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SHORT COMMUNICATION

Can relic shells be an effective settlement substrate for oyster reef restoration?

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Acute storms (e.g. hurricanes) are major stressors to eastern oysters (*Crassostrea virginica*) through burying oysters and settlement substrate. Subsequently, managers use many restoration efforts, of which one approach is bag-less dredging. This resurfaces relic shells as settlement substrate; however, buried shells turn black in anoxic sediments potentially influencing spat settlement. This study compared three shell types: sun-cured white shell utilized in oyster reef restoration and two representative black shell types for bag-less dredging. Settlement was significantly higher on sun-cured white shell suggesting that restoration activities resurfacing black shells may not provide suitable substrate and alternative methods of providing substrate should be prioritized.

Key words: bag-less dredging, black shell, *Crassostrea virginica*, hurricane restoration, oyster restoration, oyster settlement

Implications for Practice

- Acute storms (e.g. hurricanes) often kill or bury live oysters and larval settlement substrate and subsequent restoration efforts have focused on constructing new reefs, but resurfacing buried shell has been one mechanism for restoration.
- Resource managers should focus on constructing reefs with material other than buried relic shell. While this may be a readily available substrate after a storm, it may not be an effective settlement substrate for restoration practices, either ecologically or financially.

Introduction

Populations of the eastern oyster (*Crassostrea virginica*) have had historic declines along the Atlantic and Gulf coasts of the United States due to naturally occurring disturbances and numerous anthropogenic stressors (Beck et al. 2011; Kirby 2004). One acute natural disturbance impacting oysters is large-scale storm events (e.g. tropical storms, hurricanes) through rapid freshwater influx and sediment deposition. The Galveston Bay (GB) estuary, located south of Houston, Texas, United States, is a model study system for estuaries with oyster fisheries and periodic hurricanes. This estuary has a historically strong fishery providing ca. 15% of the nation's oysters and contributes $50 million annually toward Texas' economy (Rivera 2018). Hurricanes Ike and Harvey have recently impacted oysters within GB. Ike was particularly devastating when it made landfall in September 2008 as a Category Two storm, when the high storm surges (3–4 m) brought in high sediment loads and buried ca. 70% of oyster reefs in East GB (Robinson 2014). In August 2017, Harvey hit the Texas Gulf Coast as a Category Four storm, bringing 824–1,043 mm of rain in 3 days to the Houston area (Van Oldenborgh et al. 2017) and the estimated freshwater discharge was three times the bay's normal volume (Du & Park 2019). This freshwater discharge heavily sedimented GB, as Du et al. (2019) found an average of 10.5 cm of newly deposited sediment, with some areas exceeding 50 cm.

Restoration reefs are constructed by adding settlement substrate back into the estuary, often sun-cured white oyster shell (SCWS), but alternative hard substrates are used when shell availability is limited or cost prohibitive (Kennedy et al. 2011), providing similar functionality (George et al. 2015). This restoration technique is time consuming and costly, but uncovering buried shells by bag-less dredging, a practice that involves removing the collection bag from an oyster dredge and towing through the sediment, can quickly provide substrate for larval oyster (spat) settlement after a storm event (Kennedy et al. 2011; Buzan et al. 2015). After GB oyster reefs were sedimented in during Hurricane Ike, Texas Parks and Wildlife Division (TPWD) contracted commercial fishermen in 2010 to deploy bag-less dredges over 3,808 acres of buried reefs in GB, with a final cost of $741 per acre (Robinson 2014). However, quantifying the success of bag-less dredging or other methods of shell cultivation has been limited for the quantity and quality of shell uncovered. Along with

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potential habitat disturbance and sedimentation impacts, the functionality of the relic shells resurfaced by these methods is poorly understood (Buzan et al. 2015). Buried shells remain entombed within an anoxic, potentially sulfide-rich environment (Saoud & Rouse 2000), and turn black in color. It is unclear how black shell influences spat settlement and, in turn, the success of exposing relic shell. Therefore, the primary objective of this experiment was to compare three shell types for spat settlement: SCWS, preferentially used in reef restoration, and two representative types of bag-less dredging: freshly exposed black shell and dredged black shell that has been sun-cured. Ancillary background oyster abundances were also quantified to evaluate variation in settlement patterns with conspecific abundances on the reefs.

Methods

Created intertidal oyster reefs ($n = 5$) constructed in Sweetwater Lake, a semi-enclosed embayment off West Galveston Bay Texas, USA (Fig. 1), were used for this experiment. These reefs were constructed in 2014–2015 out of individual mesh bags with SCWS shell. On each reef, oyster abundances were quantified from five individual bags in early August 2019 by haphazardly selecting a bag, enumerating all oysters, then returning the bag to the original location.

