Phenotypic plasticity expands oyster survival and realized niche space across tidal elevations

Carter Lin[1,2], Benjamin A. Belgrad[2], Christa M. Russell[1,2], Jessica Lunt[2], Delbert L. Smee[1,2,*]

[1]University of South Alabama, Mobile, AL 36688, USA
[2]Dauphin Island Sea Lab, 101 Bienville Blvd, Dauphin Island, AL 36528, USA

ABSTRACT: The realized niche of many sessile intertidal organisms is constrained by different stressors that set boundaries for their distribution based on tidal elevation. Higher tidal elevation increases desiccation risk but can provide a refuge from predation. Conversely, deeper water increases feeding time and growth but also increases vulnerability to benthic predators. Eastern oysters *Crassostrea virginica* harden their shells in response to predator cues, which reduces their mortality from predation. We performed a field study to investigate if this defense mechanism could be manipulated to expand their realized niche and increase space for oyster survival and growth. We raised oysters in the presence of predators (blue crabs *Callinectes sapidus*) or in no-predator controls, measured changes in shell morphology, and then monitored oyster survival at different tidal elevations across 7 locations with different predator and salinity regimes. Oyster survival was significantly higher at the highest tidal elevations tested. Exposure to predators before deployment also significantly increased shell hardness and survival, with intertidal oysters experiencing greater improvement in survival from cue exposure than subtidal oysters. Intertidal placement (>15% exposure time) had larger effects on survival than predator exposure, but predator exposure increased oyster survival at all tidal elevations, suggesting that predator induction could help oysters both deter predators and resist abiotic stressors like desiccation, and perhaps increase the spatial areas where oysters can be restored.

KEY WORDS: Phenotypic plasticity · Realized niche · *Crassostrea virginica* · Restoration · Exposure · Blue crab · *Callinectes sapidus* · Predator—prey interactions

1. INTRODUCTION

The environmental conditions suitable to the physiological tolerances of a given organism comprise its fundamental niche (Vandermeer 1972, Whittaker et al. 1973). Yet, organisms rarely occupy their entire fundamental niche, as their niche space is confined by negative biotic interactions such as competition (Connell 1961), disease (Anagnostakis 2001), parasitism (Lafferty et al. 2006, Atkinson & Samuel 2010), and predation (Pace et al. 1999, Eastwood et al. 2007) that can limit where a species persists and forms its

realized niche (Whittaker et al. 1973, Roughgarden 1974). In contrast, the realized niche of organisms can be expanded by positive, facilitative interactions that alter conditions and increase habitat suitability (Bruno et al. 2003), or by phenotypic plasticity against negative biotic interactions that can re-open niche space (e.g. induced defenses against predation, Noor et al. 2008, Gómez et al. 2023).

Many organisms are phenotypically plastic, whereby they can adjust their phenotype in response to environmental conditions such as wave energy (Freeman & Hamer 2009, Freeman et al. 2014) or the

presence of potential predators (Relyea 2002, Miner et al. 2005). Gastropods on windward rocky shores have a larger foot area to better attach themselves and resist dislodgment by waves (Trussell et al. 1993) and a smaller foot opening and thicker shell on wave-protected shores where predators are more common (Large & Smee 2013). Like gastropods, barnacles exhibit phenotypic plasticity in response to flow, growing shorter thicker feeding appendages in faster flows (Marchinko & Palmer 2003, Reustle et al. 2023). Phenotypic plasticity is also often associated with prey reactions to predation risk and is usually triggered by predator exudates (Weissburg et al. 2014). For example, *Rana sylvatica* tadpoles will change their body size when living in ponds with dragonfly predators (Relyea 2002, Schoeppner & Relyea 2009), and mussels produce more byssal threads to resist being dislodged by predators (Leonard et al. 1999). Adaptive forms of phenotypic plasticity can allow organisms to balance costs of changes in morphology or behavior with counteracting negative conditions such as stressful environments or the presence of consumers (Miner et al. 2005). Phenotypic plasticity can increase survivorship in the face of unfavorable conditions, helping to maintain and even expand realized niche space (Bruno et al. 2003, Miner et al. 2005).

