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SHORT COMMUNICATION

TIMING OF PREDATION RISK DURING EARLY DEVELOPMENT INFLUENCES OYSTER SHELL MORPHOLOGY[§]

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INTRODUCTION

Avoiding consumers is paramount for organisms, and many species possess adaptations such as spines, thorns, hardened exoskeletons, or distasteful chemicals to deter them (Hay 2009, Scherer and Smee 2016). These adaptations often come at a cost of reduced growth or fecundity (Relyea 2002, Miner et al. 2005), and in habitats where risk of consumption is high, these types of adaptations are more common and stronger than in habitats with low consumption rates (Coley et al. 1985, Bolser and Hay 1996). Because risk of consumers is variable and responding to them is costly, many prey species use inducible or plastic defenses that are only expressed when the risk posed by consumers is high (Relyea 2002, Miner et al. 2005, Weissburg et al. 2014). Inducible defenses allow prey to avoid unnecessary costs and prioritize critical life history processes like foraging, mating, or growth when the risk from consumers is low, and concentrate on defenses to increase survival when risk is high.

Inducible defenses require prey to evaluate risk posed by consumers and react appropriately by allocating resources to defense when necessary but prioritizing other life history processes when risk is low (Relyea 2002, Miner et al. 2005). Prey overwhelmingly rely on chemical cues to evaluate and respond to risk (Weissburg et al. 2014), and these cues may emanate from predators and injured prey (Scherer and Smee 2016). Expression of predator avoidance or deterrence may be influenced by numerous factors such as prey physiological state (Lima and Dill 1990), temporal variation in risk (Ferrari et al. 2008), and environmental conditions (Smee et al. 2010) as well as the age or vulnerability of the organism (Scherer et al. 2018). However, it is not understood how life stage and development may affect the ability of prey to manage defense mechanisms.

Oysters are ecosystem engineers that build habitat for numerous other species and provide a host of benefits including shoreline protection and water filtration (Grabowski and Peterson 2007). In addition to their ecological importance, oysters remain a profitable fishery through both wild harvesting and aquaculture (Grabowski et al. 2012). However, oyster reef habitats have declined in the Gulf of Mexico (zu Ermgassen et

al. 2013), and considerable investments have been made to restore oyster populations and recover lost benefits (Grabowski and Peterson 2007, Grabowski et al. 2012). Remote setting of spat-on-shell is often employed to reestablish reefs in spat-limited areas. In this process, oysters are settled on shells or other hard substrates in a nursery, grown until they reach a suitable size to place in the field (~2 weeks), and then placed in the field. Despite these investments, reef restorations often fail (LaPeyre et al. 2014), and predation on newly settled oysters is a common source of mortality (Bisker and Castagna 1987). Previous work suggests using predator cues in the nursery can strengthen oyster shells and reduce mortality and may augment aquaculture and restoration efforts (Combs et al. 2019, Belgrad et al. 2021). However, the effectiveness of using predator cues to reduce mortality may vary with different nursery practices such as rearing time. Therefore, there is clear need to understand how temporal exposure of oyster spat to predation risk in nurseries influences oyster growth and morphology.

In this study, we assessed responses of Eastern oysters (*Crassostrea virginica*, hereafter oysters) to temporal variation in predation risk. Oysters react to predation risk by building heavier, stronger shells that effectively deter predators and increase survival (Robinson et al. 2014, Ponce et al. 2020, Belgrad et al. 2021). Predation on newly settled oysters is a common source of mortality (Bisker and Castagna 1987), but predation is variable and shell building is costly (Scherer et al. 2018). Oysters cease responding morphologically to predator risk after reaching ~3 cm in shell length (Johnson and Smee 2012, Scherer and Smee 2017). This response suggests there is a critical time in development when predator exposure induces shell changes, but the timing of defense development is unknown. In this study we asked 2 questions: 1) does predator exposure immediately after settlement have larger effects on oyster morphology compared to exposure later in development? and 2) once exposed, can oysters stop responding to predation risk if risk cues are removed? Here, we exposed oyster spat to predation risk cues using blue crabs (*Callinectes sapidus*) actively

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feeding on oysters or controls for 4 weeks, but we swapped half the oyster spat between predator and control tanks after 2 weeks to determine if the initial 2-week exposure or the second 2-week exposure had different effects on shell morphological changes.

