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Evidence for local adaptation of oysters to a within-estuary gradient in predation pressure weakens with ontogeny

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ABSTRACT

Consistent spatial variation in phenotypes within a species can reflect local adaptation to gradients in selective pressures, or plastic responses to variable conditions. In benthic marine foundation systems with long, dispersive pelagic larval stages, the usual assumption is that the plastic strategy should dominate, yet surprising examples of local adaptation are accumulating. We tested the potential for local adaptation to an environmentally-driven spatial gradient in predation pressure on the Eastern oyster (*Crassostrea virginica*) in a Florida estuary by reciprocally transplanting juvenile oysters between two sites. Experimental units included oysters in predator exclosures and controls that were exposed to both consumption by predators and nonconsumptive predator cues. One month after deployment, juvenile oysters from the upper-estuary site where more predators of adult oysters are present exhibited thicker shells in both the upper and lower estuary deployment sites, a possible signal of local adaption to a more intense predation regime. However, nine months after deployment, oysters sourced from both the upper and lower demes had thicker shells in predator-exposed treatments than in predator exclosures, regardless of location. Additionally, there was no evidence that the thicker shells in those oysters led to changes in survival or growth, as one would expect. These results suggest that while oysters from high-predator sites may initially exhibit an apparently adaptive antipredator trait, over the course of an oyster's life, trait expression is a plastic response to the perceived predation threat environment.

1. Introduction

An important area of inquiry in evolutionary ecology is whether spatial and temporal variation in phenotype reflects individual organisms optimizing fitness components (e.g., survival, growth, and reproduction) through individual plasticity in traits (i.e., defensive structures, foraging behaviors) or adaptation of traits in the population through local selection (Richardson et al., 2014; Urban et al., 2020; Warner, 1997). In general, the answer depends on the rate of migration among local populations, with high gene flow preventing local adaptation and favoring plasticity unless selection pressure is very strong (Hellberg et al., 2002; Sotka and Palumbi, 2006; Waples, 1998). Manipulative field experiments are usually the most effective way to distinguish between static, locally adapted traits and plasticity, particularly for systems in which migration is difficult to quantify (Kawecki and Ebert, 2004).

In coastal marine systems, researchers once assumed that reproductive propagules disperse so widely that there is too much gene flow to allow for local adaptation, except over very large spatial scales (Bohonak, 1999; Sotka and Palumbi, 2006) or in species without propagule dispersal (Sanford et al., 2003). Hence, marine species exhibiting phenotypic variation across fine-scale environmental differences would be assumed to be exhibiting plasticity (Warner, 1997). For example, in the sex-changing coral reef fish *Thalassoma bifasciatum*, the mating system varies with local population density, and whether newly settling juveniles initially become male or female is plastic and depends on social cues after settlement (Munday et al., 2003). However, a collection of genetic and geochemical tagging studies have consistently revealed unexpectedly high rates of larval retention in a diversity of taxa from coral reef fish to temperate bivalves (Almany et al., 2013; Becker et al., 2005; D'Aloia et al., 2015; Jones et al., 2009; Swearer et al., 1999; reviewed by White et al., 2019). Given these findings, an emerging consensus is that realized dispersal distances of marine larvae and seeds are much smaller than previously assumed because spawning adults can time larval release with shifting currents and larvae can display subtle

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yet active planktonic behaviors, both of which can favor larval retention (Morgan et al., 2009; Paris and Cowen, 2004; Shanks, 2009). Therefore, the assumption that long planktonic larval stages preclude local adaptation over fine scales in marine systems merits further investigation.

Testing this assumption may be particularly important for sessile marine foundation species where essentially all movement is limited to planktonic propagule life stages. At micro-geographic spatial scales from meters to kilometers, these populations often exist along strong environmental gradients that can favor genetic differentiation of traits. For example, sea grasses, salt marsh plants, mangroves, and corals all display local adaptation to elevation gradients and corresponding temperature stress (Hays et al., 2021). While the majority of the evidence for local adaptation in these systems comes from studies of abiotic stress, a recent meta-analysis suggests that biotic selective agents may be equally influential (Hays et al., 2021). For example, in the classic rocky shore zonation pattern (Connell, 1961), thermal tolerances at higher tidal heights and predation as well as competition selective agents at lower tidal heights would be predicted to explain any phenotypic variation in barnacles across an elevation gradient (Hays et al., 2021). Consequently, empirical tests of local adaptation may benefit from the simultaneous evaluation of abiotic and biotic selective agents across stress gradients.

A key tool in testing for the influence of local adaptation is the reciprocal transplant experiment, whereby individuals from one environment (i.e., subpopulation or *deme*; we will use the latter term hereafter) are transplanted to their home environment and to a different location (Kawecki and Ebert, 2004). When it is suspected that biological selection (e.g., by predators) is important, this experimental design can be enhanced to include a predator exclosure treatment in order to measure the strength of that selection (e.g., survival in exclosure – survival in control treatments) and to assess how selection affected trait frequency in the surviving population. This extended design, however, has a complication: in addition to selecting against traits in a population by consuming particular phenotypes, predators also produce visual, chemical, and tactile cues that can elicit plastic trait responses in individual prey (Long and Hay, 2012; Peacor et al., 2020). For example, predators may preferentially consume and select against faster growing individuals in a prey population. But the predation risk cues associated with consumption can also cause individuals to develop defensive traits (e.g., thicker shell, spines) that inhibit predation. Because these risk-induced trait responses are energetically costly, a higher frequency of prey with less tissue mass could also be explained by the costs of individual trait plasticity, not preferential selection by predators of faster growing prey. Because this predator-induced plasticity may itself be under selection, reciprocal transplant experiments should be accompanied by an attempt to differentiate the role of biological selection on the population versus risk-induced plasticity of individuals and their traits.