To test spat settlement on different shell types, we acquired SCWS from Galveston Bay Foundation's oyster shell recycling program (galvbay.org/work/habitat-restoration/). These shells were collected from restaurants, sun cured for approximately 6 months, and represent the shell type used in constructing restored reefs. Black shell was collected within GB by TPWD's Coastal Fisheries Division in February 2019 through oyster monitoring dredges and sun cured until May. Fresh black shell, representing shell exposed by bag-less dredging, was collected from the bottom of the created reefs immediately before experimental deployment. Oyster shells were placed in 0.04 m^2 caged trays constructed from 1 × 1 cm hardware cloth, filled with 10 pieces of similar-sized shells (70–90 mm) of each shell type, and closed with a hardware cloth lid. Cages were deployed on 20 June, 2019, and retrieved on 19 October, 2019, to capture the majority of the settlement within this system (Soniat & Ray 1985). On each reef, eight sets of trays were deployed approximately 3–5 m apart, with each set consisting of one tray from each treatment type. Individual trays were attached to the reef immediately next to the other two treatment trays (Fig. 2) and tray order was varied in each set. All eight sets were retrieved on two reefs and six sets retrieved on three reefs. All spat were measured and enumerated upon retrieval.

Data were analyzed using Statistical Analysis Software (SAS) version 9.4. Before using analysis of variance (ANOVA) tests using proc. GLM with random effects, each dataset was tested for homogeneity of variance with Levene's tests. Oyster abundance and settlement spat size met the assumptions and spat settlement was transformed ($\log[x + 1]$) prior to analysis. A one-way ANOVA tested how background oyster densities varied among the five different reefs and two-way ANOVAs tested how spat count and spat size differed among the shell treatments and different reefs. Pearson correlation analysis investigated potential relationships between oyster abundance among reefs and the mean and total number of spat recruited to each reef.

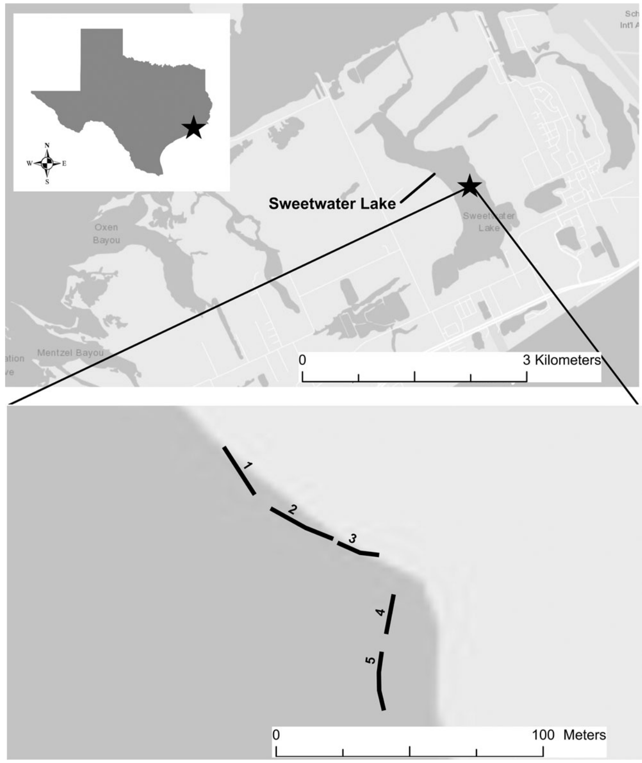

Figure 1. Locations of restored intertidal reefs in Sweetwater Lake, Galveston Bay, Texas, utilized in this study. The five reefs were built on sandy substrate and approximately 3–5 m away from the marsh edge.

Results

Background abundances significantly varied among the reefs ($F_{[1,4]} = 7.05$, $p = 0.001$), with significantly greater abundance

Figure 2. The three different shell treatment used in the study in the spat settlement trays attached to a reef restored using bagged shell. From left to right: sun-cured white shell, sun-cured black shell, and fresh black shell.

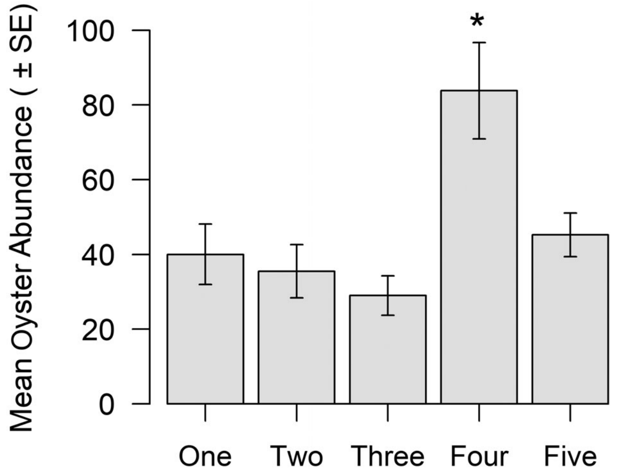

Figure 3. Mean (± SE) oyster abundance per bag ($n = 5$ per reef) collected from restored intertidal reefs in Sweetwater Lake, Galveston Bay, Texas. Star represents results significantly greater ($p < 0.05$) based on SNK posthoc tests after one-way ANOVA.