MATERIALS AND METHODS

Nursery rearing

Oyster larvae were settled onto 4.5 cm x 4.5 cm marble tiles at the Auburn University Shellfish Laboratory (AUSL) on Dauphin Island, AL in May 2021. Following settlement and metamorphosis into spat, oysters were placed into a flow-through unfiltered seawater system at the Dauphin Island Sea Lab (DISL) which pumped water directly from Mobile Bay and maintained natural abiotic water conditions. Oyster spat were exposed to predation risk from actively feeding blue crabs or a no-predator control in 4 flow-through mesocosms (2.0 m diameter, water depth = 0.4 m) with water flow ~25 L/min. Within each tank, oyster spat were held in 10 plastic cages (64 x 23 x 14 cm), and each cage contained 52 tiles with oyster spat (520 tiles per tank, 2080 total tiles). The number of oyster spat per tile varied greatly from 0–50, and we elected to maintain this variation to mimic natural settlement and reef restoration practices. Cages were suspended above the substrate to reduce sediment build-up. Tanks were drained daily, and oysters rinsed to remove sediment. Two tanks contained adult blue crabs (6 crabs per tank in 3 cages that partitioned individuals apart), while 2 tanks held empty cages and served as controls without predators. Predator cages were distributed evenly along the tank edges. Crabs were fed recently shucked oyster tissue 3 times per week (6, ~5.0 cm oysters were used per tank). Oyster spat react to both blue crab cues and cues from injured conspecifics, and thus we elected to feed crabs oysters in situ to make the cue strong and to simulate crabs actively eating oysters on a reef (Scherer et al. 2018). Crabs were replaced during the experiment as needed due to mortality. Oyster cages were rotated daily within their respective tank to prevent tank placement artifacts.

Oyster spat were reared in the DISL mesocosm for 4 weeks under these conditions. After 2 weeks, half of the oyster tiles from control tanks were moved to tanks with blue crabs and half of the oyster tiles with blue crabs were moved to controls. This created 4 treatments: controls without predators (i.e., no exposure), oysters exposed to predators for 2 weeks and then controls for 2 weeks (i.e., early predator exposure), oysters exposed to controls for 2 weeks followed by 2 weeks of predator exposure (i.e., late predator exposure), and oysters exposed to predators for 4 weeks (constant exposure).

Shell morphology measurements

After 2 weeks, 175 oyster spat from controls and another 175 from blue crab treatments were haphazardly selected for analyses for shell size and hardness. Then, after 4 weeks, 875 oyster spat were haphazardly selected for analysis: 275 from controls, 175 from early exposure, 175 from late exposure, and 250 from full exposure. At this size, oysters are roughly round (Figure 1), and shell diameter was measured to the nearest hun-

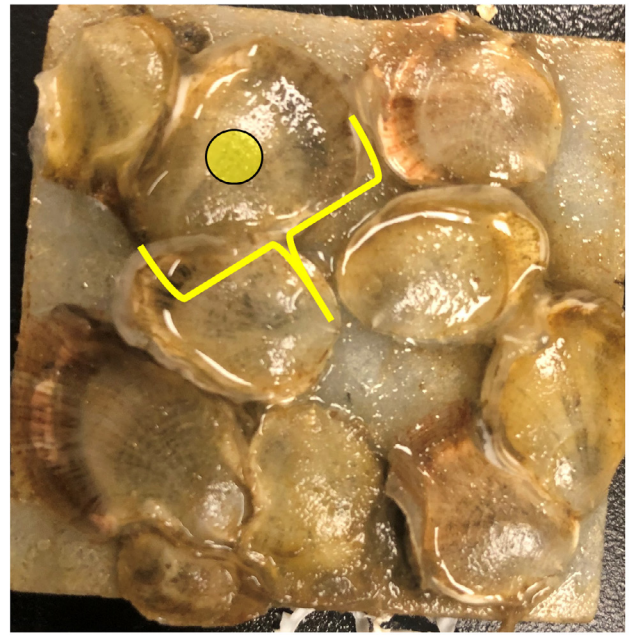


FIGURE 1. Oyster spat growing on marble tile. The line represents a typical measurement of shell size and the circle indicates the location where hardness would be measured via crushing with a penetrometer.

dredth of a mm using digital calipers from the umbo to the outer shell edge. We then quantified the force needed to break each oyster shell using a penetrometer (Kistler force sensor 9207 and a Kistler charge amplifier 5995). A small blunt probe was placed centrally to be equidistant from the shell edges and perpendicular to the shell surface (Figure 1). Gentle and consistent pressure was applied until the shell cracked, and the maximum force (N) needed to break the shell recorded, which is a standard proxy for shell hardness (Robinson et al. 2014). Because larger individuals have a stronger shell as a byproduct of their size, we divided shell crushing force by shell diameter to produce a size-standardized metric of shell strength (i.e., standardized crushing force, *sensu* Belgrad et al. 2021). Care was taken to avoid measuring oysters surrounded by other oysters to ensure shell growth or shape was not limited by space.

