

Research article

Induced defenses as a management tool: Shaping individuals to their environment

Benjamin A. Belgrad^{a,*}, William Knudson^a, Sarah H. Roney^b, William C. Walton^c, Jessica Lunt^a, Delbert L. Smee^{a,d}^a 101 Bienville Blvd, Dauphin Island Sea Lab, Dauphin Island, AL, 36528, USA^b School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, 30332, USA^c Department of Fisheries Science, Virginia Institute of Marine Science, Gloucester Pt., VA, 23062, USA^d Department of Marine Science, University of South Alabama, Mobile, AL 36688, USA

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ABSTRACT

Many prey species can adjust morphology to reduce predation risk in response to predator cues. Enhancing prey defenses using predator cues may improve survival of cultivated species and enhance species restoration efforts, but assessment of such benefits at industrially relevant scales is needed. We examined how raising a model foundation species, oysters (*Crassostrea virginica*), under commercial hatchery conditions with cues from two common predator species can improve survival across a variety of predator regimes and environmental conditions. Oysters responded to predators by growing stronger shells than controls, but had subtle variations in shell characteristics depending on the predator species. Predator-induced changes significantly increased oyster survival up to 600% and survivorship was maximized when cue source was matched with local predator regime. Overall, our findings demonstrate the utility of using predator cues to enhance the survival of target species across landscapes and highlight the opportunity to employ nontoxic methods to control pest-based mortality.

1. Introduction

The success of ecological populations depends upon the ability of individuals to adapt to local environmental conditions that can vary across a species' distribution (Grenier et al., 2016). Organisms' responses to environmental differences range from behavioral and physiological modifications, to changes in morphology and internal structure (West-Eberhard, 1989). Induced defenses, where prey alter their body morphology and chemistry to deter predators, are among the most widespread forms of phenotypic plasticity, occurring in taxa from bacteria to chordates (Tollrian and Harvell, 1999; Kaufmann and Dorhoi, 2016). These defenses are frequently stimulated by chemical cues (kairomones) released from predators and injured conspecifics (Weissburg et al., 2014). Ecological theory predicts that individuals should tailor their defenses to the local predator regime to maximize fitness (Lively et al., 1999), and many laboratory studies show that individuals can alter their defenses to guard against the particular hunting mode of different predators. For example, bivalves will increase the thickness of their shell against crushing predators like crabs and will strengthen their

adductor muscles against sea stars that pry open shells (Freeman, 2007). However, models of how induced defenses are expected to govern predator-prey dynamics in multi-predator systems are contradictory (van Velzen et al., 2018; Ramos-Jiliberto et al., 2008) and field studies exploring responses to multiple predators remain rare.

Multiple predators feeding on prey are the norm rather than the exception in natural systems, with broad food web summaries identifying a median of two to three predator taxa feeding on each prey taxon (Cohen and Briand, 1984; Schoener, 2010) while a more in-depth analyses of a "simple" food-web found, on average, 9.6 predators per prey (Polis, 1991). Simultaneously, the dominant predator species prey encounter within a region can shift across landscapes with abiotic gradients. For instance, the salinity gradients of estuaries are well-known to govern local predation pressure and species assemblages (e.g., 13). Consequently, understanding how induced defenses alter prey survival under natural predator regimes and the consistency of these relationships across space are important for predicting how populations will respond to heterogeneous landscapes and controlling the abundance of key species.

* Corresponding author.

E-mail addresses: babelgra@eckerd.edu (B.A. Belgrad), sarah.roney@gatech.edu (S.H. Roney), walton@vims.edu (W.C. Walton), lsmee@disl.org (D.L. Smee).

Managing, conserving, and restoring species within complex landscapes often involves translocating individuals. Reintroduction programs are a keystone for conservation of endangered species and habitat restoration (Gibbs et al., 2008). Similarly, the release of captive-bred individuals into the environment is one of the most popular tools for the continued large-scale exploitation of wild animals and plants in the wildlife, fishery, and forestry industries (Laikre et al., 2010). Acclimating individuals to their environment prior to release is well-known to improve their performance (Bright and Morris, 1994; White et al., 2005). Subsequently, a variety of techniques have been developed to increase the survival and reproductive success of released individuals, ranging from mimicking the physical conditions of the environments to exposing individuals to other cohorts (Kuwada et al., 2004). One of the greatest sources of mortality to released individuals is predation since individuals are often naïve to local predator regimes, and predator-aversion training is consequently growing in acceptance to address this problem (White et al., 2005; Griffin et al., 2000; Bais et al., 2003; Rowell et al., 2020). The majority of such training is conducted on the behavioral response of vertebrate species and chemically inducing plants to be resistant to disease (Rowell et al., 2020; Walters et al., 2009), while translocating individuals from different environments has shown success at increasing the predation resistance of mussels (Beadman et al., 2003; Christensen et al., 2012). However, chemically inducing individuals in mass to be resistant to predators remains a relatively unexplored field of study, primarily focused on plant chemical defenses, and has yet to be tested across multiple natural predator regimes.