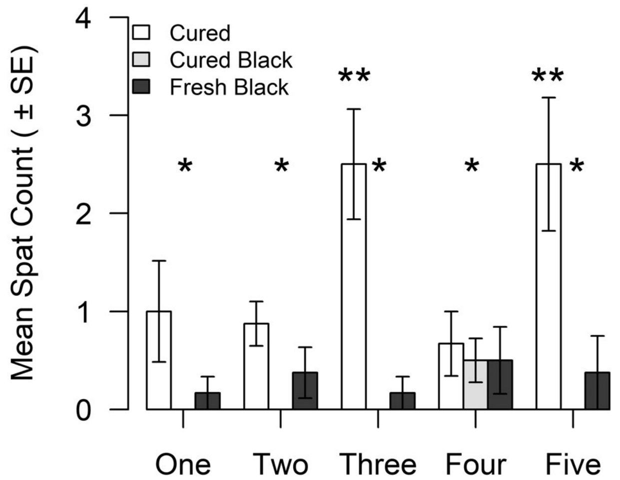

Figure 4. Mean (± SE) oyster spat recruitment for the recycled, sun-cured white shell (shown as cured in the figure), sun-cured black shell (shown as cured black in figure), and fresh black shell for each of the five bagged intertidal reefs utilized in this study. Single stars represent significant difference between treatment types, with sun-cured white shell having significantly greater ($p < 0.0001$) recruitment. Double stars represent the significant differences for interactive effect of shell treatment and individual reef ($p < 0.0001$).

on reef four (Fig. 3). Spat settlement was not significantly different between the five reefs ($F_{[4,87]} = 1.14$, $p = 0.34$), but was significantly different among the shell types ($F_{[4,87]} = 30.04$, $p < 0.0001$; Fig. 3), with the highest settlement on SCWS. There was a significant interaction between reef and shell treatment ($F_{[4,87]} = 30.04$, $p < 0.0001$) for reefs three ($F_{[2,17]} = 36.41$, $p < 0.0001$) and five ($F_{[2,21]} = 10.86$, $p = 0.0006$), which could be attributed to increased spat settlement on SCWS at these sites (Fig. 4). Spat size did not significantly vary by shell treatment type ($F_{[2,55]} = 1.83$, $p = 0.17$), individual reef ($F_{[2,55]} = 0.87$, $p = 0.49$), or shell treatment type × individual reef ($F_{[2,55]} = 0.25$, $p = 0.91$). There was no significant correlation between the mean oyster abundance on each reef with the mean number ($r = -0.26$, $p = 0.66$) or total number ($r = -0.26$, $p = 0.64$) of spat that recruited to each reef.

Discussion

The SCWS was the most effective settlement substrate, suggesting the addition of substrate should be prioritized over resurfacing substrate. Resource managers may not achieve target restoration goals using relic shell based on the limited spat settlement observed in this study. Sun-cured black shell had very minimal spat settlement and was an ineffective substrate. This substrate was only used in the experimental design as an intermediate substrate type and it is not currently used in restoration activities presumably because of the assumed habitat disturbance, logistics, and high costs associated with obtaining the shell. Fresh black shell, which would be surfaced during bagless dredging, had significantly lower spat settlement compared to SCWS. Uncovering buried shells will provide increased area for spat settlement, but our results suggest it may not provide quality settlement substrate. This decreased functionality may be from the shell's compositional change when buried in anoxic sediments, potentially reducing or inhibiting necessary biofilm growth that provides chemical cues for spat settlement (Campbell et al. 2011)

Interestingly, there was no significant difference in mean spat size among the different treatment types, suggesting that the different shell types do not influence post-settlement processes for oyster development. With growing aquaculture practices, understanding that alternative shell types may not influence growth rates could open future shell sources, provided the larvae could be attached to the shell within a controlled environment with lab-induced biofilms (Campbell et al. 2011).

While this study offered preliminary data suggesting exposed relic shells may be ineffective for larval recruitment, this experiment was limited to a suite of restored intertidal reefs. These reefs were used due to several logistical and financial constraints. These results may vary if the experiment occurred in a subtidal environment, where bag-less dredging and shell cultivation occur. Intertidal reefs are subjected to tidal fluxes, impacting larval encounter patterns, but subtidal reefs are continuously submerged allowing for continual and behavioral-driven settlement (Dame & Patten 1981; North et al. 2008; Johnson & Smee 2014). Given oyster settlement can be driven by Allee effects and conspecific cues (Moore et al. 2018), there was no significant correlation between mean oyster abundance and spat settlement to each reef, suggesting that current oyster abundance was not driving settlement within the cages and further indicating settlement was a function of shell type. Thus, the results suggest, despite tidal dynamics, restoration efforts within subtidal environments should also be highly dependent on substrate quality.

We found SCWS shell to be the most effective settlement substrate and this initial study indicated exposed relic shell receives comparatively minimal spat settlement. Future studies should be conducted within a subtidal environment; meanwhile, resource managers should construct restoration reefs from SCWS, or other reliable and cost-effective materials, before using bag-less dredging.

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