We compared shell length and standardized crushing force among predator exposure treatments for 2 and 4 weeks culturing in 4 separate generalized mixed-effects models (one model for each time period and shell characteristic; GLMMs; R package lme4). Predator exposure treatment was set as a fixed effect while holding cage nested in tank were treated as random effects to account for nonindependence among oysters. Tukey's multiple comparison tests were run on models to determine significantly different groupings using the general linear hypotheses function in the R package multcomp. All statistical analyses were conducted in R version 4.0.0 (R Core Development Team 2020).

RESULTS AND DISCUSSION

Oyster spat shells were not significantly larger (Figure 2A, difference of < -0.01 mm, $t = -0.78$, $p = 0.433$) or harder (Figure 2C, difference of 0.23 N/mm, $t = 1.34$, $p = 0.181$) after 2

weeks of predator exposure. Oyster spat had significantly harder shells when reared with blue crabs for 4 weeks (Figure 2D, 0.25 N/mm compared to controls, $t = 2.40$, $p = 0.017$), consistent with previous studies (Combs et al. 2019, Ponce et al. 2020, Belgrad et al. 2021). Conversely, oysters reared without predators were ~10% larger than those reared with predators, regardless of exposure time (Figure 2B, size difference of controls compared to predator-exposed = 0.01, $t = 1.53$, $p = 0.034$). Oysters reared with predators for 4 weeks had the hardest shells (21% stronger) but were not significantly different from spat exposed to crab cues for just the second 2-week period (Figure 2B), implying that the second 2-week period was important for shell induction. Interestingly, oysters exposed to predators for 2 weeks and then placed into tanks with no predator cues for another 2 weeks did not produce harder shells than control oysters (Figure 2D). Oysters exposed to predators for 2 weeks in both early and late treatments had significantly shorter shell lengths than controls (but not as short as the constant predator treatment, Figure 2B), suggesting that in both instances oysters were initiating reactions to predators. Yet, the early exposure did not produce harder shells despite the shorter length, suggesting that oysters can stop shell induction when predation risk is removed. In contrast, spat exposed for only 2 weeks in the later treatment produced shells that were significantly harder than controls and not significantly different from those reared with blue crabs for 4 weeks, indicating that oysters can also start shell induction if risk is introduced after settlement. In summary, at least within the first 4 weeks after settlement, oysters can initiate and cease shell changes depending upon the presence of predation risk.

Oysters can alter their shells by incorporating additional inorganic material (i.e., calcium carbonate), which increases their size and may help defend against crushing predators that may be size limited. Adding calcium carbonate to shells is often energetically cheap (Currey and Taylor 1974, Frieder et al. 2016) and can be produced quickly under favorable conditions to increase shell size (Lee et al. 2011, Frieder et al. 2016). For instance, the snail *Nucella lapillus* can rapidly increase shell size by adding a homogenous calcium carbonate layer to reach a size refuge (Avery and Etter 2006). Bivalves, including oysters, also make larger shells by adding inorganic shell material (Scherer et al. 2018). Thus, building a larger shell is likely the initial step in responding to risk, but building the shell quickly produces shells that are weaker per unit area (Scherer et al. 2018). Shell strength is increased substantially by adding additional organic material to the shell, which is twice as energetically costly as adding calcium carbonate (Lee et al. 2016, Frieder et al. 2016), and tends to be limited in oysters to situations when risk is high and con-

