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Use of predator cues to bolster oyster resilience for aquaculture and reef restoration

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ABSTRACT

Many mollusks alter their shell morphology in response to predator exudates or injured conspecifics to lower their predation risk. However, studies have yet to examine whether this predator-avoidance response can be applied to bolster reef restoration, fisheries enhancement, or aquaculture. We tested whether exposure to predator cues under hatchery conditions can increase the survival of oysters, *Crassostrea virginica*, planted in the field on the substrate. Juvenile oysters, set on shells and grown in a flow-through system, were exposed to either caged blue crabs, *Callinectes sapidus*, or controls of empty cages for either four or eight weeks then placed in the field for 30 days. We compared oyster shell strength and morphology as well as oyster survival among predator exposure time treatments. Oysters grown in the hatchery for eight weeks were 46% larger and almost 2× stronger than oysters grown for four weeks. However, predator exposure also caused a 50% increase in shell strength for both time periods. In the field, oysters suffered relatively little mortality when protected from predators using cages, and virtually all mortality was attributed to predation. Predator cue treatments significantly increased the survival probability of uncaged oysters (as would be done in reef restoration or stock enhancement) compared to unexposed treatments. Early cue exposure yielded substantially greater gains in survivorship over time as predator induced oysters nursed for four weeks exhibited 53% higher survival in the field than unexposed oysters while this survivorship gain jumped to 300% for eight weeks of cue exposure. Our findings demonstrate that predator cues can be an effective means for the industry to increase the operational efficiency of aquaculture and restoration efforts, and may potentially be applied to other bivalve fisheries.

1. Introduction

Globally, more than 15 million tons of marine bivalves are harvested each year for human consumption, 89% of which comes from aquaculture efforts (Wijsman et al., 2019). Oysters are among the most valued of these species as they not only constitute 33% of this global production (FAO, 2019), but also provide a host of ecosystem services. These services range from shoreline protection, water filtration, and habitat creation (Grabowski and Peterson, 2007) to shaping the cultural identity of regions (Michaelis et al., 2020). Yet, oysters are one of the most degraded marine habitats, with ~85% of oyster reefs lost worldwide (Beck et al., 2011). Countries, including the United States, have experienced significant declines in the wild oyster fishery (71% over the past half-century) accompanied by the loss of benefits that oysters provide (Zu Ermgassen et al., 2013, Wijsmanet al. 2019). Consequently, oyster aquaculture continues to increase considerably in an effort to both supplement the loss of the wild fishery and to facilitate the restoration of oyster reefs and their ecosystem services.

Most oyster aquaculture, restoration, and stock enhancement involves relatively extensive culture methods (rather than intensive operations) where larvae are spawned in a hatchery and juveniles are planted in natural or semi-natural settings to grow to adulthood. One of the greatest challenges to extensive operations is mortality from predation that can decimate populations since even protected stocks on farms can lose 28% of their biomass from predators (Richard et al., 2020) while unprotected regions can lose 94% of planted juveniles

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within weeks (Mackenzie, 1970). Locally, losses to predation within off-bottom containers are typically near zero when maintained properly (Walton, pers. obs.), while predation on-bottom has been observed to inflict losses ≥87% (Lappin Jr., 2018). The predominant predators that threaten stocks can vary by region and oyster age. For example, in northern latitudes, starfish are frequently considered the most destructive predators to crops (Hancock, 1955) while oyster drills are a larger threat in the Gulf of Mexico (Butler, 1985) to both juveniles and adults. Additionally, mud crabs and a wide number of fish species are common predators of juvenile oysters (McDermott, 1960; Anderson and Connell, 1999) while fish species like black drum can be a major predator to adults (Brown et al., 2008). Consequently, farmers have developed a number of practices to reduce mortality from these different predators (Matthiessen, 2001; Gosling, 2008). Such practices include selecting sites with relatively low predation intensity (Buitrago et al., 2005), mass removal of predators from sites (Calderwood et al., 2016), protecting the bivalves inside some type of container (often suspended or floating, Gosling, 2008), nursing juvenile bivalves in the hatchery until they reach a size refuge from predation (Wijsman et al., 2019), or some combination of the above. However, many of these techniques are expensive, labor intensive, and/or are not feasible at the desired scale due to conflicts with other local economical, ecological, or cultural interests. Similarly, it is not uncommon for oyster reef restoration efforts to fail (Mann and Powell, 2007; La Peyre et al., 2014) as yearly age-specific mortality rates can exceed 70% in some locations (Mann et al., 2009). Although predators are a common source of mortality in oysters, especially among juveniles (Bisker and Castagna, 1987), many of the most effective techniques in farming to prevent predation (e.g. containerized culture) are too labor intensive for large-scale commercial growers or restoration projects. These large-scale efforts typically use remote setting, where oyster larvae are allowed to set upon substrate (often oyster shell, and called spat-on-shell) and ultimately stocked into the target area.