When phenotype shifts enable better exploitation of a resource like light or food, or reduce a stressor to sublethal intensity, a formerly unfavorable environment can become usable or even favorable (Miner et al. 2005). Such is the case of plants which grow taller stems in response to shade to outgrow their neighbors and access more light (Donohue 2003). This makes phenotypic plasticity a form of niche construction, where organisms modify the biotic and abiotic conditions of their surroundings via direct manipulation, construction, or metabolic interactions to better support survival (Odling-Smee et al. 2013). Comparable to environmental engineers generating more realized niche space by directly shaping environmental qualities (Hui et al. 2004), phenotypic plasticity may increase realized niche space by shaping the organism.

Eastern oysters *Crassostrea virginica* are both ecologically and economically important and exhibit notable morphological plasticity in their shells in response to predation risk (Scherer et al. 2016). These bivalves occupy both intertidal and subtidal areas, but their populations face a gradient of different stressors across tidal depths (Fodrie et al. 2014, Johnson & Smee 2014). Oysters that live at shallower tidal depths experience increased exposure during low tides, leading to reduced feeding time and increased risk of desiccation and mortality. In contrast, oysters that live deeper in the water column remain submerged, feeding longer and growing faster, but are more accessible to benthic predators such as blue crabs *Callinectes sapidus* or southern oyster drills *Stramonita* spp. (Brown & Richardson 1988, Fodrie et al. 2014, Johnson & Smee 2014).

The exact range of oysters' realized tidal elevation niche varies by region. Even within a single estuary, interactions between environmental factors like salinity and predation can shift the depth of the realized niche (Walles et al. 2016). For example, some populations of oyster drills cannot tolerate low salinities (Pusack et al. 2019), allowing oysters residing in less saline water to grow at lower tidal elevations than their counterparts in higher-salinity areas (Fodrie et al. 2014, Johnson & Smee 2014, Walles et al. 2016). Oyster reefs throughout the US have declined from overharvesting, hypoxic events, and intense benthic predation (Lenihan et al. 2001, Beck et al. 2011, Grabowski et al. 2012). Some organisms can maintain their realized niche by adjusting their phenotype to counter adverse conditions, but applying or manipulating these responses has rarely been used (Beadman et al. 2003).

Juvenile oysters can modify their shells in response to chemical cues from predators, and naturally occurring oysters have significantly harder shells in areas with more intense predation (Lunt & Smee 2014, Scherer et al. 2018). By growing stronger shells, oysters reduce their vulnerability to predators and increase survival by 50% compared to non-induced oysters (Ponce et al. 2020, Belgrad et al. 2021, 2023). This difference in survivorship becomes more pronounced in periods of intense predation. Mature oysters have a size refuge against predation, as their shells reach a critical threshold at >30 mm where they can no longer be easily crushed by predators (Eggleston 1990). However, juvenile oysters lack that degree of protection, so this induced predator defense is essential to survive early life stage mortality.

This defense also poses an interesting possibility for niche expansion and potentially increases the area viable for oyster reef restoration efforts. If oyster survival at lower tidal elevation is constrained by predation, but the induced defense can significantly increase survival against heavy predation, then oysters with enhanced morphological defenses could survive at lower depths by resisting predation. However, the likelihood of niche expansion via this shift depends on predation intensity. At moderate to high intensity, an adaptive shift would be most likely to affect the realized niche space. However, if the predation were too intense, it could overwhelm any miti-

gation from that morphological shift (Bruno et al. 2003, Odling-Smee et al. 2013).

We compared the relative mortality rates of oysters, raised with and without exposure to predator exudates to increase oyster shell hardness, across a range of tidal elevations to examine whether predator-induced shell morphology affected the realized niche of oysters and to determine the optimal tidal elevation for oyster restoration to balance growth and survival. We hypothesized that oysters at the highest tidal elevations would grow more slowly due to reduced feeding time and be more prone to die from abiotic stress, while those in the subtidal areas would grow faster but experience higher levels of predation.