In this study we test the viability of using predator chemical cues to enhance the survivorship and restoration success of a model organism commonly cultured worldwide for both habitat restoration and human consumption: oysters. Oysters are ecological engineers that build habitat for numerous other organisms and provide a variety of ecological services such as shoreline stabilization and water filtration (Grabowski et al., 2007). While oysters remain a major fishery through both wild capture and aquaculture, with over five million tons harvested annually for consumption globally (Wijsman et al., 2019), wild harvest rates are half of those 70 years ago (FAO, 2021), and at least 85% of native reefs have been lost worldwide (Beck et al., 2011; McAfee and Connell, 2021). Considerable investments have therefore been made to restore oyster populations and return their ecological, economical, and cultural benefits, and remote setting of spat-on-shell is often implemented in larvae limited regions. In this process, larval oysters are settled onto shells or other hard substrate then grown in a nursery until they reach a size suitable for placement in the field. Despite these investments, reef restoration efforts often fail to achieve the desired oyster densities (Mann and Powell, 2007; La Peyre et al., 2014) as predators are a common source of mortality in juvenile oysters (Bisker and Castagna, 1987), and 70–100% of a cohort can be lost within one month (Gosselin and Qian, 1997).

Two major predators of oysters, *Crassostrea virginica*, are blue crabs, *Callinectes sapidus*, and oyster drills, *Stramonita haemastoma*. Although both predators are common in the field, their relative abundance can vary substantially across landscapes due to differences in salinity tolerances (Hines et al., 2007; Pusack et al., 2019). Additionally, these two predators use different mechanisms to feed on oysters: blue crabs consume oysters by crushing their shell while drills access oysters by boring holes into their shell. In turn, oysters can increase their shell hardness in the presence of predator cues from blue crabs and drills which decreases their susceptibility to predation in the lab (Ponce et al., 2020). However, field studies remain rare (but see 37), and it is unknown how cues from different predators may alter survival across landscapes that contain different abundances and species of predators, which limits our ability to predict how survival varies spatially. This study therefore also evaluates how the source of a predator cue and transplant environment can shape the efficacy of inducing organisms targeted for management as matches or mismatches between cue source

and the dominant predator regime of the planting site may offer enhanced or decreased survival benefits respectively.

2. Methods

2.1. Oyster culturing

Oysters (*Crassostrea virginica*) were raised as spat at the Auburn University Shellfish Laboratory (AUSL) on Dauphin Island, AL starting in mid-June 2020 using standard techniques (Congrove et al., 2009). Spat were allowed to settle onto 4.5 cm × 4.5 cm marble tiles to standardize settlement surface. Oysters were ~1.0 mm when the experiment began and housed in 12 circular flow-through holding tanks (radius = 2.0 m) with a water depth of 0.4 m (~8000 spat/tank). Water was supplied from Mobile Bay and flow rates in the holding tanks averaged 23.5 L/min. There was immense variation in the number of spat per tile which we elected to maintain during the experiment to mimic natural settlement and normal reef restoration practices (~5–40 spat/shell at four weeks of culturing). Oysters were suspended above the tank bottom in five oyster aquaculture baskets (64 × 23 × 14 cm with 65 spat covered tiles/cage; ~96,000 spat total) to prevent sediment buildup from suffocating oysters. These holding containers and tile densities mimicked normal nursery procedures for spat-on-shell (Matthiessen, 2001, personal communication, AUSL hatchery manager Scott Rikard) which enables large-scale settlement of spat suitable for industrial applications, but does not offer the ability for high replicate tank numbers. However, previous work by our lab has shown that oysters exposed to predator cue treatments in big industrial scale tanks exhibit the same morphology patterns observed in small scale, highly replicated experiments (Belgrad et al., 2021; Scherer et al., 2016, 2017; Robinson et al., 2014; Combs et al., 2019).

Oysters were exposed to exudates from two different predator cue treatments and an untreated (predator-free) control for one month to assess the effect predator species has on stimulating defense production. Four tanks contained adult blue crabs, four tanks contained oyster drills, and four tanks were without predators as a control. Predators were locally collected and held in three 64 × 23 × 14 cm cages distributed evenly along the tank edges. Blue crabs (13–18 cm) were grouped two per cage and partitioned to prevent cannibalism (six crabs per tank) while oyster drills (3.4–4.8 cm) were in groups of 10 within cages to be roughly equivalent to the mass of the blue crabs (30 drills per tank). Crabs and oyster drills were fed recently shucked oyster tissue 3x per week (six, ~5.0 cm oysters were used per tank). Crabs and oyster drills were replaced during the experiment as needed due to mortality. Oyster baskets were rotated daily within their respective tanks to prevent tank placement artifacts.