In southeastern U.S. estuaries, Eastern oysters (*Crassostrea virginica*) form extensive reef systems, comprising metapopulations that span several potentially strong selection gradients. Because of predictable changes in ocean tides and freshwater discharge from rivers, salinity typically increases with distance away from river input, forming the primary organizing environmental gradient. Many sessile estuarine prey species, including oysters, are most abundant in the brackish mid-estuary where they can benefit from feeding on plankton in the absence of stenohaline natural enemies. There, crab and gastropod predators cannot tolerate dynamic salinity fluctuations (Kimbro et al., 2017b; Livingston et al., 2000; Powell et al., 1996). Within a particular area of an estuary's salinity gradient, oyster reefs can span tidal elevations, from subtidal reefs (never exposed) to intertidal reefs (exposed at low tide). Whether by local adaptation or plasticity, aerial exposure can affect oyster traits and fitness through many pathways. The stress of aerial exposure can reduce oyster survival and growth (Kimbro et al., 2020), but aerial exposure may also reduce foraging success of predators that must remain immersed (Fodrie et al., 2014; Johnson and Smee, 2014). Because oysters respond phenotypically to the presence of predator cues, it is likely that aerial exposure also induces microgeographic variation in oyster growth and shell traits as a function of predation risk (Robinson et al., 2014). Thus, oysters from different demes have likely experienced unique predation and environmental stressor selection regimes.

Despite strong selection gradients, phenotypic plasticity is expected to emerge as the stable evolutionary strategy if oyster larvae are dispersed widely during their three-week planktonic stage (Richardson et al., 2014). Indeed, phenotypic variation in oysters has been assumed to represent plasticity because population genetic studies have documented extensive gene flow at regional scales, such as the South Atlantic Bight (North Carolina to Florida; Hughes et al., 2017). This would suggest that dispersal is too widespread to support local adaptation. However, new evidence collected at finer spatial scales in adjacent estuaries or within estuaries suggests that metapopulations of oysters are less well-mixed by larval dispersal than those larger-scale studies suggested (e.g., Burford et al., 2014), as well as similar patterns in the Olympia oyster *Ostrea lurida* on the U.S. Pacific coast (Bible and Sanford, 2016; Maynard et al., 2018). For example, Adrian et al. (2017) found that while there was limited population genetic structure among oyster populations spanning 200 km of South Atlantic Bight coastline, there was substantial structure and kin aggregation at small scales within individual reefs, indicating more larval retention and less genetic mixing at smaller spatial scales than previously assumed. Finally, at even smaller scales, local kinship can dictate local settlement patterns of natural larvae in the field (Hanley et al., 2016). Thus, it is possible that the realized small-scale dispersal of oyster larvae within estuaries (despite their potential for widespread mixing) may permit local adaptation to the strong spatial gradients.

We tested for local adaptation to abiotic and biotic selective agents using a manipulative experiment at two sites that span 5 km within an estuary on the Atlantic coast of Florida, USA (Fig. 1). Because the two sites differ both in mean salinity and the composition of the oyster predator community, we used a reciprocal transplant design that included predator exclosure treatments. At each site, we orthogonally manipulated the deme from which juvenile oysters were transplanted (high salinity deme and variable salinity deme) and the presence of predation (predator exclosure cage versus control). Throughout the experiment, we quantified potential abiotic (salinity, temperature, aerial exposure, and flow) and biotic selective agents (predator abundance, predation strength) at each site. During the juvenile (1 month) and adult (9 months) stages of the transplanted oysters, we tested for evidence of local adaptation to abiotic and biotic stress in each environment. The response variables were two demographic components of fitness (survival and growth) and several phenotypic traits that should be associated with fitness and anti-predator defense (e.g., body mass, shell thickness, and tissue mass).

2. Methods

2.1. Study system

This research was conducted at two sites within the Guana Tolomato Matanzas National Estuarine Research Reserve in northeastern Florida (GTM NERR, Fig. 1) from August 2019 to May 2020. The shorelines of this estuary contain emergent oyster reefs surrounded by open mudflat as well as reefs adjacent to salt marsh (*Spartina alterniflora*) and mangrove (*Avicennia germinans*) habitats. The oyster reefs of this estuary experience varying hydrodynamic conditions, with reefs closer to the inlet influenced by tidal excursion and reefs farther from the inlet influenced by freshwater input and longer water residence times (Sheng et al., 2008). In addition to directly influencing oyster physiology, these hydrodynamic conditions promote spatial variation in the abundance of predators that consume oysters. Mud crabs (*Panopeus herbstii*) consume juvenile oysters (< 25 mm shell length) and are ubiquitous throughout the estuary (Garland and Kimbro, 2015). In contrast, the crown conch (*Melongena corona*) preferentially consumes adult oysters (oyster length