2. MATERIALS AND METHODS

To determine whether predator cues can be used to expand the realized niche of oysters, we performed a field experiment in Mobile Bay, near Dauphin Island, AL, USA. We compared oyster survival across tidal elevations when initially grown with blue crab predators or in no-predator controls within the nursery prior to placement in the field. Due to oyster spawning logistics, we deployed part of our experiment in summer (June) and another in fall (September) 2021.

Seven field sites covering a range of abiotic conditions and predator regimes were selected for the experiment (Walles et al. 2016, Belgrad et al. 2023) (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m734p035_suppl.pdf). Sites comprised a mix of 4 natural sites and 3 oyster farms. Mobile Bay has a diurnal tidal cycle with < 1.0 m amplitude, although water elevation is also driven by wind and seasonal patterns (Webb & Marr 2016). To capture the full range of seasonal tidal variation, our oyster treatments remained in the field for 9 mo, until they reached ~7.5 cm in length. Thus, our treatments experienced different levels of inundation across the year and allowed us to test long-term viability, which is essential for restoration and aquaculture.

Oyster larvae were provided by the Auburn University Shellfish Laboratory in May and July 2021 and were settled onto marble tiles (4.45 × 4.45 cm) to standardize the settlement surface. Once the larvae finished settlement and metamorphosized into spat, they were kept in a flow-through seawater system for 1 mo. Water in this system was drawn from the mouth of Mobile Bay near the Dauphin Island Sea Lab. Spat-on-tile were held in 8 tanks (2 m diameter, water height = 0.4 m). Within each tank, tiles were held in 5 cages (64 × 23 × 14 cm), and each cage contained 65 tiles with oyster spat (325 tiles per tank, 2600 total tiles). Cages were rotated throughout the tanks daily to eliminate any position effects on feeding and chemical cue dispersal.

Four of the tanks had oysters induced with predator cues from 6 caged adult blue crabs (Combs et al. 2019, Belgrad et al. 2021). The cages prevented the crabs from consuming the oysters while allowing oysters to receive predator exudates. Crabs were fed shucked adult oysters 3 times per week. Chemical cues from both the crab and the injured oysters elicited oyster shell hardening (Scherer et al. 2016).

Immediately before deployment in the field, subsets of oysters were taken to assess how predator cues influenced shell growth and strength. In the June deployment, 30 tiles were haphazardly selected from each predator cue treatment evenly across the tanks with 5 spat selected from each tile for assessment (300 spat total for June), while 30 tiles were taken from the control tanks and 20 from the predator cue tanks during the September deployment (150 and 100 spat measured, respectively). We assessed effects of predator exposure on spat shell growth by measuring shell lengths using digital calipers and the force needed to crush the shell using a penetrometer (Kistler force sensor 9207 and a Kistler charge amplifier 5995). The force sensor was placed equidistantly from the shell edges and perpendicular to shell surface. Gentle and consistent pressure was applied until the shell cracked, and the maximum force (N) needed to break the shell was recorded, which is a standard proxy for shell hardness (Robinson et al. 2014). We were careful to avoid pressing the probe into the tile to which the oyster was attached. Crush force was standardized by shell length (N mm^{-1}) to account for size effects, as larger shells tend to be stronger.

Before deploying oyster tiles in the field, we randomly thinned oyster spat to standardize the number on each tile to 10 individuals. The tiles were mounted to 5.0 cm diameter PVC pipes using screws so that the tiles spanned a range of 102 cm and were spaced 8.9 cm apart (Fig. S2). At each height, a pair of tiles was placed on opposite sides of the PVC pole so that at each tidal elevation, 1 tile held spat reared with blue crab predators and the other held spat reared in no-predator controls. When mounted vertically in the field, the 102 cm range spanned from subtidal to intertidal and exposed oysters to different levels of air exposure (Table S1).