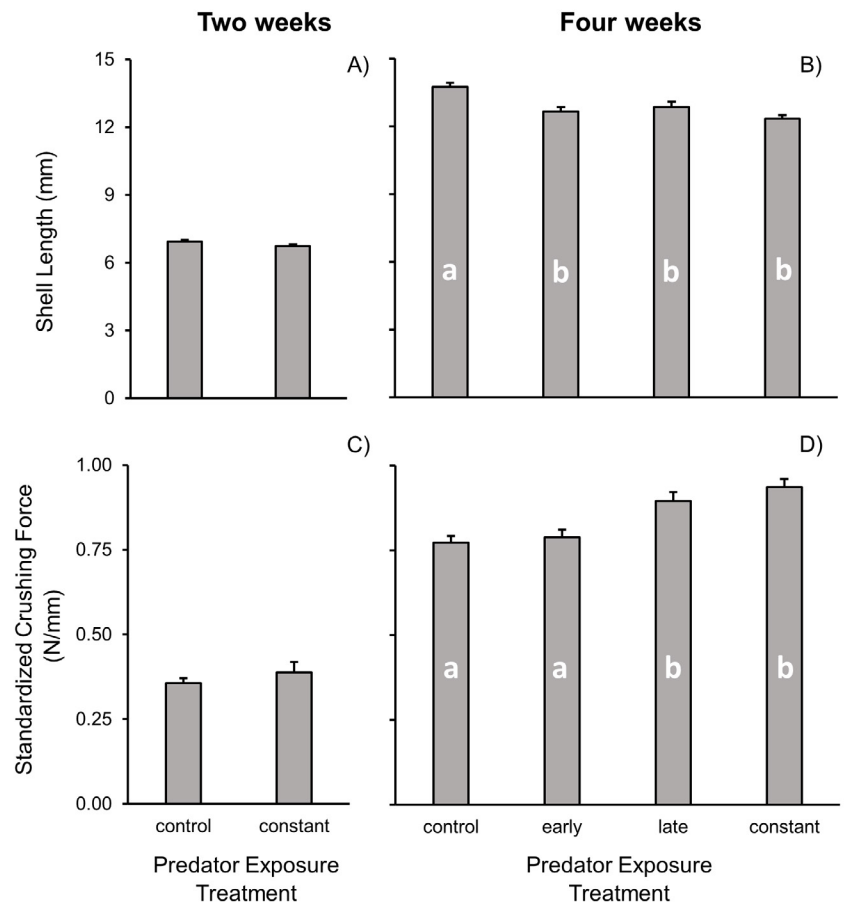


FIGURE 2. Mean ($\pm$ SE) measurements of oyster (*Crassostrea virginica*) spat cultured for 2 or 4 weeks with or without predators. A. Shell length (mm) of oysters nursed in the hatchery for 2 weeks ($n = 175$). B. Shell length (mm) of oysters nursed in the hatchery for 4 weeks ($n = 275, 175, 175$, and 250 respectively). C. Crushing force standardized by shell length (N/mm) of oysters nursed in the hatchery for 2 weeks ($n = 175$). D. Crushing force standardized by shell length (N/mm) of oysters nursed in the hatchery for 4 weeks ($n = 275, 175, 175$, and 250 respectively). Letters reflect significant differences among groups (Tukey's multiple comparison tests). constant—constant exposure to blue crab (*Callinectes sapidus*) predators; early—exposure to blue crab predators for the first 2 weeks of the study; late—exposure to blue crab predators for the last 2 weeks of the study.

stant (Newell et al. 2007, Scherer et al. 2018). Unlike previous studies, oysters had smaller shells in all risk treatments in our experiments (e.g., Robinson et al. 2014), and further experiments are necessary to better understand this discrepancy.

In nature, risk posed by consumers is commonly temporally variable, and responding to predators requires prey to evaluate risk and respond appropriately (Preisser et al. 2005, Weissburg et al. 2014). Organisms that rely on behavioral changes to avoid consumers can quickly adjust behavior to avoid predators when risk is present (Weissburg et al. 2014), while organisms that depend on morphological changes to deter consumers are less able to quickly adjust to temporal variations in risk (Scherer and Smee 2016). However, our data suggest oyster spat can start and stop a morphological response, at least in their early development, to adjust to changes in predator regime. Additional research is needed to better test oyster responses

to temporal variation in risk, and experiments rearing oysters under natural variation to reproductive age would be beneficial to better measure changes in costs and benefits of induction in terms of survival, marketability, and fecundity.

Predation can be a significant hurdle to success in remote setting programs that use spat-on-shell to rebuild oyster reefs. Preadapting oysters to predators by exposing them to predator cues in the hatchery may be a useful tool to increase effectiveness of spat-on-shell programs (Belgrad et al. 2021). However, it is common to hold spat in nurseries for 2 weeks before placement in the field as this time allows the maximum number of spawns each season. Yet, our results demonstrate that 2 weeks

is insufficient time for predator induction to work, and oysters are substantially smaller, weaker, and more vulnerable to predators after 2 weeks in the nursery vs. 4 weeks. These findings indicate that restoration operations that are limited in spawns will likely have the greatest cost-benefit when oysters are nursed for 4 weeks and future studies should investigate if the differences in survival by growing spat-on-shell for 4 weeks increases survival enough to justify having fewer spawns each year in operations that are not spawn limited. Finally, costs associated with using predator cues to induce oyster spat could be reduced by limiting exposure only during the final 2 weeks of rearing.

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