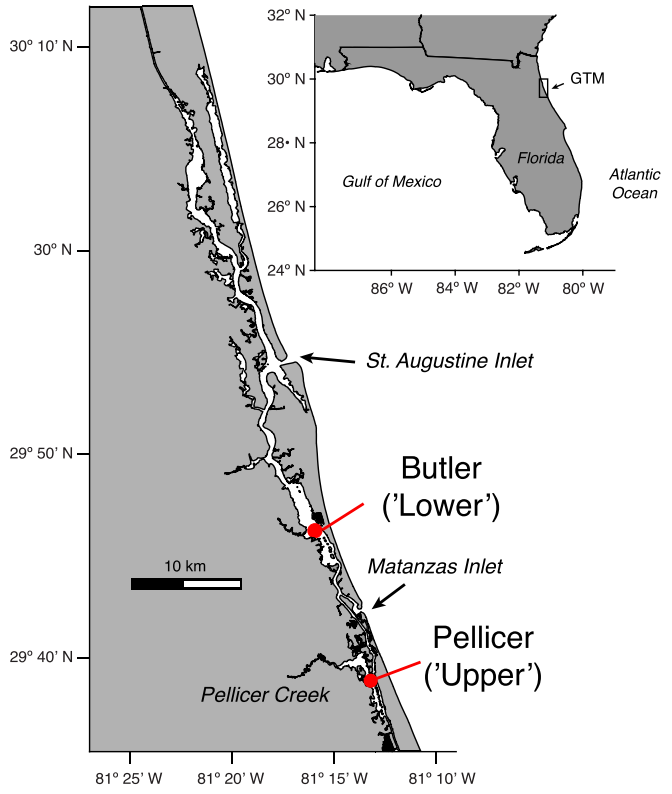

Fig. 1. Map of the study sites in the Guana-Tolomato-Matanzas estuary, Florida, USA. Inset illustrates area of Florida where research occurred. Red circles denote the two sites used in the reciprocal transplant experiment. Lower and Upper refer to a site's position in the estuary with the latter being farther from the inlet, closer to freshwater input, and having both predatory mud crabs and crown conchs, while the former contains only predatory mud crabs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

> 50 mm) and are restricted to sites south of the inlet, near Pellicer Creek (Booth et al., 2018; Garland and Kimbro, 2015). Consequently, we designed this study to focus on a site near (Butler) and far (Pellicer Creek) from the inlet (Fig. 1); for convenience hereafter we will refer to these sites – and the respective oyster demes – as *Lower* and *Upper*, respectively, referring to their position in the estuary with the latter being farther from the inlet and closer to freshwater input.

2.1.1. Environmental conditions

To quantify spatial variation in environmental conditions, we deployed a HOBO salinity logger (model no: U24–002) and a HOBO pressure gauge logger (model no: U20–001-04) at the center of one oyster reef within each site. This on-reef logger was paired with an additional pressure gauge logger that was secured above the water at a known elevation to simultaneously record atmospheric pressure. Subtracting pressure data of the latter from the former yielded site-specific measurements of hydraulic pressure. Hydraulic pressure was divided by the temperature-corrected density of water (also recorded by the Onset gauges) to yield water depth, which we used to estimate the duration and depth of reef submergence. These instruments recorded data at 15-min intervals. Pressure-converted depths greater than or equal to 0.11 m were considered submerged times while pressure-converted depths <0.11 m were considered aerial exposure times during low tide. Based on our previous work in this system (Garland and Kimbro, 2015), we expected more aerial exposure at the Upper location, but we were uncertain the degree to which the locations would differ.

Meanwhile, at monthly intervals, we obtained site-specific snapshots of water flow and phytoplankton concentration (chl *a*) by deploying a Nortex Aquadopp Profiler 2Mhz and a SeaBird Hydrocat-EP just seaward of the same reef containing the HOBO instruments. These instruments recorded samples at 60 s intervals for three hours. While these three variables may be important for oyster traits and fitness, we did not have a priori predictions on how they would vary spatially.

2.1.2. Predation risk

Prior to the experiment, predation risk was estimated by surveying three reefs within each site for residential predators, which consist of mud crabs and crown conchs. In May 2019, reef length was estimated by deploying a transect along the crest of the reef. For each reef, that transect was then partitioned into 6 intervals. In the center of each interval, a second transect was deployed from the reef crest to the seaward edge of the reef to estimate reef width. At the midpoint of the reef width within each interval, we deployed a 1 × 1 m quadrat and searched for all mud crabs and crown conchs within each quadrat ($n = 6$ quadrats per reef). Carapace widths of all mud crabs were measured to estimate crab size, while distance from siphon to pointed end of shell was used to estimate conch size. Because crown conchs are concentrated on the seaward edge of the reef at low tide, we also placed the quadrat at the seaward edge of each of the reef intervals to count and measure size of crown conchs, which resulted in 12 quadrat samples per reef: half from the interior (as describe above) and half from the reef edge.

2.2. Reciprocal transplant experiment

In August 2019, we conducted a 2 × 2 × 2 reciprocal transplant experiment with oyster deme (Upper or Lower), environment (Home or Away), and predation treatment (Exclosure and Control) as fixed effects. Before beginning the experiment, we collected juvenile oysters that were < 15 mm shell length from each site and transported them to the lab (Whitney Laboratory for Marine Bioscience and GTM NERR Marineland Field Office) for processing. Processing involved identifying valves of dead adult oysters that contained naturally settled, living juvenile oysters. The juveniles on each valve were marked with fingernail polish (red, blue, or purple) and measured for length (umbo to tip). The adult valves were attached to bird netting material with z-spar marine epoxy compound (Splash Zone A-788 kit) and allowed to cure. Then, one or two adult valves were attached to a single PVC post (0.25 m length) with a cable tie threaded through the mesh in order to have a collection of posts each with three juvenile oysters (blue, red, and purple). Posts were then marked with unique numeric identifiers. Each post held three oysters from a single deme.

We randomly assigned posts carrying oysters from Upper and Lower demes among 20 experimental units either in a Home or Away environment as well as in either a predator Exclosure cage or Control treatment, yielding $n = 5$ of each unique combination of the three factors. At the midpoint between the crest and submerged edge of the reef, experimental units were deployed along a 20 m transect running parallel to the water line, with 1 m spacing between each unit. For all treatments, we first removed surface sediment and reef material within a 0.3 × 0.3 m area to a depth of 0.2 m. These contents were sieved in the field to remove mud crabs. Next, we inserted a fully enclosed (with roof and floor) cage made of a PVC frame wrapped in Vexar plastic mesh (0.25 × 0.25 × 0.25 m; 9 mm mesh openings). The cleaned reef material was deposited back into the Exclosure. Six posts with painted oysters were placed in the middle of the experimental units equidistant from the edge of the Exclosure and the other posts. Exclosure tops were sewn shut with DeWalt nylon lawn trimmer line. To standardize the installation procedure among experimental units, the Control treatments were installed in the same manner, except for lacking Vexar plastic sides and a top (i.e., they contained a PVC frame and a buried Vexar bottom). Given the accumulation of similar experiments in this (Garland and Kimbro, 2015; Kimbro et al., 2017a, 2020) and other Florida systems (Grabowski et al., 2020; Kimbro et al., 2017b) that did not detect procedural artifacts, we elected to omit a procedural control treatment (i.e., a Partial cage) from

this experiment. This decision was reinforced by our using caging material with even larger mesh openings than the aforementioned field studies.

