Juvenile oyster shell strength measurements from predator cue bioassay experiments with treatments including blue crab urine, homarine, and trigonelline conducted at Dauphin Island Sea Lab, Dauphin Island, AL between June and August of 2020

Website: https://www.bco-dmo.org/dataset/883945
Data Type: Cruise Results
Version: 1
Version Date: 2022-11-18

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

Contributors	Affiliation	Role
Kubanek, Julia	Georgia Institute of Technology (GA Tech)	Co-Principal Investigator
Smee, Delbert Lee	Dauphin Island Sea Lab (DISL)	Co-Principal Investigator
Weissburg, Marc	Georgia Institute of Technology (GA Tech)	Co-Principal Investigator
Belgrad, Benjamin A.	Dauphin Island Sea Lab (DISL)	Scientist
Cepeda, Marisa	Georgia Institute of Technology (GA Tech)	Student
Roney, Sarah Hope	Georgia Institute of Technology (GA Tech)	Student
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
These data include measurements of juvenile oyster shell strength from predator cue bioassay experiments conducted at Dauphin Island Sea Lab, Dauphin Island, AL between June and August of 2020. Study description: Homarine and trigonelline are two blue crab urine metabolites that cause mud crabs to seek refuge, but it is unknown whether these molecules influence other species. In the current study, homarine, trigonelline, and blue crab urine of animals fed conspecific and heterospecific diets were tested on juvenile oysters to ascertain if the same molecules known to alter mud crab behavior also affect oyster morphology. Juvenile oysters were exposed to chemicals for roughly 6 weeks and their shell strength (N) was measured and standardized to the size of the animals (mm) as a proxy for understanding this defense.

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Coverage

Temporal Extent: 2020-05-05 - 2020-08-23

Dataset Description

See "Related Datasets" section for results of other predator cue bioassay experiments.

Methods & Sampling

Oyster spat were exposed to one of the following cues in individual aquaria: blue crab urine (mud crab or oyster diet), trigonelline (24.6 µM), homarine (15.1 µM), or trigonelline (24.6 µM) + homarine (15.1 µM). Additionally, we used natural seawater (settled for 3 days to remove sediment) as a negative control and predator water as a positive control.

Diploid oyster spat were purchased from the Auburn University Shellfish Laboratory and settled onto 4.5 cm × 4.5 cm marble tiles. For one week after settlement, spat on tiles were caged and kept in 1250 L mesocosms with natural flowing seawater from Mobile Bay at a flow rate of 20 L/min. We then ensured each tile had at least 15 oyster spat and used high-density polyethylene fishing line to tie tiles back-to-back. Three tile pairs were placed in each aquarium, ensuring that every tile pair was upright to maintain good water flow around the spat. An intact, sun-bleached adult oyster shell was also placed into each aquarium for spat tile pairs to lean on so that they maintained an upright position. Aquaria were filled with 2 L of natural seawater (with the exception of the predator water control, which received 1.5 L seawater + 0.5 L predator water) that had settled for at least 3 days to remove sediment particles. Seawater was supplemented with either Instant Ocean salt or deionized water to reach 20 ppt (± 2 ppt). Each aquarium contained filtered air bubblers for oxygenation. Aquaria were covered with lids to reduce evaporation and stored outside under a covered pavilion in a water bath containing ambient flow-through seawater to regulate temperature. Spat were fed Instant Algae Shellfish Diet 1800 (Reed Mariculture). At the start of the experiment, spat were fed 0.5 mL twice daily, but we increased this amount to 1 mL twice daily as spat grew larger. Complete water changes and aquarium cleanings were performed twice weekly, and 1 mL of predator chemical cues (i.e., blue crab urine and pure chemical compounds) were added to the aquaria immediately after water changes. Predator water was created by housing 6 blue crabs (carapace width 12-17 cm), in a 238 L volume mesocosm, and feeding each crab an adult oyster (length 6-7 cm). After 3-5 hours, 500 mL of this water was added to each positive control aquarium.

Homarine (15.1 µM) and trigonelline (24.6 µM) solutions used to induce oyster spat were prepared at natural concentrations found in urine of blue crabs fed an Eastern oyster diet (SI Appendix, Fig. S1 in Roney et al. (submitted)). Homarine (Santa Cruz Biotechnology Company) and trigonelline (Toronto Research Chemicals) were dissolved in deionized water at 27x and 97x (respectively) of the final concentrations used for the bioassay. These stock solutions were aliquoted and frozen at –80 °C. On days designated for cue addition, stock solutions were diluted to 1x concentration and 1 mL was added to each corresponding replicate aquarium. To prepare the trigonelline + homarine treatment, 2x concentration homarine and trigonelline solutions were mixed together in a 1:1 ratio to form a 1x concentration mixture.