One potential technique to increase the survival of oysters in large-scale aquaculture or reef restoration efforts is early exposure of juvenile oysters to predator cues. Many mollusks, including mussels (Leonard et al., 1999), clams (Nakaoka, 2000), and oysters (Robinson et al., 2014), will strengthen their shells when exposed to predators to reduce their risk of being consumed. Oysters are known to strengthen their shells in response to both crustacean (Newell et al., 2007) and gastropod predators (Lord and Whitlatch, 2012; Ponce et al., 2020), which can increase their survival under laboratory settings (Robinson et al., 2014; Ponce et al., 2020). Oysters respond to chemical exudates from injured con— and hetero—specifics as well as predator exudates by building thicker shells and altering the composition of shells (Scherer et al., 2018). However, most studies on inducible defenses of bivalves have occurred under closed laboratory conditions which can only induce dozens to hundreds of individuals simultaneously and frequently inflate exposure to predator cues beyond natural conditions. It is unknown whether predator exposure techniques can induce bivalves to grow stronger shells under large-scale settings that utilize flow-through systems and have the capacity to hold hundreds of thousands to millions of oysters. Additionally, the few studies that have investigated the effects of predator induction on bivalve survival are typically laboratory based and short-term, lasting hours to days (e.g. Robinson et al., 2014; Sherker et al., 2017). Researchers have yet to study the extent to which predator induction enhances survival in the field when encountering a natural suite of predators over longer time periods.

We tested the feasibility of using predator cues to increase the survival of oysters in aquaculture and reef restoration operations. We grew eastern oyster (*Crassostrea virginica*) juveniles set on shell under hatchery flow-through conditions, which can raise millions of juveniles per brood, to determine if 1) oysters can be induced to grow thicker shells in mass quantities and 2) predator induction affects survival in the field. We nursed oysters for four weeks (comparable to normal nursery times; Matthiessen, 2001) and eight weeks to assess the degree to which cue exposure benefits scale over time, and then assessed survival in the field.

2. Methods

2.1. Oyster culturing

Oysters (*Crassostrea virginica*) were cultured as spat-on-shell at the Auburn University Shellfish Laboratory (AUSL) on Dauphin Island, AL starting in late May 2019 using standard techniques (Congrove et al., 2009). Oysters were ~ 1.0 mm when the experiment began and housed in four flow-through holding tanks measuring 2.4 m × 0.9 m (length x width) with a water depth of 0.4 m (~20,000 spat/tank). Water flow rates in the holding tanks averaged 36.9 L/min. There was immense variation in the number of spat per shell which we elected to maintain during the experiment to mimic natural settlement and normal reef restoration practices (~5–40 spat/shell at four weeks of culturing). Oysters were suspended above the tank bottom in seven oyster aquaculture baskets (64 × 23 × 14 cm with 140 spat covered shells/cage; ~80,000 spat total) to prevent sediment buildup from suffocating oysters. These holding containers and shell densities matched normal nursery procedures for spat-on-shell (Matthiessen, 2001, personal communication, AUSL hatchery manager Scott Rikard).

Half of the oysters were exposed to predator exudates by holding four live adult blue crabs, *Callinectes sapidus,* in two of the flow-through tanks (8 crabs total) while the remaining two tanks did not have crabs and served as a control (hereafter known as induced and uninduced oysters respectively). Crabs were held in two partitioned baskets to prevent crabs from consuming the experimental oysters or each other while control tanks had empty crab cages. Each crab was fed one adult oyster daily (~5.0 cm in length) to maximize predation risk cues, causing experimental oysters to receive exudates from both crabs and injured oysters as they were being consumed. Oyster cages were rotated daily around crab cages to reduce differences in growth due to proximity to cue sources. Crabs were replaced at least every other week to ensure predators remained healthy and to replace crabs that died. After four and eight weeks in the hatchery, subsets of spat-covered shells were taken to the laboratory to measure differences in shell morphology while other subsets were planted in the field to assess effects on survival.