Each of these experimental units contained three posts that were removed one month after deployment (Sept. 2019) and three posts removed after nine months (May 2020). Those time periods correspond approximately to a post-settlement/juvenile stage (vulnerable to mud crabs), and an early adult stage (preferred by crown conch), respectively. At each sampling period, the oysters attached to the sampled post were removed, brought to the lab, and processed to quantify two fitness components: survival (live or dead) and growth (final – initial shell length). These oysters were then frozen, shipped to the Northeastern University Marine Science Center in Nahant, MA, where they were further processed to quantify three traits: shell mass (dried at 60 °C for 72 h, then weighed Mettler Toledo NewClassic MS balance), shell thickness (measured with Rexbeti 0–1" Digital Micrometer over the visible scar from the adductor muscle on the inside of the shell), and tissue mass (dried at 60 °C for 72 h, then weighed on Mettler Toledo

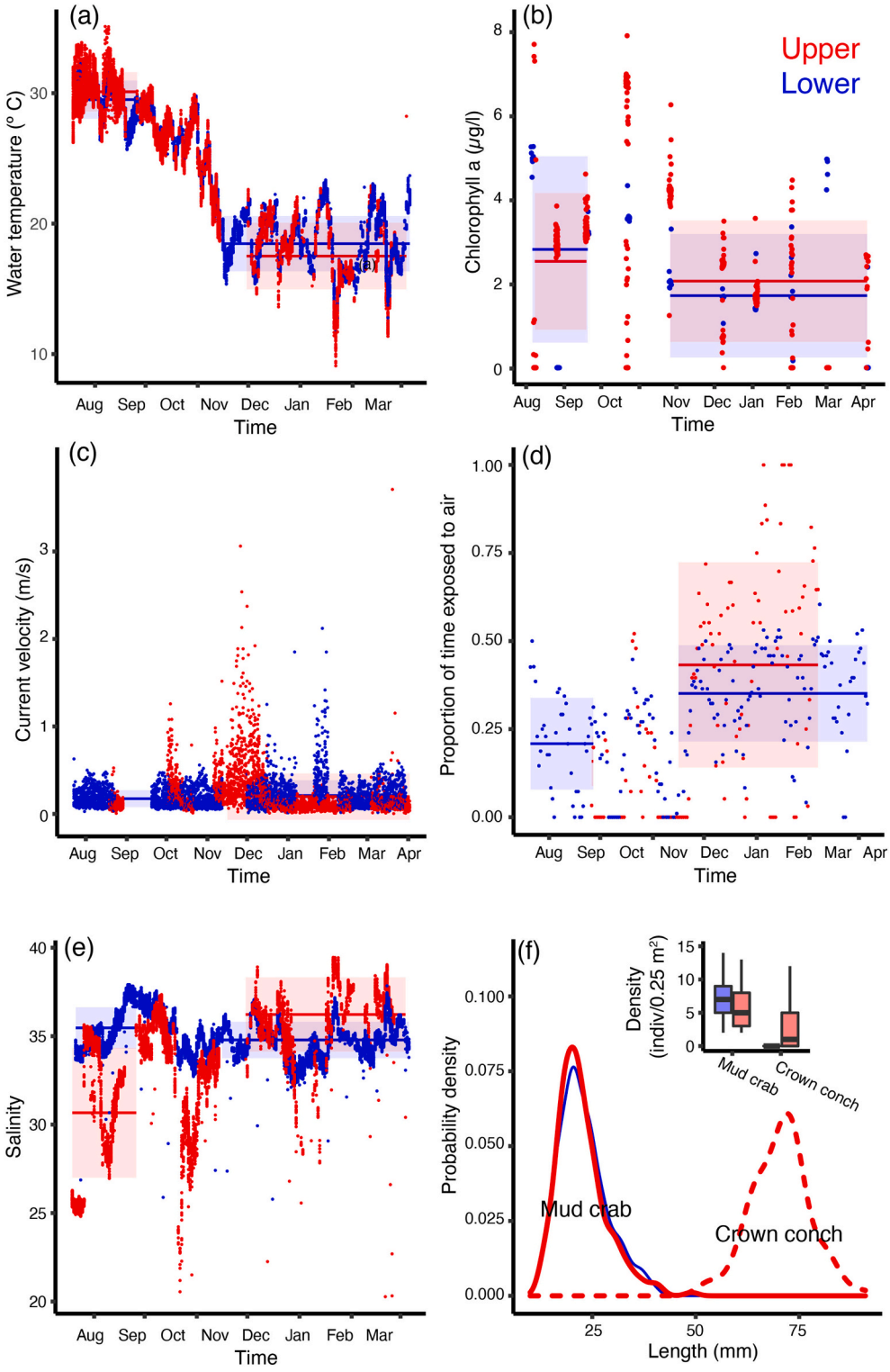

Fig. 2. Physical and biological conditions at the study sites. In all panels, results from the Upper and Lower estuary sites are illustrated with red and blue symbols, respectively. Panels show a) water temperature, b) chlorophyll *a*, c) current velocity, d) air exposure at low tide, and e) salinity, all measured over the course of the experiment. In all panels each point is one sample; different metrics were sampled at different frequencies, as explained in the text. Gaps in data collection at some sites reflect instrument malfunction or maintenance periods. Horizontal lines and shading indicate the mean ± one standard deviation of conditions at each site during the first month or final three months of the experiment. Panel f shows the size distribution of mud crabs (*Panopeus herbsteii*, solid curves) and crown conch (*Melongena corona*; dashed curves) at the two sites; inset shows the total density of both predators at both sites. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

NewClassic MS balance). The values for these traits were divided by shell length (measured with digital calipers) to standardize responses among oysters of varying sizes.

