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Recruitment of the eastern oyster, *Crassostrea virginica*, in response to settlement cues and predation in North Carolina

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ABSTRACT

We conducted two field experiments to test the hypothesis that recruitment of the eastern oyster, *Crassostrea virginica*, could be enhanced through the selective deployment of artificial settlement cues. For both experiments, either dead shell or live oysters were cemented to patio blocks. In the first experiment, half of the blocks received discs that diffused the tri-peptide Glycyl-Glycyl-Arginine (GGR), a potent analog for natural settlement inducers, and only blocks with dead shell received GGR in the second experiment. Recruitment was therefore monitored on substrata with settlement cues (live oyster or shell with GGR) and no settlement cues (dead shell only). In our preliminary experiment (Experiment 1), recruitment of oysters was lower to blocks with live oyster or GGR, counter to our expectation. We repeated the experiment with the addition of anti-predation cage treatments (with partial cage controls). Again, we found no enhancement of recruitment to blocks with live oysters or with cue added. However, recruitment was significantly higher on blocks shielded from predation. These results suggest both a strong predator control in this system and that adding chemical cues are not likely to be an effective restoration strategy.

1. Introduction

The reef-building eastern oyster *Crassostrea virginica* has seen dramatic declines along the US Atlantic and Gulf of Mexico coastlines as a result of overharvesting and disease, among other factors (Kirby, 2004; MacKenzie, 2007). This loss is especially problematic because vast oyster reefs once provided a variety of ecosystem services, including the production of a commercially valuable product (oysters), the stabilization of sediments and the physical buffering of exposed shorelines, the creation of shelter for fishes and invertebrates, and the top-down control of now pervasive phytoplankton blooms (Coen et al., 2007). Due to their high economic and ecological value, oysters have been the subject of major restoration efforts nationwide. However, restoration efforts are often hampered by a number of biotic—e.g., disease (Powell et al., 2008) and predation (Johnson and Smee, 2014; Koeppel, 2011; Nestlerode et al., 2007)—and abiotic—e.g., flow (Knights et al., 2012), temperature and salinity (Lenihan, 1999; Lenihan et al., 1999), and taphonomy (Harding et al., 2012; Powell and Klinck, 2007)—factors affecting the recruitment of larvae and subsequent establishment of healthy reefs.

Recruitment of oysters in southeastern North Carolina has been considered limited by low substrate availability, which led restoration efforts to focus primarily on increasing substrate through a series of oyster reef reserves (Geraldi et al., 2013; Marshall et al., 1999). Restoration efforts also typically include the practice of seeding reefs with juvenile or adult oysters to enhance reef development, especially in areas where larval supply may be low or recruitment otherwise limited (Brumbaugh and Coen, 2009). However, seeding oyster reefs requires considerable additional cost, and there may be limited benefits for seeding reefs (Geraldi et al., 2013). Restoration success in the Pamlico Sound region of North Carolina has been attributed to high larval availability and connectivity between sites, which are primarily subtidal (Geraldi et al., 2013; Puckett and Eggleston, 2012). In contrast, reefs in the southern estuaries of North Carolina are restricted to the intertidal zone (Coen and Grizzle, 2007), and restoration strategies successful elsewhere have been less successful at enhancing recruitment to these reefs (Finelli, *unpublished data*). Given the increased cost and limited benefits of seeding, it is important to investigate other avenues for enhancing larval settlement and recruitment.

One potential method for enhancing larval settlement may involve synthetic chemical cues. Numerous marine invertebrates respond to chemical cues for site selection and settlement (Pawlik, 1992), including oysters (Barnes et al., 2010; Bonar et al., 1990; Turner et al., 1994; Zimmer-Faust and Tamburri, 1994). There are two strategies for chemically inducing settlement. One is to modify larvae by dosing them directly with a waterborne chemical to induce settlement and metamorphosis, which has been successful in the lab, but not as successful in field applications (Coon and Fitt, 1999). A second option is to make the substrate more attractive to larvae to encourage attachment. Competent oyster veligers respond to dissolved chemical cues

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released by live adult oysters and their associated microbial community by swimming downwards toward the substrate and undergoing metamorphosis (Tamburri et al., 1992; Zimmer-Faust and Tamburri, 1994). Zimmer-Faust and Tamburri (1994) identified a compound, glycyl-glycyl-L-arginine (GGR), which could enhance oyster larval settlement at low concentrations in a lab setting. Chemical cues have been used to induce settlement and metamorphosis in aquaculture settings (Doroudi and Southgate, 2002); however, little is known about their potential utility in a field setting.

While larval availability and subsequent settlement often drive patterns in oyster recruitment (Bushek, 1988; Roegner, 1991), this is not always the case (Knights et al., 2012). Post-settlement mortality can lead to recruitment failure of numerous benthic invertebrates, regardless of settlement densities (Gosselin and Qian, 1997; Hunt and Scheibling, 1997). For oysters, some factors that could decouple settlement and recruitment include physical characteristics such as flow, habitat setting, intraspecific competition, and predation (Grabowski et al., 2005; Knights and Walters, 2010; Knights et al., 2012; Newell et al., 2000). Predation can be especially important in structuring oyster recruitment and populations (Knights et al., 2012; Newell et al., 2000; Rindone and Eggleston, 2011; Soniat et al., 2004) and may be driving the intertidal distribution of oysters in southeastern estuaries (Dame, 1979; O'Beirn et al., 1996; Ortega, 1981). Oyster restoration efforts may also be hindered by predation (Koeppel, 2011; Nestlerode et al., 2007), and since chemical cues are also used by predators to locate prey (Weissburg and Zimmer-Faust, 1994; Zimmer-Faust et al., 1995), it is possible that methods used to enhance settlement—seeding or even chemical cues—could also enhance predation.