Predator urine was collected from blue crabs fed two different diets, oysters or mud crabs (Panopeus herbstii). Blue crabs were collected from crab pots near Dauphin Island, AL, USA and housed in 1250 L mesocosms flowing with natural seawater. Crab size ranged from 11-19 cm, measured from spine to spine on the widest part of the carapace. Crabs were starved for 2-3 days and then were each fed either one adult oyster (~6-7 cm length) or ~5 g of frozen mud crabs every 48 hours to standardize diets. Mud crabs used for feeding were collected from either Priest’s Landing, Skidaway Island, GA (31°57'44.89"N, 81° 0'48.22"W) or the North Inlet Estuary, SC (33°21'52"N, 79°10'03"W). Crabs were maintained on these diets for one week prior to urine extraction to ensure all extracted metabolites were from the specified diets. Urine was collected from individuals twice a week. Crabs were cooled to quiescence, then a 23 gauge-needle was inserted approximately 2 mm into the nephropore and urine was extracted with gentle vacuum suction into clean glass vials. Urine used for the experiment was clear or yellow in color and foamy; urine was discarded if it appeared cloudy or bluish-gray in color, as this indicated contamination with hemolymph. Urine was frozen at –80 °C immediately after collection. Oyster diet urine was collected from a total of 161 crabs, each crab provided on average 4.08 ± 3.11 mL per collection, and was extracted from 2.2 ±1.0 times on average. Mud crab diet urine was collected from approximately 102 crabs; each crab provided on average 5.04 ± 4.07 mL per collection and was used for extraction 2.0 ± 1.0 times on average. We later combined the urine of different individuals into 12 mixtures using the fewest individuals possible, where each mixture was considered a biological replicate (Table S1 in Supplemental File PDF). These mixtures were then partitioned into 1 mL aliquots and stored at -80 °C until use.

In this bioassay, all treatments and controls had 12 replicates, and all treatments were run simultaneously. However, the replicates were divided among two blocks, n=3 per treatment (21 total aquaria) in the first block

and n=9 per treatment (63 total aquaria) in the second block. The blocks began approximately 4 weeks apart and both experimental blocks were planned for 8 weeks, however, the first block of the experiment ended early (June 5, 2020 through July 6, 2020, total 31 days) due to high levels of mortality and the second block of the experiment was disrupted (July 9, 2020 through August 23, 2020, total 48 days) due to heavy storms in Dauphin Island, AL.

At the completion of the experiment, spat from each aquarium were randomly selected for assessment of shell strength. Approximately 20 oysters were crushed from each aquarium (distributed across tile pairs), except for one predator water replicate (15 oysters) and one trigonelline replicate (19 oysters) due to high mortality. Individual spat width was measured for each crushed oyster to 0.01 mm using a Vernier digital caliper. The force required to crush oysters was measured using a Kistler 5995 charge amplifier and Kistler 9207 force sensor following standard protocol (Robinson et al., 2014). Crushing force was divided by spat width to produce a size-standardized metric of shell strength (i.e., standardized crushing force, N/mm) because larger individuals typically have a stronger shell as a byproduct of their size. Standardized crushing force measurements for oysters within the same aquarium were averaged, however data were removed for outliers, dead oysters, and unreliable measurements resulting in fewer spat contributing to an aquarium average. Due to a batch effect (i.e., all seawater replicates in block one were statistical outliers), all block one samples were excluded from further statistical analyses. Additionally, two predator water replicates, and one trigonelline + homarine replicate were excluded from statistical analyses due to high mortality. As a result, there were 9 replicate aquaria per treatment with the exception of predator water (n=7) and trigonelline + homarine (n=8) treatments.

Instruments:
Individual spat width was measured for each crushed oyster to 0.01 mm using a Vernier digital caliper. The force required to crush oysters was measured using a Kistler 5995 charge amplifier and Kistler 9207 force sensor following standard protocol (Robinson et al., 2014).

Problems/Issues:
Only live oysters were crushed for the experiment. If an oyster was crushed and found to be dead (no soft tissue within shell), that was noted on the data file to be excluded from the analysis. Due to this, some treatments had fewer replicates than others, but this was accounted for in data analysis. Data were only included in the statistical analysis if there was no note in the life_status column or if it was specifically labeled as alive. All of Block 1 was later excluded from the statistical analysis for this publication due to this high mortality (more detail in the analysis description of the methods above).

life_status notations and meanings:

- "a" = Spat was alive and it was not excluded from initial analysis (though block 1 data was excluded from final analysis for publication regardless of life status)
- "?" = Unclear if the spat was alive or dead. These rows were excluded from the statistical analysis
- "most dead" = each row in the data refers to a single spat. This means the spat in this row was dead
- "lots of dead" = each row in the data refers to a single spat. This means the spat in this row was dead
- "d" and "dead" = each row in the data refers to a single spat. This means the spat in this row was dead
- "tiles looked bad/dead, algae growing" = treatment excluded from analysis

Data Processing Description

Data was entered and stored in Microsoft 365 Excel for Windows Version 2011. No processing (removals, transformations, etc) occurred on this data.

BCO-DMO Processing Description

* Sheet 1 of file "Predator Cue Bioassay Oyster Crushing Data.xlsx" was imported into the BCO-DMO data system.
* Rows 641 to 650 in your originally submitted excel file had value #DIV/0!. rows were excluded from the data table since the spat were dead. (see "Problems/Issues" in the "Methods & Sampling" section.)
* Missing values are displayed differently based on the file format you download. They are blank in csv files, "NaN" in MatLab files, etc.