2.3. Data analysis

At months 1 (juvenile oysters) and 9 (adult oysters), we used separate analyses to evaluate the influence of deme, environment, predation treatment, and their interaction on fitness components (survival and growth) as well as potentially plastic phenotypic traits (shell length, shell mass, shell thickness, and tissue mass). Because individually marked oysters were scored as alive or dead, oyster survival was evaluated with a generalized linear model using a binomial error and logit link transformation. Meanwhile, the remaining response variables were evaluated with analysis of variance (ANOVA), which considered deme, environment (Home or Away), and predation treatment (Control and Exclosure) as fixed effects. For all analyses, individual oysters within each experimental unit were treated as sub-samples, with the average of the samples representing the value of each replicate.

For each response variable, if the overall model detected a statistically significant difference among treatments, we used Tukey's Honest Significant Difference test to compare means. In a prior experiment in which predators were maintained in enclosures adjacent to juvenile oysters, we detected effects of their cues on oyster growth (Kimbro et al., 2020). Because we did not detect predator-induced trait responses in the Exclosure cages in the present experiment, we presume that the cages we used were large enough for any external predator cues to be diluted before reaching the caged oysters (e.g., Gosnell et al., 2017). Thus, we considered oyster metrics from the Exclosure treatment as 'no-risk' metrics, while metrics from the Control treatment were considered to potentially represent both a risk-inducted trait response (Peacor et al., 2020) by individuals as well as predator selection on the population (removal of oysters in each treatment).

All analysis was concucted in R 4.1.3 (R Core Team, 2022): data and code are available on GitHub, DOI https://doi.org/10.5281/zenodo.6518929.

3. Results

3.1. Environmental conditions

To compare environmental conditions between the two study sites we focus on conditions during the first month of the experiment (preceding the first oyster sampling endpoint) and during the final three months (preceding the nine-month sampling endpoint; Fig. 2). The two sites were similar in most environmental conditions: water temperature and chlorophyll *a* showed expected seasonal variation and were similar at both sites throughout the experiment, as was water velocity (Fig. 2a-c). Low-tide exposure was similar at the two sites during the experiment, and was nearly twice as long during the latter months (spring) than during the initial deployment in fall (Fig. 2d). By contrast, salinity at the Upper site was consistently more variable on weekly time scales than at the Lower site, and switched from being nearly 5 psu lower at the Upper site at the beginning of the experiment to slightly more saline than the Lower site in the final three months (Fig. 2e). Finally, mud crab predators had similar abundances and size distributions at both sites, but crown conch predators were only present at the Upper site (Fig. 2f).

3.1.1. Juvenile stage

After one month, there was no evidence for an effect of location or deme on oyster survival or growth, but there was a strong effect of the predator Exclusion treatment on both fitness components (survival: GLM, $c^2 = 4.65$, $p = 0.03$; growth: ANOVA $F_{1,31} = 6.04$, $p = 0.02$; Table S1a-b, Fig. 3). In Exclosures at both locations, the mean (±sd) proportional oyster survival was 0.68 (±0.3). Averaged across both study sites, predators reduced the survival of both demes to 0.57 (±0.31)

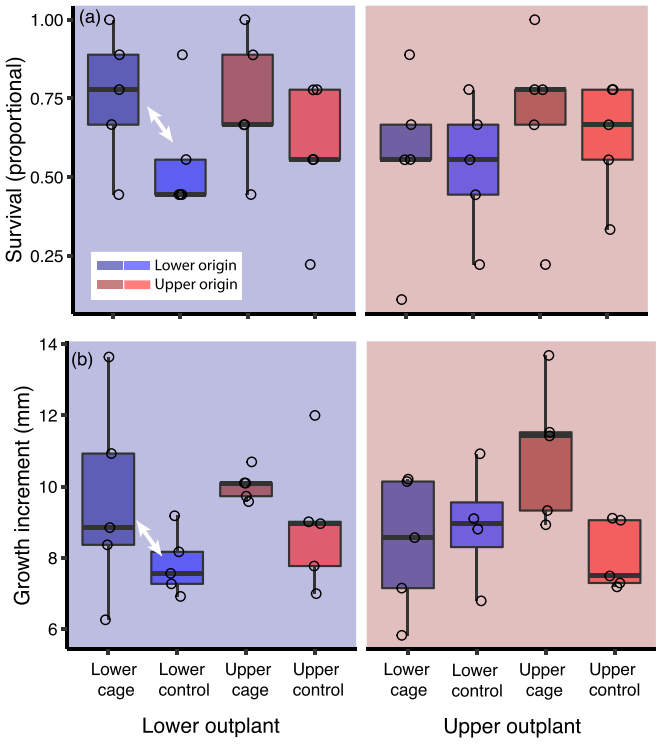

Fig. 3. Experimental results of oyster fitness components after one month. Proportional survival and growth of outplanted oysters are presented in panels (a) and (b), respectively, with growth measured as change in length of each oyster since initial deployment. In all panels, results are grouped by predator-exclosure treatment on the secondary x-axis and outplant location on the primary x-axis, with Lower site results grouped to the left and Upper site results grouped to the right. In all panels, blue and red boxplots denote oysters sourced from the Lower or Upper estuary site (respectively), with darker shading further distinguishing between predator exclusion cages (darker boxplots) or exposed controls (lighter boxplots). Blue and red backgrounds further distinguish whether oysters were outplanted at the Lower (left panels, blue background) or Upper estuary site (right panels, red background). The white arrows call attention to the strongest effects detected in this analysis, which were overall higher survival and greater growth inside cages versus in controls, across all demes and outplant sites. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in Control treatments (Fig. 3a). Of the surviving oysters, growth inside Exclosures (9.8 ± 3.4 mm) was 16% greater than growth in Controls (8.1 ± 3.1 mm), averaged across all demes and outplant locations (Fig. 3b).