2.2. Shell morphology

Two shells were taken from every basket and three live spat were selected from each shell for measuring spat shell characteristics after four and eight weeks (number of individuals = 84 for each cue exposure x time treatment; 112 shells and 336 spat total). Spat shell morphology was assessed by measuring shell size, shell weight, and shell crushing force (sensu Robinson et al., 2014, Scherer et al., 2016). Oysters are roughly round during early life stages, and shell length was measured from the umbo to the outer shell edge to the nearest 0.01 mm using digital calipers. Care was taken to only measure individuals that were not crowded by cohorts to reduce any confounding effects on growth due to space limitation, although this was not a common occurrence at these early life stages. We quantified the force needed to break each oyster shell using a penetrometer (Kistler force sensor 9203 and Kistler charge amplifier 5995). The force sensor was placed equidistant from the shell edges and perpendicular to the shell surface. Gentle, consistent pressure was applied until the shell cracked, and the maximum force needed to break the shell (N) was recorded. This technique is a standard proxy of shell hardness (Robinson et al., 2014). We divided shell crushing force by shell length to produce a size-standardized metric of shell strength (i.e. standardized crushing force, N/mm) because larger individuals naturally 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 applied to just the left valve.

We examined the effects of predator cue exposure (present vs absent) and time cultured (4 weeks vs 8 weeks) on standardized shell crushing force, shell length, and shell weight by running three separate generalized linear mixed models with Gamma distributions, one model for each of these three response variables (GLMMs; R package: lme4). Cue exposure treatment and time were set as fixed effects with an interaction term while shell 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 test was used to determine pairwise differences in shell morphology (R package: lsmeans). All statistical analyses were conducted using R v3.5.1 (R Development Core Team, 2018).

2.3. Field survival

To quantify the extent that inducing oysters alters survival in the field over time, five to six spat covered shells were selected from each basket after both four and eight weeks in the hatchery and placed in the field for 30 days (see Fig. 1 for spat sizes and shell strength). Each shell was manually thinned so only 10 spat where present on each shell to standardize predator risk exposure (number of shells used = 80 shells for each cue exposure x time treatment; 320 shells and 3200 spat total). We wished to ensure the experiment had enough replication to detect medium effect sizes on survival (h = 0.5, power = 0.999; Cohen, 1988) so we set ~4× more oysters than necessary to achieve this. Four pairs of induced and uninduced oysters were zip tied to 1-m long horizontal PVC frames (20 frames per hatchery cue exposure time; 40 frames total). One pair of shells on each frame was randomly selected to be surrounded by a mesh cage to exclude predators and control for mortality events from nonpredatory sources (e.g. disease, abiotic conditions). Initially, cages were composed of a semiflexible mesh, but after predators were repeatedly found within the cages, this setup was replaced with a stiffer inflexible cylindrical plastic cage (diameter = 18 cm, length = 22 cm) and overlain with fine mesh (2 mm pore size). The frames were set at the Point aux Pins Oyster farm (30°23′00.7"N, 88°18′46.3"W) approximately 150 m from shore in the same environmental conditions that the farm raises its oysters. Oysters cultured commercially on the farm are normally caged within industry baskets suspended above a mudflat that is frequented by oyster drills (*Stramonita sp.*), black drum (*Pogonias cromis*), sheepshead (*Archosargus probatocephalus*), and a variety of brachyuran crabs including mud crabs (*Panopeus sp.*), stone crabs (*Menippe adina*), and blue crabs (*Callinectes sapidus*). Here, predators, particularly oyster drills, are most prevalent in the summer months, but species like blue crabs can also be common throughout the year (Laughlin, 1982; Butler, 1985). The oyster frames were designed to keep spat ~15 cm above the sediment surface to prevent sediment from covering and suffocating individuals (observations of frames showed that numerous crabs, fish, and oyster drills were still able to reach all spat locations using this setup). Frames were set parallel to the shoreline with at least 0.5 m separating each frame. Oysters raised in the hatchery for four weeks were placed in the field on June 25, 2019 while oysters raised for eight weeks were planted on July 26, 2019 adjacent to the oysters planted earlier. Once planted, all spat were checked for individual survival approximately every 48–72 h for 30 days by counting the number of spat still alive on each shell. The experiment was concluded after this timeframe due to the high mortality experienced in the field.