2.2. Induction effects on shell morphology

Two tiles were haphazardly taken from each basket and two oysters from each tile were selected for measuring shell characteristics after one month of culturing (4 oyster spat/basket, 20 oysters/tank, 80 oysters/cue treatment). Spat shell morphology was assessed by quantifying shell size, shell crushing force, and shell weight. Oysters are roughly round at this life stage, and shell diameter was measured to the nearest 0.01 mm using digital calipers from the umbo to the outer shell edge. Care was taken to avoid measuring oysters surrounded by cohorts to ensure shell growth or shape was not limited by space. We then quantified the force needed to break each oyster 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 recorded, which is a standard proxy for shell hardness (Robinson et al., 2014). We divided the crushing force by the shell diameter to produce a size-standardized metric of shell strength (i.e., standardized crushing

force) because larger individuals have a stronger shell as a byproduct of their size. After crushing, oyster shell dry weight was obtained by collecting all the shell fragments and removing any soft-tissue before desiccating in an oven at 70 °C for 48 h. Only the left oyster valves were weighed as the right valves were bonded to the underlying substrate and because crushing force was just applied to the left valve.

We examined the effects predator cue exposure (blue crab cues, oyster drill cues, and no cues) has on shell standardized crushing force, diameter, and weight using three separate generalized linear mixed models with Gamma distributions, one model for each of these three response variables (GLMMs; R package: lme4). Cue exposure was set as a fixed effect while tile spat settled on, nested in basket, nested in tank were treated as random effects to control for nonindependence among individuals (Bolker et al., 2009). Tukey's multiple comparison tests were run on models where necessary to determine groups deemed significantly different using the general linear hypotheses function in the R package: multcomp. All statistical analyses were conducted using R v4.0.0 (R Development Core Team, 2020).

2.3. Induction effects on field survival

After one month of culturing, oysters were planted at seven field sites along the Alabama coastline to quantify the extent that oyster survival is dependent on predator induction treatment and local environmental conditions. Sites spanned 25.8 km and were in three clusters with an average of 0.61 km between sites within a cluster (Supplementary Material: Fig. S1). Salinity spanned 6–24 ppt while water temperature ranged from summer highs of 33 °C to fall lows of 26 °C. Five of the sites were along natural shorelines (Sites B, C, E, F, G) while the remaining two sites were within 2-ha oyster farms run by AUSL (high salinity Site A, brackish Site D). Three of the sites (E, F, G) were within sheltered bays while the remaining sites were along exposed coastline that experienced a gradient of wave and current energy.

Twenty-one tiles were haphazardly selected from each basket and manually thinned to 10 spat/tile to standardize predator risk exposure (415 tiles per cue exposure treatment; 1245 tiles and 12,450 spat total). One oyster tile of each cue exposure treatment was affixed to 90 cm long rebar poles with zip ties in triads (30 spat/pole). All spat poles were planted in the field within one week in early July so that the oyster tiles were just above the sediment surface to prevent smothering. The five natural sites each had 23 poles placed at their locations on the same day while the two farm sites each had 90 and 120 poles planted at their locations on two separate days over that same week due to space constraints. Poles were placed in the water subtidally parallel to shore with ~1 m separating each pole. A fraction of the poles at each site were enclosed in an inflexible cylindrical plastic cage (diameter = 18 cm, length = 22 cm; pore size = 1 cm) to exclude predators and control for mortality events from nonpredatory sources (e.g., disease, abiotic conditions). Natural sites each had 3 random poles caged while both farm sites had 35 caged poles (15 and 20 random poles caged on the two respective planting days) to confirm that mortality was due to predation rather than disease or an abiotic factor. Once planted, spat were checked for individual survival after one, two, and three weeks in the field by counting the number of spat still alive on each tile. We ceased to check the natural sites after two weeks and one of the farm sites after three weeks due to the high mortality experienced by the oysters during this timeframe while the second farm site was checked again after spat were in the field for approximately one and three months. Seven stakes were lost before they were checked on the first week and were subsequently excluded from all analyses.

Environmental characteristics thought to be important for oyster survival were also measured during each site visit. Water depth was estimated using a depth pole while salinity, water temperature, and dissolved oxygen were measured using a handheld multiparameter instrument (Pro, 2030; YSI Inc.) to obtain a qualitative description of site conditions. As a rough assessment of the local predator regime, we

recorded the presence of any oyster drills, crabs, or fish found on the oyster stakes or within the cages during the first month of the experiment. Predator presence was standardized by the number of stakes checked to account for differences in sampling effort.

We examined whether the different predator cue exposure treatments (blue crab cues, oyster drill cues, and no cues) and the planting site affected oyster survival (proportion of spat alive on each tile) after just one week in the field. We ran a generalized linear mixed model (GLMM) with a binomial distribution, designating predator cue exposure treatment, site, and caging status as fixed effects. All interactions were initially included in the model and nonsignificant interactions 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 and achieve the lowest Akaike information criterion (AIC) value. Spat tile nested in rebar pole were treated as random effects to control for nonindependence among individuals.

Longer term survival of individual spat located on the two farm sites was assessed using a mixed-effects Cox proportional hazards model (i.e., a survival analysis; R package: coxme). Predator cue treatment, farm location, and caging status were treated as fixed effects while spat tile nested in rebar pole, nested in day deployed were treated as random effects. All interaction terms were assessed in the same manner as the GLMM above. Pairwise comparisons among survivorship curves were made using log-rank tests in the R package: survminer with a Bonferroni correction for multiple tests.

