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

Website: https://www.bco-dmo.org/dataset/884015
Data Type: Other Field Results
Version: 1
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Project
» Collaborative Research: Keystone chemicals: Identifying general and universal molecules of fear (Identifying molecules of fear)

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Abstract
These data include measurements of juvenile oyster shell strength from a dose response experiment of varying blue crab urine concentrations conducted at Dauphin Island Sea Lab, Dauphin Island, AL in August - Oct 2022. Study description: Metabolites from blue crab urine are known to cause shell strengthening in juvenile oysters as a defensive response. Previous studies have identified several bioactive molecules in urine that induce this response in oysters, but others have yet to be identified. In the current study, an array of concentrations of blue crab urine was used to treat oyster juveniles in order to assess the dose-dependency of this response. Oysters were exposed to urine treatments for 8 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: 2022-08-08 - 2022-10-03

Dataset Description

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

Methods & Sampling

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. Four 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 predator chemical cues were added to the aquaria immediately after water changes.

Blue crabs were collected from crab pots near Dauphin Island, AL and stored in the same facility and conditions as described for the predator cue bioassay in 2020 (See related dataset: https://www.bco-dmo.org/dataset/883945). Urine collection methods remained the same as for previous experiments (2020 predator cue bioassay) except urine was pooled from all crabs for this experiment. All crabs for this experiment were fed an oyster diet (i.e., one adult oyster (~6-7 cm in length) twice weekly). Crab were kept in aquaria for a one week acclimation period before beginning a urine extraction regimen. Urine was collected twice weekly from 22 crabs that ranged 13 – 18 cm in size and each crab produced 1.31 ± 0.18 mL. Urine dose concentrations were determined based on the average concentrations of homarine and trigonelline quantified in blue crab urine in the 2020 predator cue bioassay (homarine, 13 ± 21 µM; trigonelline, 3.6 ± 6.9 µM). It was assumed that 1 mL of pooled blue crab urine contained these concentrations, and doses were adjusted by half-log steps until approximated concentrations of homarine and trigonelline spanned four orders of magnitude (Table S3 in Supplemental File PDF). Though initial experimental design was based on an assumed concentration of homarine and trigonelline, all urine doses were reported as urine volume divided by the total volume of seawater within an experimental aquaria. For the six lowest doses, 1 mL aliquots of blue crab urine were prepared via serial dilution. The two highest doses received 5.00 mL and 1.25 mL aliquots of pure urine respectively. All aliquots were frozen at –80 °C to be used on the day of cue addition.

The preparation and maintenance of the urine dose response experiment was identical to the previous homarine and trigonelline dose response experiment. The experiment was conducted for 56 days (8 weeks) from 08 Aug. 2022 – 03 Oct., 2022 and at the completion of the experiment tile pairs were removed from their jars, measured, and crushed according to previous methodology (Robinson et al. 2014). There were five replicate aquaria per concentration, and within these replicate aquaria, we took an average of 32 crushed oysters.

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: None.

Data Processing Description

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

BCO-DMO Data Manager Processing Notes:
* Sheet 1 of file "BC Urine Dose Response Oyster Crushing Data.xlsx" was imported into the BCO-DMO data system with values "NA" interpreted as a missing data identifier.

** Missing data values are displayed differently based on the file format you download. They are blank in csv files, "NaN" in MatLab files, etc.
* stand_crushing_force rounded to two decimal places as indicated as the correct precision in provided metadata.

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Data Files

File
oyster_predator_bioassay_bluecrab.csv (Comma Separated Values (.csv), 71.22 KB) MD5:9a0b8e6e0c803ae2a382ef17301a462a Primary data file of dataset 884015 version 1.

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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 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. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-11-18 doi:10.26008/1912/bco-dmo.883945.1 [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
ID	identification given to each individual replicate within the experiment	unitless
Diet	type of food that the predator received before urine was extracted to be used in the experiment	unitless
Treatment	sw = seawater (control), BC = blue crab urine, PW = predator water (positive control)	unitless
Dose	assigned dose number corresponding to appropriate urine volumes	unitless
Replicate	the replicate number assigned to an individual aquaria within a treatment	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
Individual	count of the number of individual spat crushed	unitless
size	length of each individual spat sampled, measured by Vernier digital calipers to nearest 0.01 decimals	millimeters (mm)
crushing_force_N	force to crush spat (N), as measured by Kistler force sensor, limited to nearest 0.01 decimals	newtons (N)
stand_crushing_force	standardized crushing force = crushing force/length	newtons per millimeter (N/mm)

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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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