We assessed whether oyster survival was influenced by the fixed effects of predator cue exposure, culture time, and caging status using a mixed-effects Cox proportional hazards model (i.e. a survival analysis; R package: frailtyHL). 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. Oyster shells, nested in shell pair, nested in PVC frame were treated as random effects to control for nonindependence among individuals. This model allowed us to right censor the data to account for spat that were not dead by the end of the trial. A Cox proportional hazards analysis is a statistical model which recognizes that the highest values in a study may simply be the maximum possible value, because a result did not occur by the end of the observation period, so the model weighs the data points accordingly (i.e. the data are right censored).

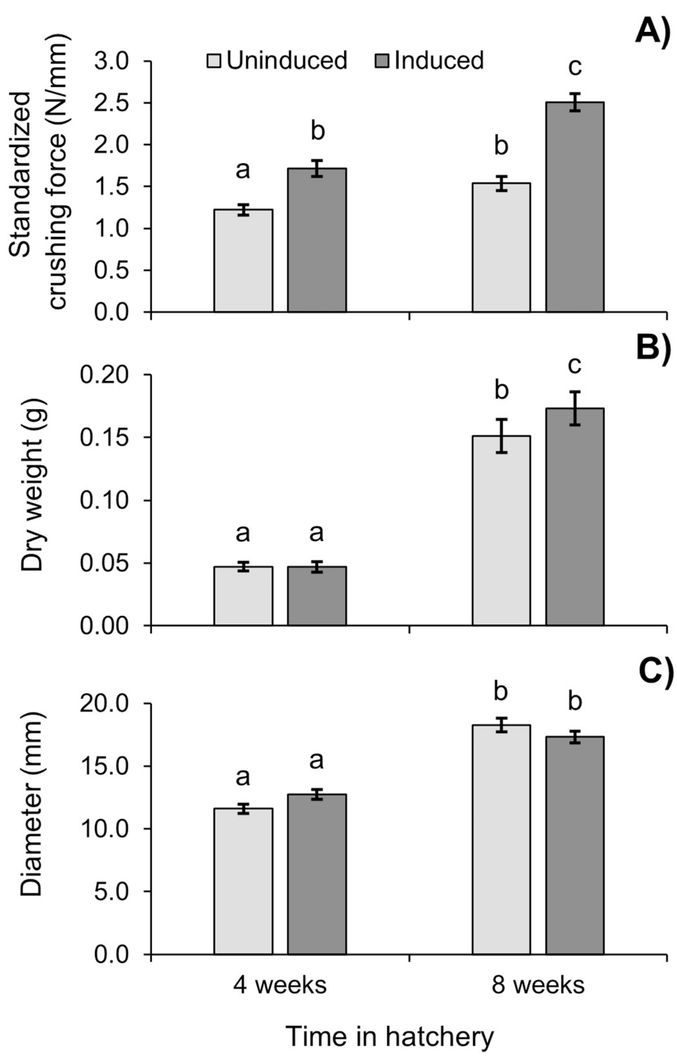

Fig. 1. Oyster spat shell characteristics when reared in the hatchery for four and eight weeks in either the presence (induced) or absence of predator cues (uninduced)(n = 84 per treatment). Mean ± SE A) shell crushing force standardized by shell size (N/mm), B) shell weight (g), and C) shell diameter (mm). Letters denote significant differences.

3. Results

3.1. Shell morphology

Oyster spat shells were significantly stronger when grown with predator cues than controls grown without predator cues (estimate = 0.23, $t = 3.76$, $p < 0.0001$). After four weeks of cue exposure, shells were on average 41% stronger than comparable control shells and 63% stronger than comparable controls after eight weeks of cue exposure (Fig. 1a). Time grown in the hatchery also had a significant effect on shell strength. Oysters raised for 8 weeks were 34% stronger than those grown for 4 weeks (estimate = 0.18, $t = 4.71$, $p < 0.001$). Thus, oysters grown for four weeks with predator cues had shell strengths comparable to growing oysters eight weeks without cues. There was not a significant interaction between cue exposure and time in the hatchery (estimate = 0.01, $t = 0.20$, $p = 0.840$).

Interestingly, shell weight exhibited a significant interaction between cue exposure treatment and growth time (estimate = 5.28, $t = 2.29$, $p = 0.022$). Although oysters grown with and without predator cues had the same weight shells after four weeks of growth, shells of oysters grown with predator cues for eight weeks were 15% heavier than those grown without cues (Fig. 1b). On average, shells became 2.5× heavier after an additional four weeks of growth (estimate = 6.50, $t = 2.80$, $p = 0.005$).