We tested whether sites were dominated by different predators or had different predator abundances to help verify if some predator cue exposure treatments conferred greater survivorship under a particular predator regime. As the majority of predation occurred within the first two weeks of deployment, we compared total predator abundance (number of predators at each stake) across all seven sites at the one-week mark using a generalized linear model with a Poisson distribution, treating site as a categorical explanatory variable.

3. Results

3.1. Induction effects on shell morphology

Exposure to predator cues substantially altered shell morphology. Oysters exposed to oyster drills had the strongest relative shell strength as their standardized crushing force was 55% stronger than controls ($n = 80$; estimate = 0.36, $df = 240$, $z = 4.45$, $p < 0.0001$; GLMM) whereas blue crab exposure produced 47% stronger shells (Fig. 1a; estimate = 0.33, $df = 240$, $z = 4.11$, $p = 0.0001$). Conversely, oysters grown with blue crabs had 15% larger and 74% heavier shells than controls (estimate = 0.01, $df = 240$, $z = 2.14$, $p = 0.0325$; estimate = 6.42, $df = 240$, $z = 2.91$, $p = 0.0037$ respectively) while oysters exposed to drills were not significantly larger and only 36% heavier than controls (Fig. 1a and b; estimate < 0.01, $df = 240$, $z = 0.90$, $p = 0.3682$; estimate = 4.78, $df = 240$, $z = 2.19$, $p = 0.0284$ respectively).

3.2. Induction effects on field survival

After one week, 7461 uncaged oysters died (56%; estimate = 7.19, $z = 17.93$, $p < 0.0001$; GLMM) while only 60 oysters within two broken cages had died (~3%). Among the uncaged oysters, 2291 (51%) blue crab exposed oysters and 2393 (53%) drill exposed oysters died, whereas 2777 (62%) control oysters died (estimate = 1.01, $df = 1583$, $z = 3.56$, $p = 0.0004$). Oyster survivorship was strongly dependent on the field site with average mortality ranging from 18 to 86% (Fig. 2; estimate = 2.04, $df = 1583$, $z = 3.71$, $p = 0.0002$). Site also significantly interacted with the predator cue treatment to govern the effectiveness of different species predator cues in enhancing survivorship over controls which varied from -0.75 – 617% change, depending on the location (estimate = -1.02, $df = 1583$, $z = -2.14$, $p = 0.0320$). Exposure to blue crab cues usually produced marginally higher survivorship than oyster

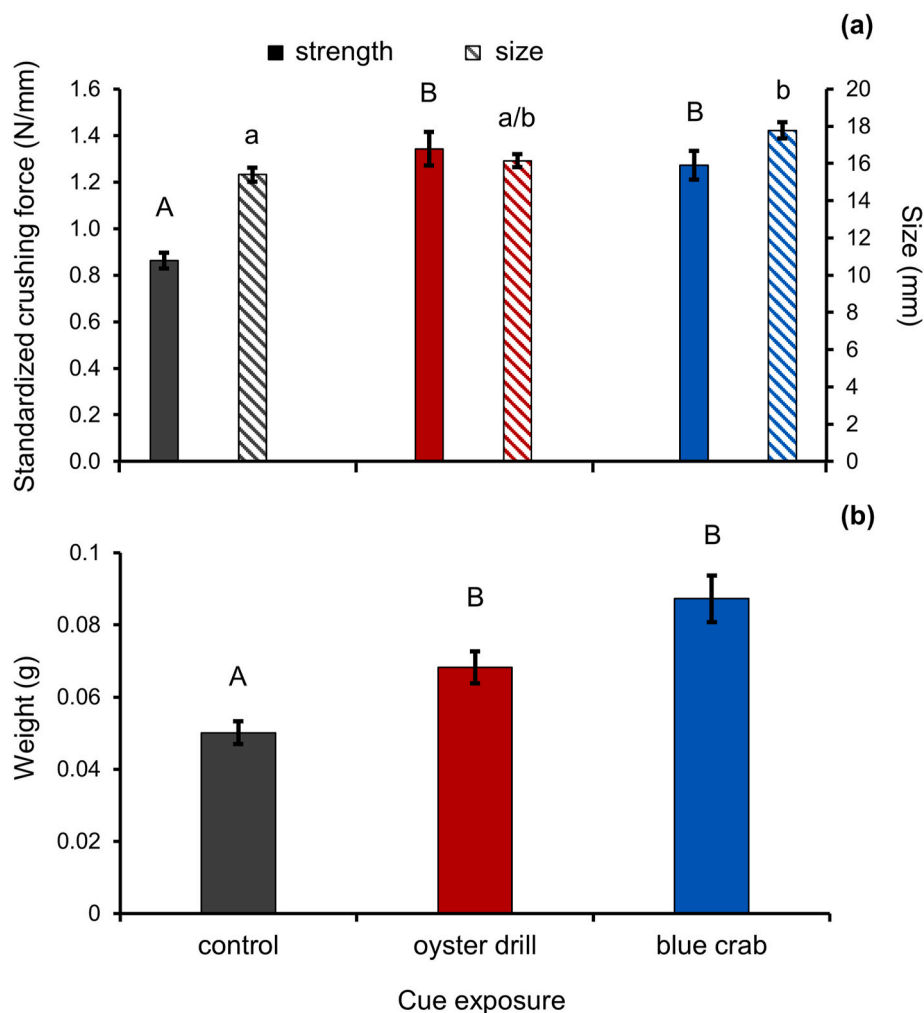


Fig. 1. Oyster shell morphological characteristics. Mean $\pm$ SE oyster shell **a)** standardized crushing force (N/mm), size (mm), and **b)** weight (g) of individuals grown in the presence of predator cues from blue crabs, oyster drills, or controls of no predator cues ($n = 80$). Letters denote significant differences among treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