PVC poles were secured to existing pilings at each site using zip ties. Two oyster farm sites and 1 natural site were selected for summer deployment, and then 3 natural sites and 1 additional farm site were used for

fall deployment (n = 3900 spat per induction treatment for summer deployment and n = 3800 spat per induction treatment for fall deployment; 15 400 spat total; 1540 tiles total). We placed 8—10 poles at each site, with the number determined by availability of attachment areas. The orientation of the poles was haphazard at each site, with the goal of ensuring that tiles at each site were oriented in different directions (e.g. on both the north and south side of pilings) and that the center of each pole was placed at mean low water. At each site, depending upon space availability, we covered 2 or 3 poles with plastic mesh to deter predators to assess the source of oyster mortality (900 spat caged per treatment during summer and 800 spat caged per treatment during fall). Previous work suggests that predators are the largest source of oyster mortality in the area (Belgrad et al. 2021, 2023).

Survival was assessed at each site periodically, with more frequent checks performed shortly after deployment when most of the mortality was anticipated, and then less frequently as the experiment progressed (Table S2). At each check, we removed the PVC poles from the pilings, counted survivors on each tile, and then remounted them back at the same height. A total of 322 tiles (21%) were lost by the end of the experiment due to failure of materials most likely from hydrodynamic forces during storms.

Water temperature and salinity were measured at each field site using a handheld YSI™ during each survival check. We were also able to gather longer-term data from established monitoring stations through the Alabama Real-time Coastal Observation Stations (www.arcos.disl.edu). Water level was measured using Solinst™ level loggers at each site and recorded water level at 15 min intervals from Fall 2021 to Summer 2022. These data were used to calculate the percentage of time each oyster tile was exposed to air at low tide. All analyses were performed using R statistical software v4.1.3. Individual shell strength data were analyzed using a generalized linear mixed-effects model (GLMM) (R package 'nlme'), with treatment and deployment time as fixed effects, while tiles within cages within tanks were set as nested random effects.

The effects of submergence time (tidal position), predator cue treatment (blue crab exposure or control), and deployment time (June or September) on the number of surviving oysters on each tile at the end of the experiment were compared using GLMMs with a Type II negative binomial distribution (R package 'glmmTMB'; Brooks et al. 2017). Only data from uncaged poles at the final sampling period of each site were used in this model, as this allowed us to test whether these treatments produced survivorship differences even after experiencing the naturally high mortality rate associated with oysters and at a time-point that would represent oyster maturity in the region or initial success of a restoration effort. The percentage of time submerged, induction treatment (blue crab exposure or control), and deployment (summer or fall) were treated as fixed effects, while attachment poles nested in sites were treated as random effects. All interactions were initially included in the model, and nonsignificant interactions ($p \geq 0.05$) were removed stepwise, from the most complex interaction terms to the simplest, following the protocol of Crawley (2013) to help resolve the significance of main effects. This left an interaction between submergence time and deployment. Assumptions of GLMMs were validated using the R package 'DHARMa' (Hartig 2022).

Individual survivorship through time over the entire experiment was analyzed using a mixed-effects Cox proportional hazards model (R package 'coxme'), and hazard ratios (survivorship probability of treatment/survivorship probability of control) were estimated with this R package. This model allowed us to right-censor the data to account for oysters that were not consumed or tiles that had gone missing over the study period. A Cox proportional hazards analysis is a statistical model which recognizes that the highest values for a sample may simply be the maximum possible value, because a result did not occur by the end of the observation period for that sample, so the model weighs the data points accordingly (i.e. the data are right-censored). The Cox model treated submergence time, predator cue treatment, and deployment time as fixed effects, while site, support piling, and settlement tile were treated as nested random effects. All interactions were initially included in the model, and nonsignificant interactions ($p \geq 0.05$) were sequentially removed, leaving an interaction between submergence time and deployment as well as deployment and cue treatment. Only data from uncaged poles were used in this model.