After one month, there was no evidence for an effect of predation treatment, environment, or deme on two of the traits we measured in juvenile oysters: standardized shell mass and standardized tissue mass (ANOVA, all $p > 0.05$; Table S1c, e; Fig. 4a,b). In contrast, there was evidence for an interactive effect of deme and predation treatment on the standardized thickness of oyster shell (ANOVA $F_{1,31} = 4.07$, $p = 0.05$, Table S1d, Fig. 4c), because shell thickness of oysters from the Upper deme – but not the Lower deme – was 38% higher in the Control treatment than inside Exclosures, regardless of outplant environment (Tukey HSD, p = 0.03). Exposure to predators and their cues also had an overall effect on shell thickness, with 14% thicker shell in Controls than inside Exclosures across both demes and sites ($F_{1,31} = 5.00$, p = 0.03, Fig. 4c).

3.1.2. Adult stage

After 9 months, there remained no evidence for an effect of source deme on oyster survival, but strong evidence for an effect of predator treatment (GLM, $c^2 = 4.15$, $p = 0.04$, Table S2a, Fig. 5a). Survival of oysters from both demes inside Exclosures was 92% greater than that in

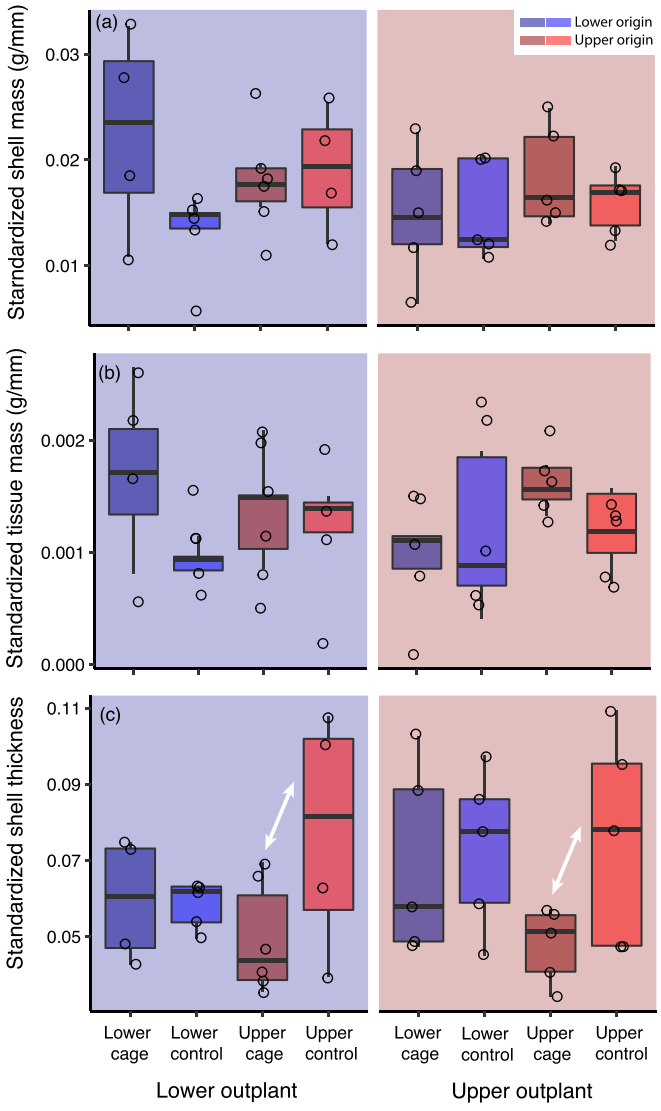

Fig. 4. Experimental results of oyster traits after one month. Results of shell mass, tissue mass, and shell thickness are presented in panels (a), (b), and (c). Each trait was standardized by dividing by oyster length; hence standardized shell thickness is dimensionless. In all panels, results are grouped by predator-exclosure treatment on the secondary x-axis and outplant location on the primary x-axis, with Lower site results grouped to the left and Upper site results grouped to the right. In all panels, blue and red boxplots denote oysters sourced from the Lower or Upper estuary site (respectively), with darker shading further distinguishing between predator exclusion cages (darker boxplots) or exposed controls (lighter boxplots). Blue and red backgrounds further distinguish whether oysters were outplanted at the Lower (left panels, blue background) or Upper estuary site (right panels, red background). The white arrows call attention to the strongest effects detected in this analysis, which was that Upper deme oysters grew thicker shells when exposed to predators versus in cages, regardless of outplant environment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