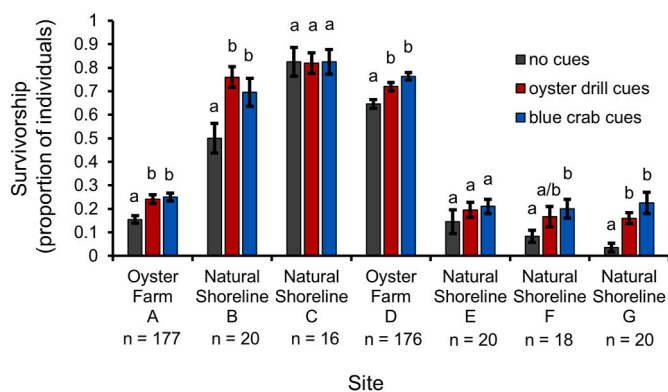


Fig. 2. Oyster survivorship. Mean $\pm$ SE survivorship of uncaged clusters of oysters staked across seven field sites after one week in the field. Oysters were exposed to different treatments of predator cues (blue crabs, oyster drills, no cue control) prior to being released in the field ($n = 16$ –177 stakes depending on field site). Letters denote significant differences between cue treatments within each site. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

drill cues (averaging 10% greater survivorship among sites), but at Site B oyster drill exposure produced 9% higher survivorship than blue crab exposure while at Site C mortality rates were virtually equal across all three predator cue treatments (Fig. 2).

Longer-term assessment of uncaged oysters (3.5 months) yielded starkly different survivorship patterns between the high salinity (Site A) and brackish (Site D) oyster farm with a significant interaction between site and predator cue treatment (mean salinity $\pm$ SD = 20.1 ± 3.1 versus 15.1 ± 5.3 ; Table 1). Oysters continued to remain at the brackish site after 104 days in the field with gradual declines in survivorship over time across all three treatments (Fig. 3a). Initially, both oyster drill and

Table 1

Descriptive statistics of a mixed-effects Cox proportional hazards model examining the influence of predator cue exposure treatment, site, and caging status on *Crassostrea virginica* survival.

Fixed effect	Coefficient (b)	exp(b)	Z	p
Predator cue	0.23	0.79	6.81	<0.0001
Site	2.03	0.13	37.93	<0.0001
Cage	2.68	0.07	25.07	<0.0001
Predator cue x site	−0.11	1.12	−2.29	0.0220
Predator cue x cage	−0.23	1.26	−2.78	0.0054
Site x cage	−0.94	2.56	−5.69	<0.0001
Predator cue x site x cage	0.04	0.96	0.31	0.7600
Penalized log-likelihood $X^2 = 11547.42$, df = 382.65, $p = 0$				

