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SHORT COMMUNICATION

COMPARISON OF NURSERY METHODS TO STRENGTHEN OYSTERS FOR AQUACULTURE[§]

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KEY WORDS: *Crassostrea virginica*, predator, chemical cue, seed oyster, shell morphology

INTRODUCTION

To reduce their risk of being consumed, prey possess traits to thwart predators, including morphological structures such as spines or hard shells as well as chemical defenses (Hay 2009, Weissburg et al. 2014). Producing predator defenses often incurs costs, such as reductions in growth or fecundity (Relyea 2002), and in habitats where predation risk is low, prey defenses may be reduced or absent (Smee and Weissburg 2008, Hay 2009, Large and Smee 2013). Prey may also minimize predator avoidance costs by using induced or plastic defenses that only develop when predation risk is high (Harvell 1990). These types of inducible defenses are appropriate when defense production is costly, predator presence is temporally variable, and prey can reliably detect and react to predators to minimize their risk of being consumed (Cronin 2001). Inducible defenses in response to predation risk are effective and are well known from many different taxa including tadpoles (Relyea 2002, Schoeppner and Relyea 2009), snails (Freeman and Hamer 2009, Large and Smee 2013), corals (Gochfeld 2004), and bivalves (Leonard et al. 1999, Nakaoka 2000, Scherer et al. 2016).

Eastern oysters (*Crassostrea virginica*) are commercially and ecologically important sessile bivalves that are preyed upon by a variety of crustacean and gastropod predators (Menzel and Nichy 1958, Grabowski 2012). Because they are vulnerable and immobile, oysters can defend against predation by changing their morphology to strengthen their shells. Oysters strengthen their shells in response to both crustacean (Newell et al. 2007) and gastropod predators (Lord and Whitlatch 2012), which can reduce their likelihood of being consumed (Robinson et al. 2014). Oysters respond to chemical exudates from injured con- and hetero-specifics as well as predator exudates by building thicker shells and altering the composition of shells (Scherer et al. 2016). Bivalves, including oysters, may increase the addition of calcium carbonate to make shells larger, add protein to their shells to increase its strength, or both (Currey and Taylor 1974, Frieder et al. 2016, Scherer et al. 2018).

The purpose of this study was to investigate morphological changes of post-metamorphic oysters in flow-through tanks located within an oyster hatchery/nursery to evaluate the po-

tential for creating predator-resistant oysters for use in aquaculture and restoration. Oyster hatcheries may spawn oysters, and in a subsequent nursery setting, grow oysters individually as seed which are commonly reared off-bottom and used in the half shell market. Alternatively, oysters may be spawned and then allowed to settle onto hard substrates (e.g., shells, crushed concrete) in the hatchery, and grown in clusters and/or groups. This technique is called spat-on-shell and is often used in oyster reef restoration as it more closely mimics natural conditions and promotes greater reef building. To date, previous studies examining oyster responses to predation risk were performed using spat-on-shell, but responses of seed oysters have not been investigated. Because seed oysters are single and do not have a large, hard substrate to grow on, their growth rate and response to predators may be different than spat-on-shell oysters. Moreover, seed oysters are used in nearshore aquaculture and are prone to predation from predators which recruit into cages, and there is commercial interest in having seed oysters produce thicker, cup-shaped shells that increases their value on the half-shell market. However, most studies on predator-induced defenses of oysters are laboratory based using small, closed systems that can concentrate predator cues and only stimulate small numbers of oysters. We therefore sought to compare responses of both spat-on-shell and oyster seed to predation risk cues in a flow-through nursery system to determine if the rearing technique could be replicated at other oyster nurseries on an industrial scale.