We analyzed effects of mesh caging on individual oyster survival across summer and fall deployments using a mixed-effects Cox proportional hazards model using predator cue exposure, deployment time, and cage as fixed effects (R package 'coxme'). Due to space availability, we only mounted 2—3 cages per site, and the cages were compromised within 6 wk of placement in the field and became accessible to predators over time as the mesh cages degraded. Thus, data from caged poles were only included in

this last model to test if most of the initial mortality was associated with predation events.

3. RESULTS

Oyster shells were significantly harder when reared with blue crabs (estimate = 0.14, t = 3.94, p < 0.001). Oysters reared with blue crab predators had harder shells after both spawns, with blue crab exposure increasing crushing force by 39.9% in spring and 20.0% in fall (Fig. S3). We did not find significant differences between spring or fall inductions (estimate = −0.03, t = −0.45, p = 0.656) or an interaction among predator exposure and induction season (estimate = 0.06, t = 0.98. p = 0.326).

Oyster survival at the conclusion of the experiment (9 mo in the field) was significantly affected by both tidal elevation and predator cue exposure (Fig. 1). Induced oysters had 51% higher survivorship than control oysters reared without predator cues at the end of the experiment (0.99 vs. 0.65 oysters per tile when averaged across all sites and tidal elevations; Table 1). Water depth at each site ranged from 0.9 to 1.6 m, and tidal range was ~1.3 m over the duration of the experiment. Increased exposure time also dramatically increased survival (Table 1), as tiles exposed between 5 and 10% of the time had 1.56-fold greater survival than those submerged 100% of the time (1.13 vs. 0.73 mean oysters per tile), and tiles

Table 1. Generalized linear mixed model examining the effects of predator cue treatment, (blue crab exposure, no cue control), submergence time (percent of time submerged), and deployment (June, September) on overall oyster survival after 9 mo in the field

Source of variation	Coefficient estimate	z	p
Predator cue treatment	0.47	4.51	<0.0001
Submergence time	4.38	8.57	<0.0001
Deployment	0.99	1.05	0.2940
Submergence time × Deployment	3.42	4.47	<0.0001

exposed >20% had a 3.16-fold increase in survivorship over those constantly submerged (2.29 vs. 0.73 mean oysters per tile; Fig. 1). The difference in survival between predator cue treatments also increased with greater exposure time to air (Fig. 1). Both induced oysters and those grown in controls without blue crabs exhibited similar trends, with survival increasing with air exposure, but the induced oysters overall had higher survival rates regardless of tidal elevation and exposure time.

Overall mortality for all sites was high, with only 8.7% of all oysters surviving after 9 mo (Fig. 2). Predator cue exposure (hazard ratio = 1.71, z = 9.12, p < 0.001) and submergence time (hazard ratio = 2.89, z = 3.73, p < 0.001) significantly affected oyster survival throughout the experiment, with induction and air exposure increasing survival by 71 and 289%, respectively, across the entire study period (Fig. 2). Additionally, the effects of deployment season were significant (hazard ratio = 0.19, z = −2.75, p = 0.006), with more oysters surviving generally during the fall deployment, while a significant interaction between season and predator cue exposure was found (hazard ratio = 0.72, z = −3.55, p < 0.001), possibly because induction had more than twice as large an effect on survivorship during the summer deployment compared to the fall deployment at the end of the experiment. There was also a significant interaction between deployment season and submergence time throughout the experiment (hazard ratio = 4.60, z = 3.53, p < 0.001).