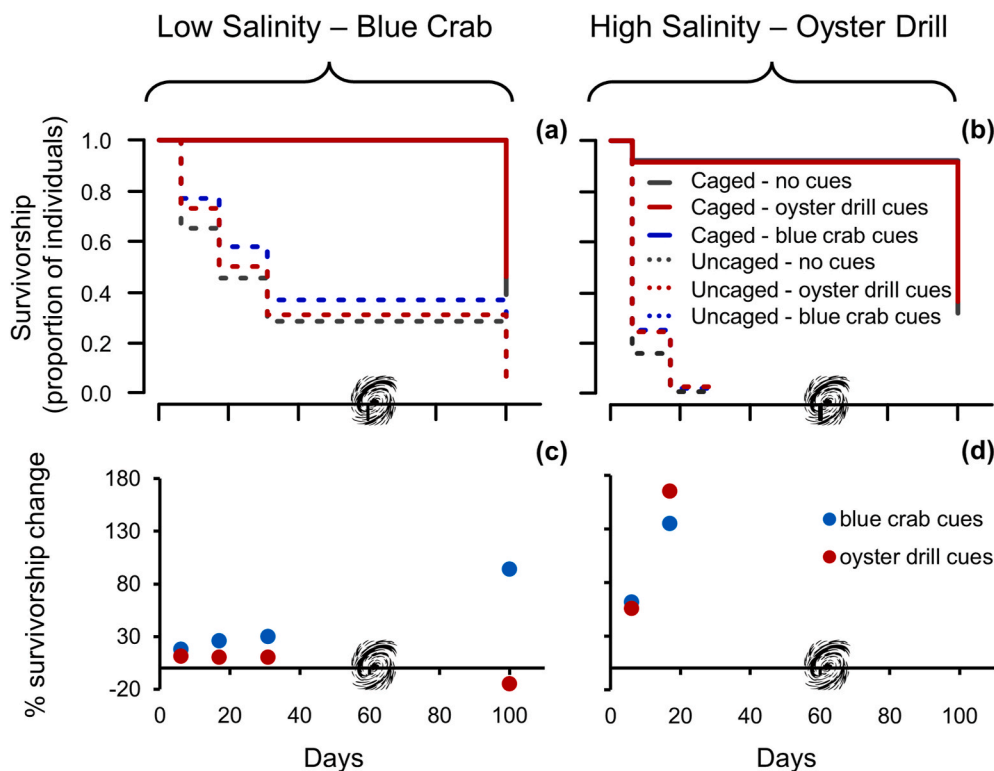


Fig. 3. Oyster survivorship curves and associated predator cue survivorship benefits. Survivorship of oysters (*Crassostrea virginica*) over time within a field site characterized by **left column**) low salinity and a blue crab dominated predator regime and **right column**) a site with high salinity and oyster drills as the dominate predator. **Top panels:** Survivorship curve of the proportion of individual oysters which survived as the experiment progressed. Oysters were reared in the hatchery with predator cues prior to being planted in the field. Line color denotes whether oysters were exposed to cues from blue crabs (blue), oyster drills (red) or control of no cues (black) while line shape denotes whether oysters were caged (solid lines; $n = 350$ per treatment in each site) or uncaged (dashed lines; $n = 1750$ per treatment in each site) in the field. Lines overlap when survivorship was the same. Cyclone symbol represents when Hurricane Sally hit field sites. **Bottom panels:** Percent increase in survivorship of uncaged oysters exposed to predator cues over uncaged control oysters during 104 days in the field. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

blue crab predator cues caused oysters to exhibit marginally higher survivorship than control oysters. However, drill exposed oysters had ~14% lower survivorship than control individuals by the end of the experiment, whereas the survivorship of crab exposed oysters steadily increased to be 94% higher than controls (Fig. 3c; Table 1). In contrast, oyster survivorship quickly plummeted at the high salinity site, where all individuals were dead within 27 days (Fig. 3b). However, prior to this, both species predator cues caused oyster survivorship to rapidly increase over control oysters with blue crab exposure producing 135% higher survivorship and oyster drill exposure producing 165% higher survivorship (Fig. 3d; Table 1). Only 4% of caged oysters had died across both sites prior to Hurricane Sally hitting our field sites 65 days into the field study (Fig. 3a and b). After the hurricane, nearly all the cages were damaged with 24 (34%) of the cages found with predators trapped inside. This damage and ability for predators to enter the cages is likely why 62% of the caged oysters were dead by the end of the study. There was a significant interaction between predator cue treatment and caging status as predator cue treatment did not affect survivorship within the cages (Table 1).

3.3. Site predator regime

The local predator regime varied substantially across site (estimate = 1.35, $z = 3.18$, $p = 0.0015$) with oyster drills dominating at high salinity sites and only blue crabs found at the low salinity site (Fig. 4). Sites within close proximity to each other (<2 km) exhibited similar predator compositions with the exception of Site C where no predators were found and which was exposed on the point of a peninsula with high wave energy.

4. Discussion

4.1. Applying predator cues at industrial scales generally increases survivorship across heterogeneous environments

Phenotypic plasticity has large ecological and evolutionary

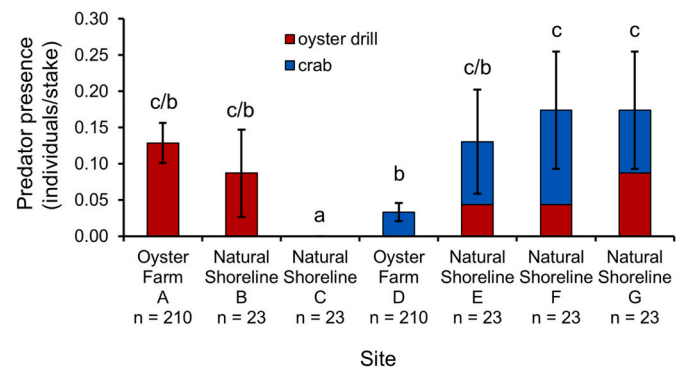


Fig. 4. Predator prevalence at field sites. Mean $\pm$ SE number of oyster drills and crabs found per stake at each field site during the first week of deployment ($n = 23$ –210). Letters denote significant differences in total abundance.

consequences (Miner et al., 2005), but only recently have studies begun exploring the potential for chemically manipulating phenotypic plasticity for practical applications (Espeland et al., 2018; Wang et al., 2020). We were able to substantially increase the survivorship of oysters in mass quantities by exposing individuals to predator cues in a nursery. Oyster shells grew markedly dissimilar in the presence of predator cues from different species, and the survival advantages of these morphological changes were site-specific and associated with salinity driven predator regimes. These results provide a window into how phenotypic plasticity can structure populations and highlight the viability of using predator chemical cues as a nontoxic means to increase crop productivity and species restoration efforts.