MATERIALS AND METHODS

Oysters were cultured as spat-on-shell or single seed at the Auburn University Shellfish Laboratory (AUSL) on Dauphin Island, AL starting in late May 2019. Both types of oysters were raised from the same brood stock and housed together in 4 flow-through holding tanks measuring 2.4 m x 0.9 m (length x width) and held in a water depth of 0.4 m. Spat-on-shell oysters were ~1.0 mm and seed oysters ~2.5 mm when the experiment began. There was immense variation in the number of spat per shell which we elected to maintain during the experiment to mimic natural settlement and normal reef restoration practices (~5 - 40 spat/shell). Seed oysters were

[§]The first author conducted this research as part of the Dauphin Island Sea Lab's Research Experience for Undergraduates program.

grown as individuals. Water flow rates in the holding tanks averaged 36.9 L/min. Spat-on-shell oysters were suspended above the tank bottom in 7 plastic cages 64 x 23 x 14 cm (140 spat covered shells/cage), and seed oysters were housed in the same tanks within 2 Vexar mesh bags 24 cm x 35 cm x 2 cm (100 seeds/bag) suspended above the bottom to prevent sediment buildup from suffocating oysters. Structural and morphological differences between spat-on-shell and single seed oysters require that different suspension methods are used during rearing, and the containers as well as the subsequent oyster densities followed typical nursery procedures (Matthiessen 2001, pers. comm., AUSL hatchery manager Scott Rikard). Half of the oysters were exposed to predator exudates by holding 4 adult blue crabs, *Callinectes sapidus*, in 2 of the flow-through tanks (8 crabs total) while the remaining 2 tanks did not have crabs and served as a control. Crabs were held in 2 partitioned cages 64 x 23 x 14 cm to prevent crabs from consuming the experimental oysters or each other while control tanks had empty crab cages. Each crab was fed one adult oyster daily (~5.0 cm in length) to maximize predation risk cues, causing experimental oysters to receive exudates from both crabs and injured oysters as they were being consumed. Crabs were replaced at least every other week.

After 4 weeks of predator cue exposure, both spat-on-shell and seed oyster shell size (mm), shell strength (N), and shell weight (g) were measured (number of individuals = 84 spat-on-shell and 40 seed oysters for each treatment). We assessed the effect of predator cue exposure on shell morphology by measuring shell size, shell weight, and shell crushing force (sensu Robinson et al. 2014, Scherer et al. 2016). At this size, oysters are roughly round, and shell length was measured to the nearest 0.01 mm using digital calipers from the umbo to the outer shell edge. For spat-on-shell, care was taken to only measure individuals which were not crowded by cohorts to reduce any confounding effects on growth from space limitation, although this was not a large concern during such early life stages. We then quantified the force needed to break each oyster shell using a penetrometer (Kistler force sensor 9203 and a Kistler charge amplifier 5995). A small blunt probe was placed centrally to be equidistant from the shell edges and perpendicular to shell surface. Gentle and consist pressure was applied until the shell cracked, and the maximum force (N) needed to break the shell recorded, a standard proxy for shell hardness (Robinson et al. 2014). Because larger individuals will naturally have a stronger shell as a byproduct of their size, we divided shell crushing force by shell length to produce a size-standardized metric of shell strength (i.e., standardized crushing force). After oysters were crushed using the penetrom-

eter, shell weight from each crushed oyster was recorded by separating the shell pieces from any attached soft tissue and drying at 70°C for 72 hours before weighing. For spat-on-shell, only the top valves were measured because the bottom valves were bonded to the underlying hard substrate and because crushing force was applied to the top valves (sensu Robinson et al. 2014). For seed oysters, the entire shell was measured once the tissue was removed.

We examined the effects predator cue exposure (present vs absent) and oyster culture method (spat-on-shell vs single seed) have on standardized shell crushing force and shell diameter by running 2 separate mixed-effects generalized linear models with a Gamma distribution (GLM; R package: lme4). Cue exposure treatment and culture method were treated as fixed effects with an interaction term while oyster holding container nested in tank were treated as random effects to control for nonindependence of oysters within the same container (Bolker et al. 2009). Tukey's multiple comparison test was used to determine pairwise differences in shell morphology (R package: lsmeans). Two separate mixed-effects GLMs were run for spat-on-shell and single seed oysters to examine the effect of predator cue exposure on shell weight because different proportions of the shell were measured for these growing methods. This prevented direct statistical comparisons between spat-on-shell and single seed oyster weight. Here, statistical models had cue exposure treatment as a fixed effect and holding container nested in tank as random effects. All statistical analyses were conducted using R v3.5.1 (R Development Core Team, 2018; <http://www.R-project.org/>).