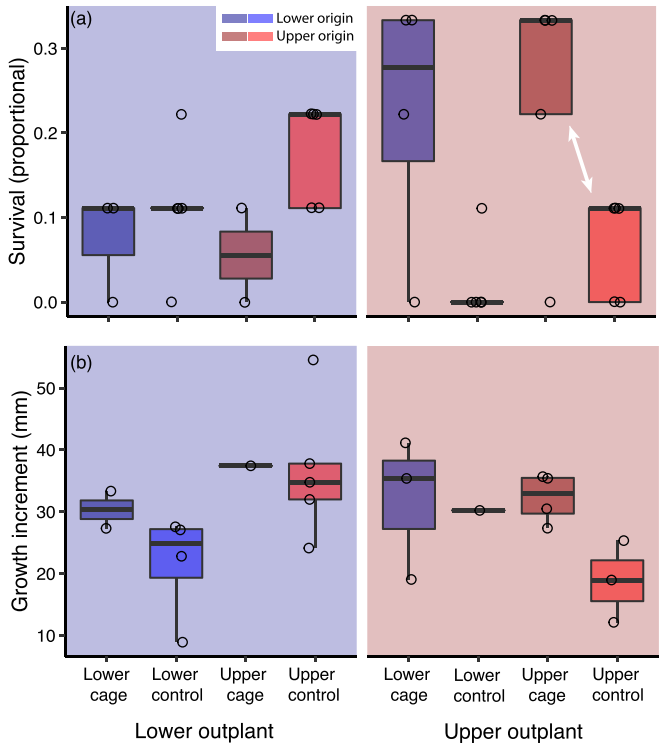

Fig. 5. Experimental results of oyster fitness components after nine months. Proportional survival and growth of outplanted oysters are presented in panels (a) and (b), respectively, with growth measured as change in length of each oyster since initial deployment. In all panels, results are grouped by predator-exclosure treatment on the secondary x-axis and outplant location on the primary x-axis, with Lower site results grouped to the left and Upper site results grouped to the right. In all panels, blue and red boxplots denote oysters sourced from the Lower or Upper estuary site (respectively), with darker shading further distinguishing between predator exclusion cages (darker boxplots) or exposed controls (lighter boxplots). Blue and red backgrounds further distinguish whether oysters were outplanted at the Lower (left panels, blue background) or Upper estuary site (right panels, red background). The white arrow calls attention to the strongest effect detected in this analysis, which was overall higher survival in the cages versus controls, regardless of source deme or outplant site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Control treatments, across both environments. Oyster survival also depended on an interaction between predation treatment and environment (GLM, $c^2 = 12.05$, $p < 0.0001$, Table S2a, Fig. 5a), as survival inside Exclosures at the Upper environment exceeded that both in Controls at the Upper site (Tukey contrast, $p < 0.001$) and in Exclosures at the Lower environment (Tukey contrast, $p = 0.02$). Of the surviving oysters, there was no evidence for an effect of predator treatment, location, or deme on oyster growth after nine months (ANOVA, $p > 0.06$, Table S2b, Fig. 5b).

Similar to the results for juvenile oysters, after nine months there was no evidence for the effects of predation treatment, location, or deme on the standardized shell mass or standardized tissue mass of surviving oysters (ANOVA, $p > 0.05$; Table S2c, e, Fig. 6a,b). In contrast, there was strong evidence for an effect of predators (but not deme or location) on the standardized thickness of oyster shell (ANOVA $F_{1,20} = 11.04$, $p = 0.003$, Table S2d, Fig. 6c), with oysters exposed to predators in Control treatments having shells 25% thicker than that of oysters inside Exclosures (0.06 ± 0.01 vs 0.048 ± 0.01; these length-standardized values are a dimensionless ratio).

4. Discussion

We used a reciprocal transplant design to test whether a sessile benthic foundation species with the potential for high larval dispersal could nonetheless exhibit local adaptation over small spatial scales. Across 5 km of varying abiotic and biotic conditions in a Florida estuary, we found some support – albeit limited – for this hypothesis. After one month, outplanted juvenile oysters from the Upper estuarine deme (the sole location of voracious conch predators of adult oysters) grew thicker shells when exposed to any predator and their cues, regardless of the