The size of shells also exhibited a significant interaction between cue exposure treatment and growth time (estimate = −0.01, $t = -2.80$, $p = 0.005$). Oysters induced with predator cues for four weeks were, on average, 10% larger than controls not exposed to cues, but after eight weeks in the hatchery, predator induced oysters were 10% smaller than controls (Fig. 1c). However, there was not a significant difference in shell size between predator cue treatments for either time period (estimate = 0.01, $t = 1.94$, $p = 0.052$). Shells, on average, grew 46% larger with an additional four weeks of culture time (estimate = 0.02, $t = 27.74$, $p < 0.001$).

3.2. Field survival

In total, only 102 (13%) of caged oyster spat died, while 2124 (88%) of the uncaged oysters died after 30 days in the field (hazard ratio = 28.06, 95% CI = 21.11–37.31, z = 22.93, $p < 0.001$). Most cage mortality could easily be attributed to predators that had breached the cage and were contained therein. Exposure to predator cues in the hatchery significantly affected oyster survivorship, regardless of exposure time (hazard ratio = 1.50, 95% CI = 1.12–2.02, z = 2.71, $p = 0.007$; analysis of full dataset; Fig. 2). However, predator cues only substantially enhanced survival over uninduced oysters when individuals were unprotected. Caged oysters exhibited relatively similar survival rates across induction treatments. This difference in survival of uncaged cue induced oysters over uninduced oysters grew geometrically over time in the field (Fig. 3). Additionally, the survival benefits from cue exposure were more pronounced when oysters were induced with cues for 8 weeks rather than 4 weeks. While survivorship of oysters induced with cues for four weeks was ~50% greater than uninduced oysters, eight weeks of cue exposure produced a nearly 300% increase in survival after 30 days in the field. Interestingly, oysters that were grown in the hatchery for eight weeks had 21% greater overall mortality after 30 days in the field than those grown for only four weeks in the hatchery (hazard ratio = 3.5495% CI = 2.49–5.04, z = 7.02, $p < 0.001$; Fig. 2; Table 1). There was not a significant interaction between cue exposure treatment and time in the hatchery on oyster survival (hazard ratio = 1.08, 95% CI = 0.72–1.63, z = −0.37, $p = 0.710$).

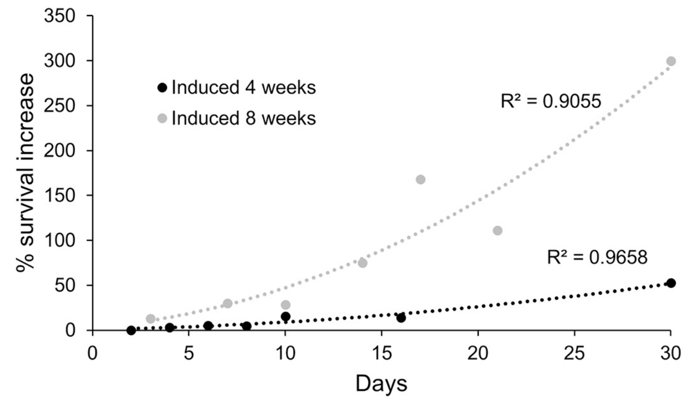

Fig. 3. Percent increase in survivorship of uncaged induced oysters over uncaged uninduced oysters after a month in the field. Oysters were exposed (induced) to predator cues for either one or two months.

4. Discussion

These results demonstrate that oysters can readily be induced to grow stronger shells in mass quantities and that this treatment can substantially increase survival rates in the field. The difference in

Table 1
Proportion of spat surviving after 30 days in the field for each experimental treatment.