RESULTS AND DISCUSSION

Both spat-on-shell and seed oysters substantially changed their morphology in response to blue crab exudates (seed

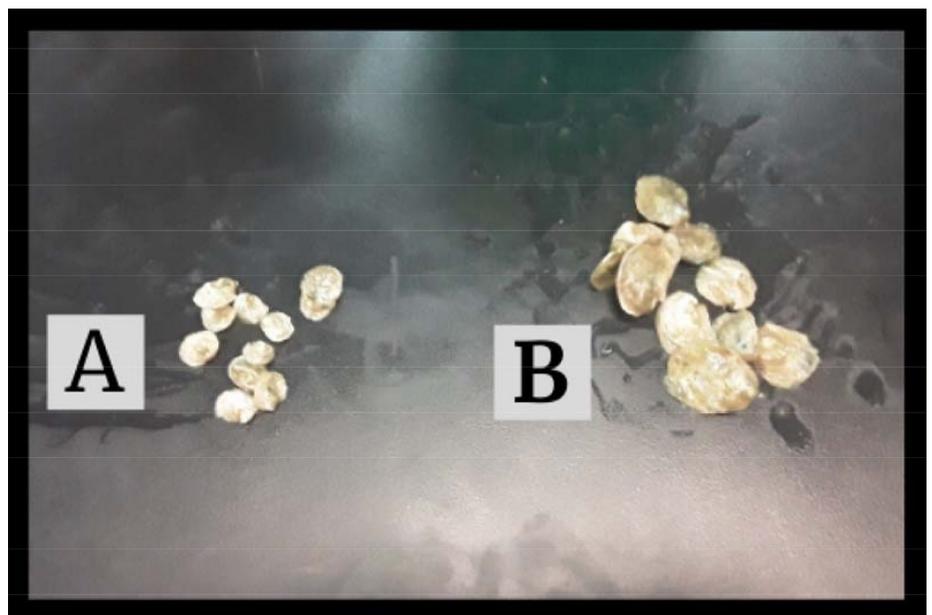


FIGURE 1. Oysters (*Crassasteroa virginica*) grown in a flow-through aquaculture nursery for 4 weeks. A. With blue crab (*Callinectes sapidus*) exudates. B. Controls without predator cues.