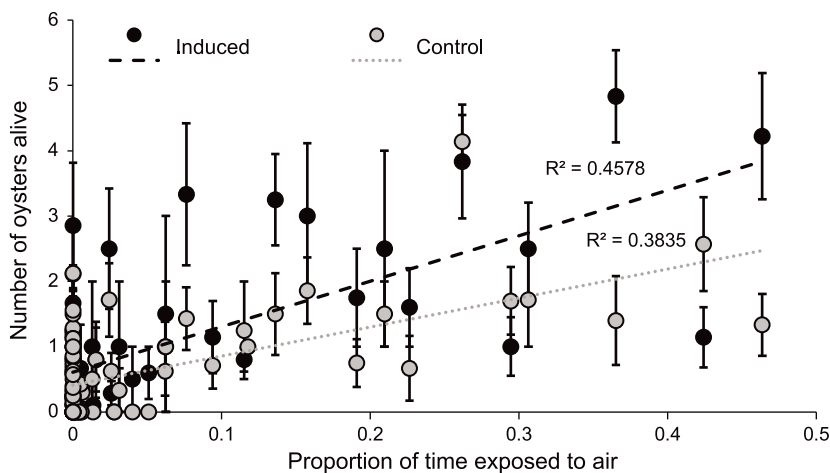Survival was higher among oysters protected with mesh cages (hazard ratio = 3.75, z = 6.28, p < 0.001), par-

Fig. 1. Oyster survival over proportion of time exposed to air. Each point represents mean ± SE oyster survival at a single exposure time from one site. Each exposure time (i.e. tidal elevation) has a pair of tiles containing oyster spat that were reared with blue crabs (induced) or controls without predators (typically n = 6−10 for each point, depending on the site and position, but n = 2−5 for 19% of the points due to lost poles or tiles). A significant positive relationship was found between exposure time and oyster survival, and oysters reared with blue crabs had lower mortality
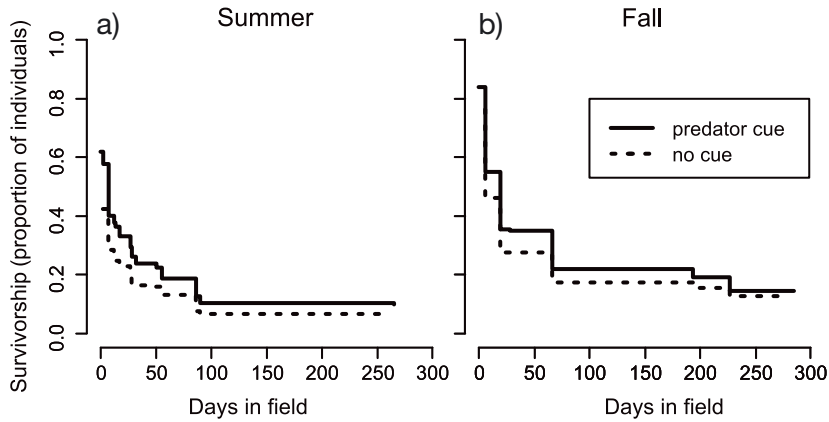
Fig. 2. Cox proportional hazards model of oyster survival during (a) summer and (b) fall deployments, showing survivorship between oysters reared with blue crabs and those raised in controls without predators. Induced oysters survived significantly more than control oysters, and survivorship was significantly higher in the fall

ticularly during the first month when oysters were most susceptible to predators (Fig. S4). Although the main effect of deployment season did not produce a significant effect on survival in the model including caging treatment (hazard ratio = 1.21, z = 0.43, p = 0.660), there was a significant interaction between cage and deployment time (hazard ratio = 0.50, z = −2.33, p = 0.020) as well as between predator cue and cage treatments (hazard ratio = 1.32, z = 3.02, p = 0.003).

During the experiment, average water temperature was 19.3°C and ranged from 7.2 to 34.6°C, and average salinity was 11.6 and ranged from 0.07 to 34.6 at Cedar Point. At Dauphin Island, the average water temperature was 22.9°C and ranged from 8.2 to 32.6°C, and average salinity was 15.3 and ranged from 0.02 to 23.5.

