive relationships between either the composition of prey flesh or the predator taxon and the signal molecule. Understanding the molecular nature of these cues can determine if there are general rules governing likely signal molecules. Once identified, investigators will have the ability to precisely manipulate or control these molecules in ecological or other types of studies. Oysters are critical to estuarine health, and they are important social, cultural and economic resources. Broader impacts of the project include training of undergraduate and graduate students from diverse backgrounds and working with aquaculture facilities and conservation managers to improve growth and survival of oysters. One response to predator cues involves creating stronger shells to deter predation. Determining the identity of cues used by oysters to detect predators can provide management options to produce oysters that either grow faster or are more resistant to predators. Project personnel is working with oystermen to increase yields of farmed oysters by managing chemical cues.

For marine prey, waterborne chemical cues are important sources of information regarding the threat of predation, thus, modulating non-consumptive effects of predation in many systems. Often such cues are produced when the predators consume the flesh of that prey. In nearly all cases, the specific bioactive molecules responsible for modulating these interactions are unknown, raising the question whether there is a universal molecule of fear that prey respond to. Thus, the focus of the project is to determine the generality of fear-inducing metabolites released by predators and prey in estuarine food webs. The project combines metabolomics analysis of diet-derived urinary metabolites with bioassays to identify the bioactive molecules producing responses in two prey species from different taxonomic groups and trophic levels (oysters, mud crabs). Metabolites are sampled from three types of predators, fish, gastropods or crustaceans. This project aims to: 1) identify bioactive molecules produced by several common estuarine predators from different taxa; 2) compare cues from predators that induce defenses in prey vs. changes in prey behavior; and 3) contrast the identities and effects of predator-released cues with fear-inducing molecules from injured conspecifics. By identifying and contrasting the effects of waterborne molecules that induce prey responses from six predators and injured prey, this project is yielding insights into the mechanisms that mediate non-lethal predator effects, while addressing long-standing questions related to predator-prey interactions. In addition to the search of a universal molecule of fear, the experiments are exploring the role of complementary and distinct chemical information on the specificity of prey responses to different types of predators.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1948423

[table of contents | back to top]