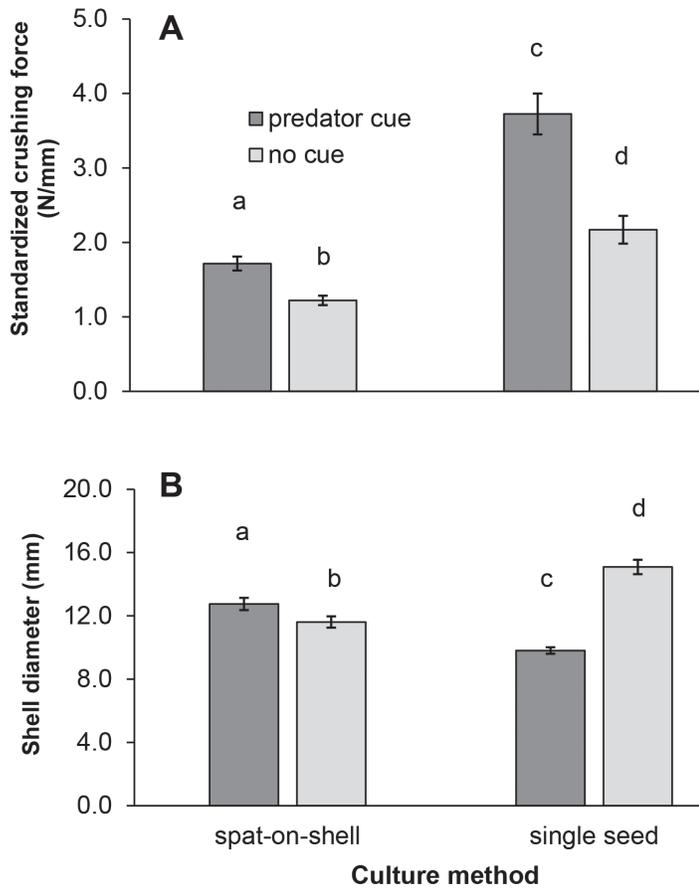


FIGURE 2. Mean (\pm se) of shell characteristics of oysters (*Crassostrea virginica*) cultured as spat-on-shell ($n = 168$ total) or single seed ($n = 80$ total) and exposed to either blue crab (*Callinectes sapidus*) predator cues or a control of no cues. A. Standardized crushing force (N/mm). B. Shell diameter (mm). Spat-on-shell oysters, $n = 84$ per treatment cue; single seed oysters, $n = 40$ per cue treatment. Letters denote significant differences (Tukey's multiple comparison test, $p < 0.05$). N = Newtons of force.

oysters pictured in Figure 1). Consistent with previous studies (Robinson et al. 2014, Scherer et al. 2016), oysters in blue crab treatments produced shells that required significantly more force to break, regardless if they were raised as spat-on-shell or as seed (Figure 2A; estimate = 0.19, $t = 4.20$, $p < 0.001$). Oyster culture method also had a strong effect on the standardized crushing force of shells which was on average 2x greater for seed oysters than spat-on-shell (estimate = 0.31, $t = 7.54$, $p < 0.001$). Interestingly, predator cues seemed to have a stronger influence on seed oysters as their standardized crushing force increased 71% when exposed to cues versus a 41% increase for spat-on-shell, although there was not a significant interaction between cue exposure treatment and oyster culture method on standardized crushing force (estimate = 0.04, $t = 0.59$, $p = 0.557$).

Oyster sizes and shell weights showed a different type of response. Seed oysters exposed to predator cues were 35% smaller than unexposed individuals (Figure 1), while spat-on-shell oysters grew 10% larger when exposed to predator cues (Figure 2B; cue exposure treatment estimate = -0.04 , $t = -7.45$, $p < 0.001$; culture method estimate = -0.02 , $t = -5.15$, $p < 0.001$; interaction term estimate = 0.04, $t = 7.64$, $p <$

0.001). Similarly, spat-on-shell oysters also grew 35% heavier after rearing in the presence of predator cues (Figure 3A; estimate = 6.50, $t = 2.82$, $p = 0.005$) while seed oysters were 58% lighter when exposed to predator cues (Figure 3B; estimate = -4.38 , $t = -4.81$, $p < 0.001$). These drastic differences in growth patterns are partially responsible for why the standardized crushing force of oysters was much greater for seed oysters exposed to predator cues than their spat-on-shell counterparts. However, even the raw unstandardized crushing force of cue-exposed oysters was 66% greater for seed oysters than spat-on-shell (unpublished data). Interestingly, seed oysters achieved greater shell strength despite being 23% smaller than oysters cultured as spat-on-shell.