	4 weeks in hatchery		8 weeks in hatchery	
	Induced	Uninduced	Induced	Uninduced
Uncaged	0.24	0.15	0.04	0.01
Caged	0.81	0.79	0.97	0.96

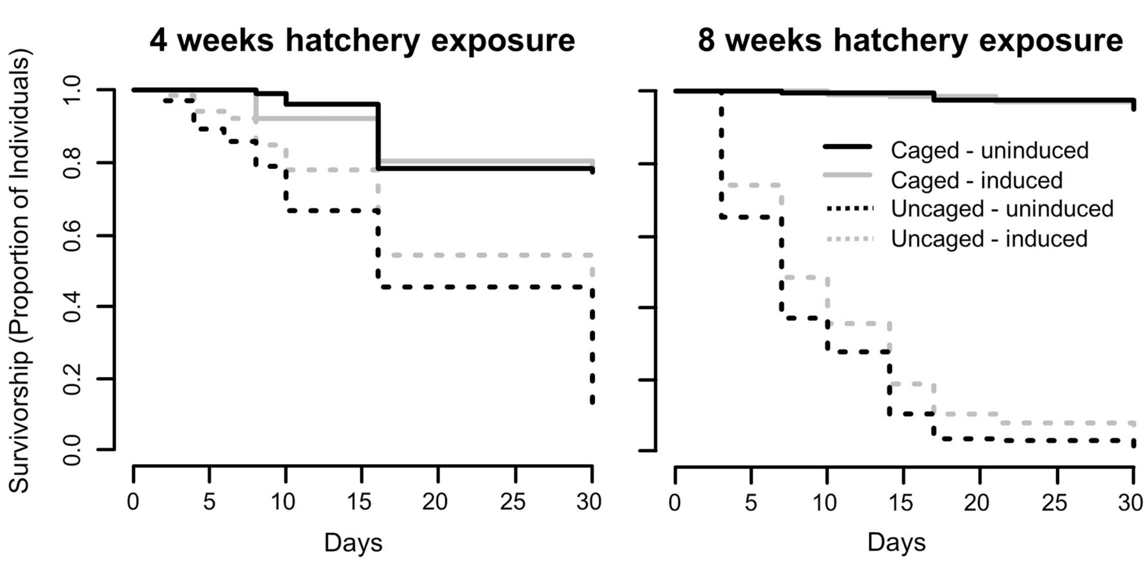

Fig. 2. Survivorship curve of the proportion of individual oysters (*Crassostrea virginica*) which survived each day in the field as the experiment progressed. Oysters were reared in the hatchery for either four weeks or eight weeks prior to being released into the field. Line color denotes whether oysters were exposed to predator cues (induced) or no cues (uninduced) in the hatchery while line shape denotes whether oysters were caged ($n = 200$ per treatment) or uncaged ($n = 600$ per treatment) in the field.

survival rates between caged and uncaged oysters indicates the primary source of mortality for our oysters was predation. Indeed, most instances of mortality in the cages coincided with predators also being found trapped within the cages. These findings, coupled with the reduced benefits of induction in the caged treatment, also suggest that differences in survival rate between induced and uninduced oysters was due to differences in predation rate, consistent with previous laboratory studies (Robinson et al., 2014; Sherker et al., 2017; Ponce et al., 2020).

Surprisingly, absolute survivorship was lowest for oysters grown in the hatchery for eight weeks rather than four weeks (Fig. 2), despite the larger size and stronger shells of the eight-week old oysters (Fig. 1). This is likely due to a seasonal shift in the local predator regime. When assessing survival of the eight-week oysters, we frequently observed oyster drills among our samples but rarely encountered them when surveying the four-week oysters that had been deployed a month earlier (personal observations). Oyster drills are considered one of the main impediments to profitable oyster aquaculture in many otherwise suitable regions of the northern Gulf of Mexico and are generally more abundant later in the summer, after spring rains (Butler, 1985). Critically, even in the presence of high levels of this voracious predator, we observed a 300% increase in survival of induced oysters over uninduced oysters. However, the oyster drills' sudden appearance here and subsequent drastic increase in overall oyster mortality highlights the importance of extended field assessments when estimating species survival probability or the suitability of a region for aquaculture or restoration.

Extremely high juvenile mortality is a common phenomenon among r-selected species, like oysters, which often rely on producing enough offspring so that they can overwhelm predators (Pianka, 1970; Bishop and Peterson, 2006). Consequently, reef restoration efforts frequently involve planting millions to billions of oyster spat to increase the likelihood of establishment of new reefs in regions where recruitment is limited (Brumbaugh and Coen, 2009; La Peyre et al., 2014). Although few of our oysters survived longer than one month in the field, the 50–300% greater survivorship of induced oysters over uninduced oysters, coupled with these differences growing progressively larger over time, indicate that applying predator cues in the hatchery can potentially cause dramatic increases in the efficiency of oyster aquaculture, particularly when utilized at the scale of commercial bottom production or reef restoration projects. This technique was effective in increasing survival even when predation pressure was intense (Figs. 2 and 3). Applying predator cues in the nursery may allow oysters to be grown cost-effectively in some regions which would normally have prohibitively high predation, although more research is necessary to determine the extent to which return on investment for cue exposure varies over space and time. Additionally, further assessment is necessary to determine oyster survival when only induced spat are available. While many prey species such as small crabs will likely have trouble breaking toughened shells and will cease feeding on stocks, species like oyster drills that can bore into shells may simply just expend more effort consuming induced spat.