* stand_force, length, crushing_force columns rounded to four decimal places as indicated as the correct precision in provided metadata.
* commas in comment column changed to semicolon for better csv format support.

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Data Files

File
oyster_predator_bioassay_2020.csv (Comma Separated Values (.csv), 59.43 KB) MD5:123558bfb9d12e57bc6ec274143ff519 Primary data file for dataset ID 883945

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Supplemental Files

File
Supplemental information for oyster strengthening data in response to various predator cues filename: Supplemental_Information_Roney_and_Cepeda.pdf (Portable Document Format (.pdf), 174.34 KB) MD5:ab3833cd4686e7af861982181dbd237b This file contains information related to the data of oyster strengthening response to predator cues such as blue crab urine, homarine and trigonelline. Tables in this file include more information on the animals comprising each cue mixture and the concentration range of chemical cue doses within each dose response experiment.

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Related Publications

Robinson, E., Lunt, J., Marshall, C., & Smee, D. (2014). Eastern oysters Crassostrea virginica deter crab predators by altering their morphology in response to crab cues. Aquatic Biology, 20(2), 111–118. https://doi.org/10.3354/ab00549
Methods

Roney, S. H., Cepeda, M. R., Belgrad, B. A., Moore, S. G., Smee, D. L., Kubanek, J., & Weissburg, M. J. (2023). Common fear molecules induce defensive responses in marine prey across trophic levels. Oecologia, 202(4), 655–667. https://doi.org/10.1007/s00442-023-05438-2
Results

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Related Datasets

IsRelatedTo

Roney, S. H., Cepeda, M., Belgrad, B. A., Smee, D. L., Kubanek, J., Weissburg, M. (2022) **Juvenile oyster shell strength measurements from a dose response assay of chemical cues homarine and trigonelline conducted at Dauphin Island Sea Lab, Dauphin Island, AL in June - August 2021.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-11-18 doi:10.26008/1912/bco-dmo.883999.1 [view at BCO-DMO]
Relationship Description: Same testing method performed on different individual oysters in different scenarios. Different individuals oysters exposed to different predator cues.

Roney, S. H., Cepeda, M., Belgrad, B. A., Smee, D. L., Kubanek, J., Weissburg, M. (2022) **Juvenile oyster shell strength measurements from a dose response experiment with an array of blue crab urine concentrations conducted at Dauphin Island Sea Lab, Dauphin Island, AL in August - Oct 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-

11-18 doi:10.26008/1912/bco-dmo.884015.1 [view at BCO-DMO]
Relationship Description: Same testing method performed on different individual oysters in different scenarios. Different individuals oysters exposed to different predator cues.

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Parameters

Parameter	Description	Units
block	the experimental block in which these replicate aquaria were tested	unitless
tank	the mesocosm in which the experimental tanks were housed for the duration of the experiment.	unitless
overall_treatment	bc-o = blue crab urine fed an oyster diet, bc-m = blue crab urine fed a mud crab diet, pw = predator water, h = homarine, t = trigonelline, t+h = trigonelline + homarine, sw = seawater (control)	unitless
mixture	The Mixture column is an individual identifying label for each of the 12 mixtures of different animals' waste products creating a treatment. Every mixture contained exudates from different individual animals. For example, bc-m1 contained urine from blue crabs fed a mud crab diet, while bc-m2 contained urine from different blue crabs fed a mud crab diet. More information on the animals that made up each mixture can be found in the Methods section and the supplemental information.	unitless
pair	count of tile pairs used for data collection	unitless
tile	count of tiles within pairs sampled from (samples crushed were from both tiles within a pair)	unitless
spat_ID	count of the number of individual spat crushed	unitless
length	length in mm of each individual spat sampled, measured by Vernier digital calipers to nearest 0.01 decimals	millimeters (mm)
crushing_force	force to crush spat (N), as measured by Kistler force sensor, limited to nearest 0.01 decimals	newtons (N)
stand_force	standardized crushing force = crushing force/length	newtons per millimeter (N/mm)
life_status	spat were removed from replicate average if they were marked as dead. Replicate treatments with less than 6 live spat were removed from analysis. (See Acquistion Description section for details of what life_status values mean and whether they were included in the statistical analyses).	unitless

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Instruments

Dataset-specific Instrument Name	Vernier digital caliper
Generic Instrument Name	calipers
Generic Instrument Description	A caliper (or "pair of calipers") is a device used to measure the distance between two opposite sides of an object. Many types of calipers permit reading out a measurement on a ruled scale, a dial, or a digital display.

Dataset-specific Instrument Name	Kistler 5995 charge amplifier and Kistler 9207 force sensor
Generic Instrument Name	Force sensor
Dataset-specific Description	The force required to crush oysters was measured using a Kistler 5995 charge amplifier and Kistler 9207 force sensor following standard protocol (Robinson et al., 2014).
Generic Instrument Description	Instrument that measures forces such as dynamic and quasistatic tensile and compression forces. Units commonly as Newtons (N).

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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 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.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1948423
NSF Division of Ocean Sciences (NSF OCE)	OCE-1948441

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