The high mortality of uncaged oysters, coupled with the similar survival rates of induction treatments among caged oysters, indicate that early exposure to predator cues can increase oyster survival by reducing their predation risk, consistent with previous studies (Ponce et al., 2020; Belgrad et al., 2021; Robinson et al., 2014). Predation was the dominant

cause of mortality as caged oysters persisted at all sites despite wide variations in environmental conditions and only exhibited substantial mortality when predators breached the cages. Concurrently, predator induction produced the highest increases in oyster survival at locations where predation risk was most intense (denoted by regions with rapid loss of uncaged oysters and high presence of predators; Figs. 2 and 4). Indeed, the lack of predators found at Site C (Fig. 4) is likely why induction appeared to have no effect on oyster survival at the site (Fig. 2). This may ultimately be a consequence of the high wave action at Site C since wave shock is well-known to control predator abundance and predation rates on fouling organisms (Menge, 1976). Although oyster survivorship was quite variable over the entire region sampled, sites within 2 km of each other showed remarkably similar patterns of survivorship (Fig. 2 Sites E – G). More importantly, predator cue induction consistently increased the survival of oysters at every site where predators were found, and usually produced progressively larger gains in survivorship over time that were persistent even after a major disturbance event (Fig. 3c and d). This suggests early exposure to predator cues is a viable tool across a wide range of environmental contexts.

Salinity had a strong indirect effect on oyster survival and the benefits of induction by altering the predator regime. High salinity sites likely exhibited the greatest oyster mortality and largest increase in survival from shell induction because the salinity supported oyster drills, which are frequently the main impediment to establishing reefs (Butler, 1985) and are intolerant of low salinity (Pusack et al., 2019). This is further supported by our predator survey which found a strong presence of oyster drills on the high salinity farm (site A) and only a modest presence of euryhaline blue crabs on the low salinity farm (site D). Although fish may also have fed on these spat, predatory fish are typically responsible for only a small fraction of predation on oysters compared to crabs and drills (Muthiah et al., 1987). Interestingly, our long-term results indicate the effectiveness of induction depended on the species used as a predator cue source matching with the local predator regime; where drill cues were highly effective at increasing survival in environments with drills but produced lower survivorship than control oysters at the site with only blue crab predators after 3.5 months in the field. In contrast, blue crab cues were generally effective at all sites although less than drill cues at drill dominated sites (sites A and B).

The performance reversal of oyster drill and blue crab induction across drill or blue crab dominated sites (Fig. 3) is likely a consequence of how these two predators feed and the morphological response of oysters. Oysters, in the presence of blue crabs that rely on crushing shells and are therefore size limited, developed shells that were both stronger and larger than control oysters (Fig. 1). Conversely, oysters exposed to drills that experience no size limitations because they bore through animal exoskeletons, grew shells that were stronger than those from blue crab induction, but were also smaller (Fig. 1). This smaller shell size probably caused the oysters to be more susceptible to crab predation whereas the modestly weaker shells of blue crab induction were more vulnerable to drill boring. Such prey preferences coincide with results from drill and blue crab predator choice experiments of induced oysters in the laboratory (Ponce et al., 2020). These findings highlight how local maladaptation may develop from prey defenses mismatching with the resident predator regime, providing field evidence for why predator-prey relationships do not always coevolve to successively exaggerated traits (i.e. geographical mosaics of co-evolution, 52, 53), and indicate that such mismatches have the potential to develop at close spatial scales (<10 km). This finding also highlights the importance of a firm understanding of prey responses to different predators in a system when seeking to manipulate these relationships for practical purposes.

4.2. Practical applications of phenotypic plasticity

The increased survival of induced oysters over a wide range of field conditions shows promise for using predator cues and induced defenses as an aquaculture and restoration tool and is consistent with previous

work (Belgrad et al., 2021). Although few of our oysters survived longer than three months in the field, such extremely high juvenile mortality is a common feature of r-selected species like oysters which typically depend on producing enough offspring that they overwhelm predators (Pianka, 1970; Bishop and Peterson, 2006). Consequently, reef restoration projects frequently involve planting millions to billions of oyster spat to increase the probability that new reefs will be established in areas where recruitment is low (La Peyre et al., 2014). The up to six-fold increase in survivorship of induced oysters over uninduced oysters, coupled with these differences growing progressively greater over time, indicate that adding predator cues in the hatchery can likely produce dramatic increases in the efficiency of oyster aquaculture, especially when applied at the scale of commercial bottom production or reef restoration. This technique was effective at increasing survival even when predation pressure was intense (Figs. 3 and 4). Utilizing predator cues in the hatchery may therefore allow oysters to be grown cost-effectively in some sites that would otherwise have prohibitively high predation, particularly if cues are matched to the local predator regime.

Given that there can be mismatches between the predator cue source and the local predator regime that can reduce the performance of induction, it is important for managers to have a good understanding of the predator field before employing predator cues. In the case where the predator regime is unknown, blue crabs are likely to be the best cue source for oysters since the crab cues increased survivorship at every site tested and across a wide range of different predator regimes.