The objectives of this study were to investigate how chemical settlement cues and predation affected intertidal oyster recruitment in southeast North Carolina. Specifically, we compared live oysters to conditioned oyster shells with and without GGR in either caged, uncaged, or cage control plots. For our first experiment, we hypothesized that oyster recruitment would be enhanced on plots, which received a chemical cue treatment. In contrast to our expectation, however, oyster recruitment was reduced in the presence of chemical cues and live oysters. We repeated the experiment with the inclusion of a caging treatment to limit access by oyster predators. We expected higher recruitment of oysters to our chemical cue treatments inside predator exclusion cages during the second experiment, while recruitment to treatments exposed to predation would not differ. Again, however, patterns in settlement were not affected by chemical cue, and recruitment was only affected by the presence or absence of the cage treatment, suggesting strong predator control in our study system.

2. Methods

2.1. Study Site

All field work was conducted on an intertidal mudflat adjacent to the research dock at the Center for Marine Science, University of North Carolina Wilmington, Wilmington, North Carolina, USA (34°8′ 25.79″N 7751′50.52″W). The site is characterized by large tidal ranges (1.5-2 m), extensive salt marshes, and natural oyster populations. In addition, the site is situated near active oyster aquaculture cages maintained by the UNCW Shellfish Research Hatchery.

2.2. Experiment 1

A 2 × 2 factorial design was used to test the effects of synthetic chemical cues and oyster substrate on settlement. Plots were established by cementing sets of live oysters or dead oyster shells, used as a settlement substrate, to 40 cm × 40 cm concrete patio blocks. Single live oysters were harvested from sites adjacent to our experimental location. All macrofauna were removed, and oysters were subsequently scrubbed clean with a scrub brush and freshwater and held in the lab for 24 h prior to use. Sun-bleached oyster shells were also scrubbed clean to remove any dirt or debris that might have accumulated. 24 oyster shells or live oysters were cemented in a vertical position, to minimize sedimentation effects (e.g., Soniat et al., 2004) using the marine epoxy (Z-Spar A-788 Splash Zone Compound); shells or oysters were arranged in a rough 5x5 grid (vertices ~8 cm apart) leaving the center position open to accommodate a gel disc used for diffusing chemical cues. Each settlement substrate was assigned either a chemical cue treatment or no chemical cue treatment. Chemical cue plots received a 10 cm diameter gel disc that was placed into the center of the patio block as the source of waterborne GGR (Browne and Zimmer, 2001). Gel discs were created using the methods of Browne and Zimmer (2001). Briefly, a 2×10^{-4} M solution of GGR (Sigma-Aldrich Chemical Company #G6887) was added to a premixed solution of 8% acrylamide catalyzed with 0.05% ammonium persulfate and 0.05% TEMED. Aliquots were dispensed into circular molds, 10 cm diameter × 1 cm tall, and allowed to harden. Once hardened, the discs were attached to the settlement substrates for deployment in the field. GGR has been shown to stimulate oyster settlement at concentrations between 10^{-10} and 10^{-6} M (Browne et al., 1998; Tamburri et al., 1996; Zimmer-Faust and Tamburri, 1994), such that source concentration within the gel is 100 to 1,000,000 times more concentrated than those shown to be active. This enhanced concentration is necessary to account for dilution of the released compound. For example, data presented by Browne and Zimmer (2001) show a 3700 fold dilution of GGR was realized 1 cm above the gel within their collectors. Our own modelling of average concentrations within a turbulent plume show an additional 5 to 10 fold dilution 25 cm from a solute release point (e.g., Finelli et al., 1999). Given the dimensions of the recruitment block with a maximum distance of shell from center of the block ~25 cm, concentrations of GGR within the shell matrix of our recruitment blocks were likely diluted from source concentrations by a factor of 10^3 to 10^4, but still in the stimulatory range. Finally, modelling completed by Browne and Zimmer (2001) showed that a 1 cm thick cylindrical gel will be exhausted of its soluble cue in 5 days or more; therefore, the discs were changed biweekly to maintain a GGR cue throughout the duration of the experiment.

Plots were placed under the pier at UNCW's Center for Marine Science in rows perpendicular to shore, and thus across a tidal gradient, and allowed to recruit oysters and barnacles for ~8 weeks, from 3 August to 29 September 2007. Due to potential differences in tidal elevation affecting recruitment, sets of 4 blocks (one from each treatment) were grouped into 3 zones (see Fig. 1). Plots were then collected, and live oyster spat were enumerated and standardized per unit shell area using ImageJ image analysis software.

2.3. Experiment 2

Because the results of experiment 1 were equivocal regarding the effectiveness of chemical cues and further suggested that predation may be driving patterns in recruitment (see results), the experiment was repeated using a 3 × 3 factorial design with 3 cue treatments (live oyster, shell with synthetic cue, shell without synthetic cue) and 3 cage treatments (full, partial and open). As above, live oysters were harvested from local reefs and cleaned of all epibionts via scraping and scrubbing with freshwater rinse. Six live oysters or oyster shells were cemented in a vertical orientation to 30 cm × 30 cm patio blocks. To eliminate positional affects on the patio blocks, shells and oysters were arranged in a circular fashion with each oyster equidistant from the center (and any chemical cue source) using Z-Spar epoxy. Gel discs, as described above, were placed at the center of substrates receiving a chemical cue treatment (Fig. 2).

Cages were constructed using 0.3 cm × 0.3 cm plastic mesh and were fastened to the concrete patio blocks by tie wrapping the cage to nuts fastened to the concrete blocks. Full cages consisted of mesh around the entire perimeter and a lid across the top. Partial cages were half cages (including partial sidewall and lid) and served as a control for