README File

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Method(s) –

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 m x 0.9 m) filled to a water depth of 0.4 m and flowing at a rate of 36.9 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 cm 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 °C and 25 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 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 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 μm^2 for four-week-old, and 200 μm^2 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.

ASTM Designation C1327–15R19. "Standard Test Method for Vickers Indentation Hardness of Advanced Ceramics," 2019. https://www.astm.org/c1327-15r19.html

Belgrad, Benjamin, Emily M. Combs, William C. Walton, and Delbert L. Smee. "Use of Predator Cues to Bolster Oyster Resilience for Aquaculture and Reef Restoration." *Aquaculture* 538 (May 30, 2021): 736553-. https://doi.org/10.1016/j.aquaculture.2021.736553.

Prezant, Robert S., Gary H. Dickinson, Eric J. Chapman, Raymond Mugno, Miranda N. Rosen, and Maxx B. Cadmus. "Comparative Assessment of Shell Properties in Eight Species of Cohabiting Unionid Bivalves." *Freshwater Mollusk Biology and Conservation* 25, no. 1 (March 2022): 27–36. https://doi.org/10.31931/FMBC-D-21-00001.

Schindelin, Johannes, Ignacio Arganda-Carreras, Erwin Frise, Verena Kaynig, Mark Longair, Tobias Pietzsch, Stephan Preibisch, et al. "Fiji: An Open-Source Platform for Biological-Image Analysis." *Nature Methods* 9, no. 7 (July 2012): 676–82. https://doi.org/10.1038/nmeth.2019.

Location where data were collected – Oyster culturing took place at the Dauphin Island Sea Lab in Dauphin Island, AL, USA and shell processing and data collection took place at The College of New Jersey in Ewing, NJ, USA

Time period during which data were collected - Oysters were raised in May – July 2019 and stored from 2019 – 2023. Shell processing and data collection took place from February 2023 – December 2023.

Environmental or experimental conditions – N/A

Description of parameters/variables –

Sheet	Description
8 Week Thickness	eight-week-old oysters overall shell thickness, foliated layer thickness, and prismatic layer thickness

4 Week Thickness	four-week-old oysters overall shell thickness, foliated layer thickness, and prismatic layer thickness

Header	Description
ID	identification number of each shell replicate. Identification number was given based on Treatment - Age - Shell Number - Sample Number, where BC = induced and N = non-induced
Measurement #	number of technical replicate measurements taken per shell sample
Grid #	total number of squares in a grid overlaid on each shell sample image
Overall Angle	angle of the line measuring overall shell length per measurement
Overall Length	length of the line measuring overall shell length per measurement
Foliated Angle	angle of the line measuring foliated shell length per measurement
Foliated Length	length of the line measuring foliated shell length per measurement
Prismatic Angle	angle of the line measuring prismatic shell length per measurement
Prismatic Length	length of the line measuring prismatic shell length per measurement

missing data was denoted by NONE or NA or left blank

File Information – raw data values are included in "Raw Thickness Measurements" datafile (.csv)

Software – Shell images were collected in Zeiss Zen 3.6 Blue edition software and length (thickness) measurements were performed in Fiji is Just ImageJ (FIJI) 1.24 data was recorded in Microsoft Excel.

Data source – N/A

Related materials – This data is associated with the study "Eastern oysters minimize costs of inducible defenses by changing shell strengthening mechanism with age".

Additional data included in this study are available as "Raw Microhardess Measurements".

Limitations to reuse – N/A

Date dataset was last modified 7/26/2024

Any other important information about your data

This data is associated with the study "Eastern oysters minimize costs of inducible defenses by changing shell strengthening mechanism with age". This study tested which mechanism, hardness or thickness, juvenile eastern oysters use to strengthen their shells in response to chemical cues from predators. Data was collected from eastern oysters, *Crassostrea virginica*, grown in a nursery in Dauphin Island, AL with or without exposure to chemical cues from blue crabs, *Callinectes sapidus*. Two age groups (four-week and eight-week-old post-settlement) of juveniles were included in this study. Oyster shell thickness overall and within both shell layers was measured.