Interestingly, caging oysters caused the most dramatic increases in survival, highlighting the value of this well-established practice. Although caging oysters and situating operations in locations with low predation pressure are common techniques (Matthiessen, 2001; Wijsman et al., 2019), these options are not always feasible. Maintaining cages is labor intensive and does not lend itself to large-scale production necessary to meet market demand. Choosing sites with low predation pressure is often a goal of aquaculture and reef restoration but has its own difficulties as such sites may be unavailable or have poor growing conditions. Further, predation pressure within areas can vary substantially among seasons and years making site selection challenging. Our results on oyster survival indicate that cue induction may therefore be best suited for these scenarios where oysters are kept uncaged (e.g. restoration projects, on-bottom stock supplementation) or when predation pressure is high or unknown.

This is one of the first attempts to induce a bivalve species to grow stronger shells under aquaculture conditions. As such, we sought to maximize the potential oyster induction response by feeding oysters to predators daily and using blue crabs. However, a number of different common, noncommercial predator species are known to induce oysters to grow stronger shells, including mud crabs (Robinson et al., 2014), oyster drills (Lord and Whitlatch, 2012), and conchs (Gosnell et al., 2017). Induction responses can also be obtained by feeding predators tissue from a variety of different animals (Scherer et al., 2016). Thus, the cost and efficiency of applying cues to oysters may readily be improved upon by using locally available resources and through additional studies comparing feeding regimes and predator species. Maintaining oysters in a nursery system with predator cues would incur additional economic outlay on top of normal farming practices. Nevertheless, many hatcheries maintain spat for about two weeks before leaving the facility and a number of nursery operations already hold spat for a month to help oysters reach a size refuge from predation (Matthiessen, 2001; Mao et al., 2019). For these existing time frames, the costs of also providing cues should be minimal, but cost-benefit analyses are necessary to evaluate the economic viability of this technique, especially if facility holding times are to be altered as a result.

Induced defenses frequently arise at the costs of reduced growth (Kats and Dill, 1998; Cronin, 2001), slower development (Steiner, 2007), and decreased reproductive effort (Lima, 2009) as resources are shunted towards avoiding predation. Few studies have investigated the amount induced defenses alter oyster somatic tissue production or reproductive output. Gosnell et al. (2017) found that after 58 days of continuous predator exposure, oysters exhibited 20% lower soft tissue mass than controls, but no significant change in the percent composition of soft tissue versus shell. Our oysters after both one and two months of cue exposure had the same sized shells, but appeared to be exhibiting slight reductions in growth after two months exposure (Fig. 1). As oysters take one to three years to reach harvestable size depending primarily on food availability and water temperature (Matthiessen, 2001), any early decreases in soft tissue have a good probability of becoming negligible. However, more research is necessary to quantify the degree cue induction affects oysters at adulthood and pinpoint the predator exposure time which maximizes total oyster production.

In conclusion, high mortality from predation plagues the bivalve aquaculture industry (Matthiessen, 2001; Gosling, 2008; Wijsman et al., 2019) and hinders reef restoration efforts (Mann and Powell, 2007). Additionally, many bivalve species commonly cultured by the industry are known to grow stronger shells in the presence of predators (Leonard et al., 1999; Nakaoka, 2000; Bishop and Peterson, 2006; Robinson et al., 2014). Exposing juvenile bivalves to predator cues in the nursery stage is therefore a promising tool which likely can provide a variety of benefits across the industry as even a small relative increase in survival can change the economics of bivalve aquaculture; causing private operations to be more profitable (or profitable at all) as well as improve the return on investment in restoration efforts.

CRediT authorship contribution statement

Benjamin A. Belgrad: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Emily M. Combs:** Data curation, Formal analysis, Investigation, Methodology. **William C. Walton:** Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing - review & editing. **Delbert L. Smee:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - review & editing.

Declaration of Competing Interest

None.

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