We expect this technique to be especially beneficial in regions where recruitment is limited and where restoration operations are seeking to establish new reefs as predators often focus on new sources of food availability, and predation pressure on new reefs can be intense (Kulp and Peterson, 2016). While low-salinity (<10 ppt) and wave swept sites may have relatively few oyster predators, and would therefore likely not benefit greatly from induction treatments, such sites are often physiologically stressful for oysters and hinder oyster growth and survival (Ortega, 1981; Wang et al., 2008). However, in regions that are not characterized by physiologically stressful conditions that limit predators, but instead support conditions that maximize oyster growth and reproduction, our results indicate induction is expected to confer notable survivorship increases (Figs. 2 and 3). Predation of newly settled oysters often dominates post-settlement mortality (Kulp and Peterson, 2016) and inducing spat to grow stronger shells is predicted to be most beneficial at these young life stages, when a wide variety of predators can consume oysters, thereby enabling oysters to live long enough to achieve a size-refuge where only a few predators (e.g. adult stone crabs, black drum) can consume individuals. The threshold values at which an initial local oyster population persists or becomes extinct can be relatively low, on the order of several hundred thousand individuals (Moore et al., 2018). Given that restoration operations often begin with millions to billions of spat, the percent increases in survival observed here from induction easily have the capacity to boost populations above extinction thresholds in some circumstances. Future research would therefore benefit from exploring the benefits and costs of induction at restoration scales.

Induced defenses frequently develop at the cost of slower development (Steiner, 2007), reduced growth (Cronin, 2001), or lower reproductive output (Lima, 2009) as resources are shifted towards avoiding predation. Few studies have examined the costs of induction in oysters, but (Combs et al., 2019; Gosnell et al., 2017) found that individuals can be smaller and had less somatic tissue immediately after 1–2 months of induction. Our oysters, after one month of induction did not exhibit differences in shell size across predator cue treatments (Fig. 1a). As oysters take one to three years to reach market size depending predominantly on food availability and water temperature (Matthiessen, 2001), any initial reduction in energy investment towards somatic tissue or reproductive output may become trivial by the time reproductive maturity is reached. Indeed, preliminary research by our lab indicates

that 10.5 months after induction there is no significant difference in somatic or reproductive tissue mass (Supplementary Material: Fig. S2). However, more research is necessary to quantify the costs of induction over the lifetime of individuals, and such diminishment in induction costs at adulthood is likely to vary depending on the species.

Using predator cues to manage individuals has the potential to work in many systems. For instance, numerous commonly cultured bivalve species also strengthen their shells in the presence of predators (Nakaoka, 2000; Neo and Todd, 2011). Additionally, fish species can increase the size of their eggs (Segers and Taborsky, 2012) and body depth (Vinterstare et al., 2020), or length of time they remember predator cues (Brown et al., 2011) if they are exposed at a young life stage, which may benefit fish stocking programs. Furthermore, employing predator cues opens options for managing populations by exploiting endogenous defense mechanisms rather than using other laborious or environmentally toxic means to suppress predation rates. In terrestrial agriculture, traditional methods of controlling predators and other pests frequently involve the application of toxic compounds to repel or kill them. Yet, such methods often also strongly impact nontarget species and can lead to biodiversity loss, altered ecosystem function, and adverse human health effects (Mahmood et al., 2016). Predator chemical cues offer a nontoxic alternative method to regulate predation rates. The use of such compounds to shape the success of restoration efforts and crop production remains an infantile area of study, but initial research shows promise of a broad array of possible uses. For instance (Wang et al., 2020), demonstrate that rice yields can be increased by stimulating shoots to develop defenses against herbivory through the application of chemical cues associated with tissue damage, while (Zhou et al., 2021; Zhang et al., 2022) have identified compounds that can stimulate herbivory defenses in wheat and tea respectively. Chemically mediated induced defenses are a common phenomenon among vertebrates, invertebrates, plants, fungi, and microbes (Tollrian and Harvell, 1999; Kaufmann and Dorhoi, 2016; Künzler, 2018). To our knowledge this is among the first experiments to test the practical applications of chemically induced defenses in an animal system and one of only a few field studies on the subject in any system. However, targeted investigation of the molecular determinants of these defenses and the dispersal of such cues will likely find a number of applications ranging from the conservation of endangered species and habitat restoration to crop production and animal husbandry for a variety of systems.

Credit author statement

Benjamin Belgrad: Conceptualization, Methodology, Investigation, Validation, Formal Analysis, Data Curation, Supervision, Project Administration, Writing - Original Draft, Funding Acquisition **William Knudson:** Investigation, Writing - Review & Editing **Sarah Roney:** Investigation, Writing - Review & Editing **William Walton:** Methodology, Supervision, Resources, Writing - Review & Editing **Jessica Lunt:** Investigation, Writing - Review & Editing **Delbert Smee:** Conceptualization, Methodology, Investigation, Validation, Formal Analysis, Data Curation, Supervision, Writing - Review & Editing, Funding Acquisition, Resources.

Data statement

Data is archived at the Dauphin Island Sea Lab data management center where it can be accessed at the following link: <https://data.disl.edu/dataset/scared-strong-enhancing-oyster-resilience-for-aquaculture-and-restoration-by-inducing-oysters>. Data will also be made available on the NOAA National Centers for Environmental Information (NCEI). Requests for the data should be submitted to: Ben Belgrad, babe.lgra@eckerd.edu.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2023.117808>.

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