4. DISCUSSION

Intertidal oysters experience lower predation rates from benthic predators than subtidal oysters, particularly from oyster drills (Fodrie et al. 2014, Johnson & Smee 2014). In this study, we also found oyster survival to increase with tidal elevation (and air exposure during low tide) and predator induction at the elevations tested. Consistent with earlier research (Belgrad et al. 2021, 2023), rearing oysters with blue crabs prior to placement in the field increased oyster shell strength and individual survival. Additionally, these induced defenses led to higher survivorship at all tidal elevations. While the effects of tidal elevation exceeded those benefits gained from blue crab induc-

tion, the change in survival from predator induction increased as exposure time during low tide increased.

Oyster mortality was high at all locations, and we attributed mortality primarily to predation because salinity and temperature were within oyster tolerance limits, food was plentiful in the study area, the most common oyster disease in the region, *Perkinsus marinus* (i.e. dermo), is rarely fatal to juvenile oysters, and predator exclusion substantially increased oyster survival in the area (Belgrad et al. 2021, 2023). We attempted to deter predators using mesh cages and compare mortality in caged and uncaged treatments to assess predation, but our cages failed within the first 6 wk and did not fully exclude predators. However, after 1 mo, mortality in the caged oysters was less than 10%, while mortality was nearly 40% in uncaged oysters.

While not statistically significant, the difference in shell strength between induced and control oysters was ~2 times greater in the summer deployment than in the fall (Fig. S3). This increased shell strength in summer may account for why induction had a substantially greater effect on survivorship in the summer than in the fall and why there was a significant interaction between induction treatment and deployment season. Another non-mutually exclusive explanation for the survivorship differences between seasons is that predation intensity typically decreases in the fall as temperatures decrease, and predators are less active. Indeed, overall survivorship was highest during the fall deployment (Fig. 2), and predators were less frequently found climbing on our experimental poles during the fall. The survivorship benefits of induction likely increase with higher predation rates (Belgrad et al. 2023), which may also explain why induction was more beneficial in the summer deployment with higher predation rates. We also found significant interactions between caging treatments and predator induction, which we attributed to predators accessing the caged oyster spat and slightly preferring to consume the uninduced oyster spat (Fig. S4). Despite the interactions, both tidal elevation and predator induction significantly influenced oyster survival, consistent with earlier findings (Johnson & Smee 2014, Belgrad et al. 2021, 2023).

Optimal growth for intertidal oysters occurs when they are exposed to air for 20–40% of the tidal cycle (Walles et al. 2016). The top 2–3 tiles on each pole

were exposed for that amount of time, while some oysters were submerged for over 90% of the time. Our initial hypothesis posited that crab-induced oysters would have the largest survival increase over controls in the lowest elevations where predation would be highest, as the survival benefit from predator induction generally increases with increased predation pressure (Fordyce 2006, Belgrad et al. 2023). However, our data suggested induction was more beneficial as tidal elevation increased. Repeating this experiment in estuaries with diurnal tides and larger tidal amplitudes is a logical next step toward investigating the potential for phenotypic plasticity to increase niche space.

The zero-growth boundary from excess exposure begins at 55% exposure time (Ridge et al. 2015). Due to variations in site bathymetry, only the highest tiles at one site experienced greater than 55% exposure time throughout the annual tidal cycle. Those tiles exhibited the greatest difference in survival between oysters exposed to predators and those reared in controls. This suggests that predator induction might improve resistance to desiccation or other abiotic stressors, but additional research is necessary to test this hypothesis.

Oysters adjust their shell morphology in 2 ways when exposed to predation risk. They can quickly increase shell size by depositing calcium carbonate, and they can invest more energy and resources into developing the organic matrix of the shell, which increases shell strength (Scherer et al. 2018). The exact pathway depends on situational factors such as food availability and perceived risk level. Increased levels of perceived risk resulted in increased investment into the organic matrix that incurs higher costs (Scherer et al. 2018). Shell dimensions can also change in response to substrate type. Induced oysters unattached to substrate will grow smaller and more curved, taking on a 'cannonball' shape, while oysters settled onto flat surfaces, like oyster shells or the marble tiles that were used in this study, will grow larger and flatter (Combs et al. 2019, Belgrad et al. 2021). Different mollusk species have different trends between size and desiccation tolerance. Although some species are more susceptible to desiccation at larger sizes (Byrne et al. 1988) or have the same tolerance regardless of size (Guareschi & Wood 2020), most species increase their desiccation tolerance with bigger shell size, especially as the organism matures (Jenewein & Gosselin 2013, Coughlan et al. 2018). If our induced oysters increased their shell size faster, they may have benefitted from better desiccation tolerance. Further study of desiccation and induced shell structure could prove helpful to test these suppositions.