We attribute these findings to growth differences related to the amount of substrate used for settlement. Spat-on-shell, already being attached to a shell, are protected on one side. Much of their initial growth is lateral, and thus both their diameter and weight increase as they grow. Spat-on-shell oysters tended to grow broader and heavier in response to predator treatments, accounting for their significant increases in these characteristics. In contrast, seed oysters exposed to crab cues produced smaller shells that were lighter than those grown in control tanks, although their shell hardness significantly increased. Seed oysters settle onto tiny shell fragments that are only suitable for a single oyster to settle upon. Thus, from the outset, these oysters are vulnerable from predation on both sides and must invest more in shell growth to protect themselves than their spat-on-shell counterparts. With-

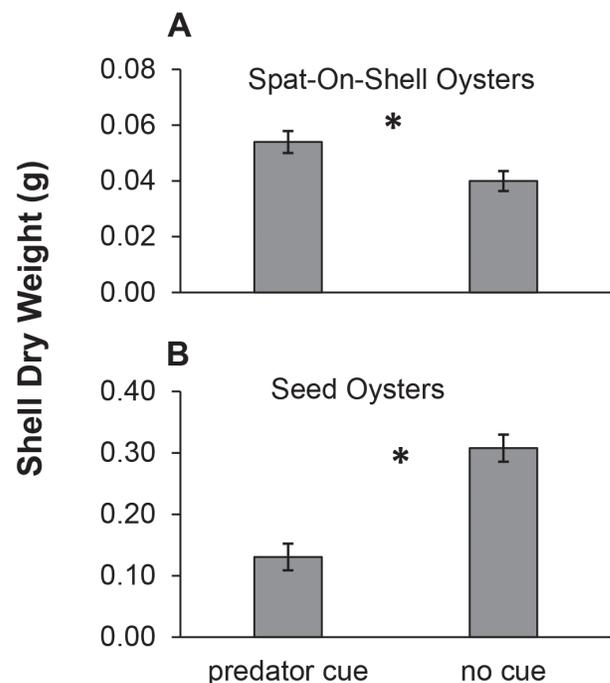


FIGURE 3. Mean (\pm se) shell dry weight (g) of oysters (*Crassostrea virginica*) exposed to either blue crab (*Callinectes sapidus*) predator cues or a control of no cues. A. Cultured as spat-on-shell ($n = 168$ total). B. Cultured as single seed ($n = 80$ total). Spat-on-shell, $n = 84$ per treatment cue; single seed, $n = 40$ per cue treatment. Asterisks denote significant differences determined by mixed-effects GLMs, $p < 0.05$.

out predator cues, seed oysters had sharp, thin edges, but in predator cue treatments, seed oysters resembled marbles that were rounded, which may be an active response to predators or a consequence of shell growth changes. Regardless, we hypothesize that the rounder edges make shells more resistant to crushing predators and less susceptible to breaking off, but additional studies are needed to verify this claim.

Organisms may add calcium carbonate to their shells in response to predation risk, which can be added quickly to reach a size refuge and is energetically less expensive than adding organic tissue (Currey and Taylor 1974, Currey and Hughes 1982). In contrast, adding organic tissue increases shell strength, but is costlier for growth and fecundity (Frieder et al. 2016, Scherer et al. 2018). Oysters react to low risk levels by adding calcium carbonate and increasing in size and weight, without increasing in shell strength, but at higher risk levels, also add protein to their shells to increase strength (Scherer et al. 2018). Differences in shell responses to predator cues between spat-on-shell and seed oysters may therefore be a consequence of different perceptions of predation risk as spat-on-shell exhibited characteristics of a low to moder-

ate threat. Although both oyster cultures were in the same tanks and occupied the same spaces, spat-on-shell creates a matrix of overlapping shells that may reduce local water flow and could subsequently reduce predator cue detection in comparison to single seed oysters. Alternatively, growth of oysters attached to substrate they can build upon is likely very different than growing without such a substrate. Further research is necessary to determine the cause behind the different responses of the two culture methods.

Overall, these findings demonstrate predator cues can be used to induce oysters to grow stronger shells in an aquaculture setting, and that regardless of culturing method, oysters will respond to predation risk by growing stronger shells. Additionally, these results show that culture method has a significant effect on oyster growth and can also govern the response of oysters to predator cues. While further research is required to evaluate the benefits of shell induction as well as rearing oysters using different culture methods, our preliminary results are promising for aquaculture and restoration efforts and may reduce predation risk in natural settings.

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