# 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

**Website**: https://www.bco-dmo.org/dataset/883999
**Data Type**: Other Field Results
**Version**: 1
**Version Date**: 2022-11-18

**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 conducted at Dauphin Island Sea Lab, Dauphin Island, AL in June - August of 2021. Study description: Homarine and trigonelline are two blue crab urine metabolites that cause juvenile oysters to strengthen their shells are a defensive response. We evaluated the dose dependency of this response with a dose-response experiment where homarine and trigonelline concentrations (of each individual chemical and a combination of the two) spanned 5 log half-steps. Juvenile oysters were exposed to chemicals 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**: 2021-05 - 2021-08

## 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 1 mL of predator chemical cues (i.e., homarine, trigonelline, or homarine + trigonelline at the correct assigned dose) were added to the aquaria immediately after water changes.

Stock solutions for homarine (7.4 mM) (Santa Cruz Biotechnology Company), trigonelline (6.6 mM) (Toronto Research Chemicals), and homarine + trigonelline were prepared in LCMS grade water, aliquoted to avoid freezing and thawing the solutions more than once, and stored at -80 °C. Serial dilutions for each cue were prepared in deionized water the same day they were added to their respective aquaria (Table S2 in Supplemental File PDF). Concentrations were determined by calculating half-log steps encompassing the natural concentrations of homarine and trigonelline found in the urine of blue crabs fed an oyster diet (SI Appendix, Fig. S1 in Roney et al. (submitted)). On the day of cue addition, serial dilutions were performed with deionized water and using a micropipette (for volumes under 5 mL) and 10 mL graduated cylinder (for volumes greater than 5 mL). Mixtures were vortexed for 10 seconds and manually agitated for 10 seconds before continuing with the serial dilution. Dilutions for treatments of individual compounds and the homarine + trigonelline treatments were prepared at the same half-log concentrations (Table S2 in Supplemental File PDF). The same stock solutions were used to prepare both the individual compound and homarine + trigonelline treatments. All serial dilutions were done in tandem. Once chemical mixtures were made, they were stored in glass bottles, and 1 mL of each solution was pipetted into the appropriate experimental aquaria with a clean pipette tip. Chemical solutions were added after water changes, which were performed twice weekly. This experiment also included a seawater control group, which received the same care (without cue addition) as all other experimental aquaria.

The experiment was conducted for 56 days (8 weeks), and at the completion of the experiment tile pairs were removed from their jars, measured, and crushed according to the same methodology from the predator cue bioassay (See related dataset: https://www.bco-dmo.org/dataset/883945). There were four replicate aquaria per concentration, and within these replicate aquaria, we took an average of 17 crushed oysters. Replicates with high mortality (less than 6 spat alive) were excluded from statistical analyses. In total, 14 aquaria from the homarine dose array, 16 aquaria from the trigonelline dose array, and 17 aquaria from the trigonelline + homarine dose array were included in the statistical analysis.

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 (see methods above).

**Data Processing Description**

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

BCO-DMO Data Manager Processing Notes:
* Sheet 1 of file "H and T Dose Response Oyster Crushing Data.xlsx" was imported into the BCO-DMO data system with values "na" interpreted as a missing data identifier.
* stand_crushing_force values "#VALUE!" were replaced with missing data identifiers.
** 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.
* column "Diet" removed. It was empty and not described in column metadata.

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## Data Files

| File |
|---|
| **oyster_predator_bioassay_ht.csv** (Comma Separated Values (.csv), 46.45 KB) MD5:f148dc96d211e46c20362466e562180d<br>Primary data file for dataset 883999 version 1. |

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## Supplemental Files

| File |
|---|
| **Supplemental information for oyster strengthening data in response to various predator cues**<br>filename: Supplemental_Information_Roney_and_Cepeda.pdf (Portable Document Format (.pdf), 174.34 KB) MD5:ab3833cd4686e7af861982181dbd237b<br>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 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.*

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 |
| Block | the experimental block in which these replicate aquaria were tested | unitless |
| Treatment | h = homarine, t = trigonelline, t+h = trigonelline + homarine, sw = seawater (control) | 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 | 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 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.

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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