We expected sites to differ in predator type and predation intensity as well as in abiotic conditions. Off-bottom oyster farming has been successful in the area, and we inferred that these sites would be favorable for oyster growth. Instead, farm sites exhibited much higher initial and overall mortality compared to natural sites (Fig. S5). Off-bottom oyster farms house growing oysters in floating cages that are air dried weekly to reduce fouling and limit predation. The higher mortality rate we observed could be driven by the increased apparency of the farm site replicates. Oyster aquaculture farms have a high density of oysters that can attract a similarly high density of predators, which we observed climbing on the poles or actively feeding on the tiles. All of our farm site poles were mounted on pilings next to floating oyster aquaculture cages containing farmed oysters. Predators would have a harder time accessing the farmed oysters inside their floating cages, while our uncaged farm site poles posed no barriers to access aside from the height of the tiles. Thus, the farmed oysters may have drawn predators to our tiles, which subsequently consumed our experimental oysters that were vulnerable. Despite higher mortality at the farm sites, the same trends emerged in both natural and farm sites, with oyster survival significantly increased both by higher tidal elevations and by being reared with blue crabs prior to placement in the field (Fig. S5).

Our findings offer potentially useful guidance for oyster restoration projects. First, tidal elevation matters for restoration success. Tidal elevation has a significant impact on reef health and survival, particularly in areas with intense benthic stressors (Fodrie et al. 2014, Johnson & Smee 2014, Walles et al. 2016). Oyster survival increased with exposure (and shallower tidal elevations), and oysters incurred increased survival benefits from predator induction at elevations with as little as 5% exposure time, progressing to steadily higher survivorship benefits up to elevations with ~40% exposure time. Whether this benefit continues in even shallower elevations with >40% exposure time requires further investigation.

Marine stock enhancement should be performed responsibly (Lorenzen et al. 2010). For oysters in the northern Gulf of Mexico, using spat-on-shell, where oyster larvae are settled on shells or other hard substrate in a nursery, is becoming more commonly used for oyster restoration. These oysters are locally sourced to follow responsible practices (Lorenzen et al. 2010). Unfortunately, the effectiveness of spat-on-shell is compromised by large-scale mortality from predators. Spat-on-shell restoration may be improved by increasing oyster shell hardness via exposure to

predator cues during the nursery grow-out period (Belgrad et al. 2021, 2023). Consistent with earlier findings, exposure of oyster spat to blue crab predators significantly increased their survival in the field. Many restoration projects using living oysters suffer intense mortality when juvenile oysters are released at a site due to the vulnerability of this early life stage. Induction of these oysters while in the hatchery could help them survive past this initial bottleneck. Our sites had an initial survival increase of 14% after 1 wk (n = 532 more total induced spat than control) and a final survival increase of 45% (n = 138 more induced spat), consistent with earlier findings (Belgrad et al. 2021). However, for spat-on-shell restoration projects that deploy metric tons of oyster shells containing millions of oyster spat, even a small increase in survival of 1–2% could be substantial. Oyster reefs need to maintain specific population thresholds to achieve reproductive self-sufficiency, based on characteristics such as total population size, volume of dead shell, and the presence or absence of fishing (Moore et al. 2018). Reefs that do not meet these thresholds will experience a population collapse. The difference in starting population size between a successful reef and a failed reef can be on the scale of a couple hundred thousand individuals, which is well within a small increase in survivorship. Raising oysters with predators and selecting proper tidal elevations can improve survival and bolster restoration outcomes.

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