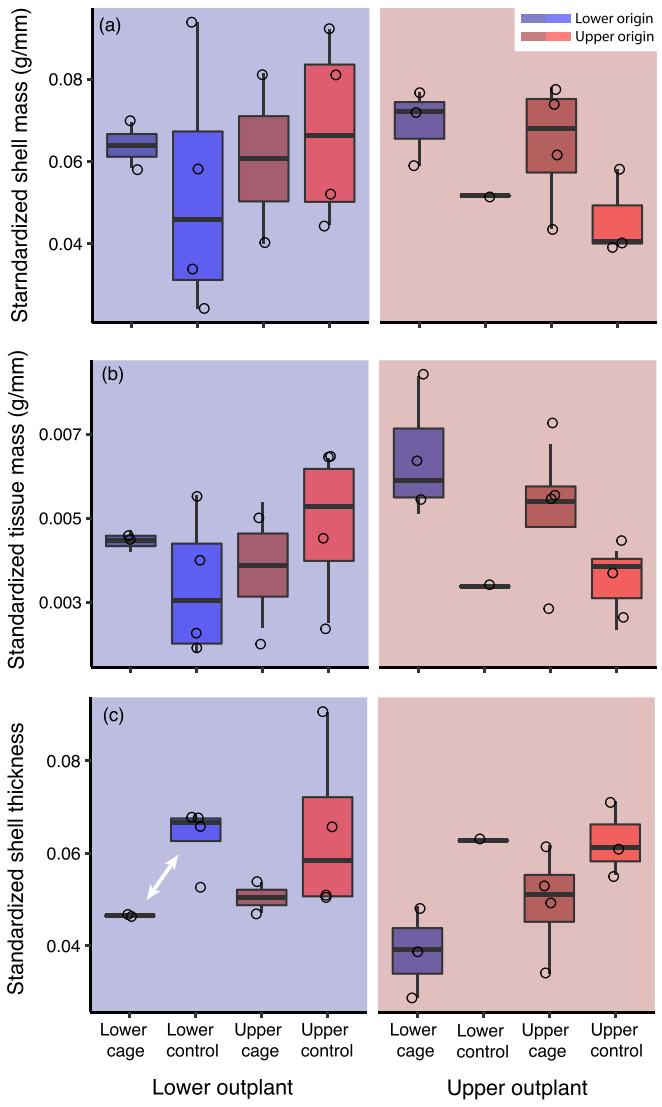

Fig. 6. Experimental results of oyster traits after nine months. Results of shell mass, tissue mass, and shell thickness are presented in panels (a), (b), and (c). Each trait was standardized by dividing by oyster length; hence standardized shell thickness is dimensionless. In all panels, results are grouped by predator-exclosure treatment on the secondary x-axis and outplant location on the primary x-axis, with Lower site results grouped to the left and Upper site results grouped to the right. In all panels, blue and red boxplots denote oysters sourced from the Lower or Upper estuary site (respectively), with darker shading further distinguishing between predator exclusion cages (darker boxplots) or exposed controls (lighter boxplots). Blue and red backgrounds further distinguish whether oysters were outplanted at the Lower (left panels, blue background) or Upper estuary site (right panels, red background). The white arrow calls attention to the strongest effect detected in this analysis, which was overall thicker shells in the cages versus controls, regardless of source deme or outplant site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

outplant location. Thicker shells are an antipredator trait (Robinson et al., 2014) so this suggests a local adaptation for early development of that trait. Oysters from the Lower estuarine deme (where conchs are not present) did not exhibit this trait. However, the evidence for local adaptation was no longer present after nine months of deployment in the field, at which point shell thickness depended only on exposure to predators and their cues, regardless of the source deme or outplant location of the oysters. This suggests that despite the initial adaptive expression of an antipredator trait by oysters from the Upper deme, oysters from the Lower estuary deme ultimately also expressed a similar trait, when induced by sustained cues of any predator.

In contrast to the anti-predator trait response, there was no evidence for corresponding adaptive responses in two components of oyster fitness (survival and growth) at either the juvenile or adult stage. Instead, variation in those fitness components was explained primarily by exposure to predation. After one month, environmental stress at both locations reduced the survival of the two oyster demes by 30%. During the same time period, the mud crab predator (ubiquitous at both sites) reduced the survival of oysters in Control treatments by an additional 20% when compared to survival in Exclosure treatments. After nine months, predation by crown conchs further exacerbated the difference in survival of adult oysters between Exclosure and Control treatments of both demes at the Upper location. Meanwhile, at the Lower location, it appears an environmental stress further reduced the survival of all adult oysters, except for those originating from the Upper deme.

Curiously, the expression of a presumably adaptive trait (thicker shell) by Upper deme juvenile oysters did not lead to a statistically detectable improvement in survival of those oysters. Because separate studies have repeatedly demonstrated that anti-predator traits such as thicker and stronger shell increase oyster survival (Robinson et al., 2014; Ponce et al., 2020), it is likely that our result represents a lack of statistical power. We found a trend towards higher survival of Upper oysters relative to Lower oysters in Control treatments in the Upper outplant location (Fig. 3a, 5a) but high variability in survival and the binomial nature of that endpoint cause us to be cautious in ascribing ecological meaning to that trend. Additional research could shed light on this detail. Another interesting result was that the increase in shell thickness after one month was not associated with a detectable cost to growth (the other fitness component we measured) as we expected according to theory of predation risk effects (Peacor et al., 2020). Rather, differences in juvenile oyster growth were driven by predator exposure, with slower growth in the exposed Control treatments regardless of source deme or outplant location.

There are two non-mutually exclusive potential explanations for the observed difference in growth between Exclosure and Control treatments after one month. On the one hand, the differences in growth could have resulted from mud crabs selectively consuming the larger and faster growing individuals in the control treatments. Alternatively, the foraging cues and tactile cues perceived by juvenile oysters in the Control treatment—not the Exclosure treatment— could have caused oysters to reduce time spent feeding with their shell open (Smee and Weissburg, 2006), or to thicken shell with a nonconsumptive cost to growth. The first and second scenarios are difficult to test without more detailed observations of crab and oyster foraging behavior, but Dodd et al. (2017) did not find support for the former explanation. The latter explanation is partially supported by the observation that towards the end of the experiment, surviving oysters exposed to predation had shells 25% thicker than those in Exclosures. In any case, when the surviving oysters reached the adult life stage, some combination of processes appear to have erased the differences in growth observed at one month. Other studies have found that mud crabs do not induce trait changes in oysters larger than 25 mm (Johnson and Smee, 2012; Scherer and Smee, 2017). This aligns with our result, though in our study oysters were also exposed to crown conchs, which preferentially feed on larger oysters and would be expected to elicit trait changes in those larger sizes. Therefore we hypothesize that oysters in the Control treatment experienced compensatory growth due to the absence of intraspecific competition for space and resources, as we observed in an experiment with simulated predator consumption in the same study system (Kimbro et al., 2020). This may also help explain the disappearance of the local-adaptation signal in shell thickness observed in juveniles.

The study sites used in this experiment were chosen to test the conclusion of a recent meta-analysis on local selection in marine systems: biotic agents of selection (e.g., predators) may be equally influential on local adaption as abiotic factors, albeit with the abiotic

variable causing spatial segregation in the two selection pressures (e.g., abiotic stresses exclude predators but not prey from certain locations; Briscoe Runquist et al., 2020). In contrast to our expectations, the experimental locations in our study were sufficiently similar in biotic and abiotic stresses on juvenile oysters that survival and growth did not substantially differ between them, thereby explaining the absence of strong signals of local adaptation in our experiment. For euryhaline species such as oysters, the environmental variability encompassed by these two experimental locations may have been insufficient to cause stress-induced variation in juvenile survival. Although we detected substantial seasonal variation in salinity at both locations – and greater variability in the Upper estuary site relative to the Lower site, reflecting the degree of ocean influence – the differences in salinity between sites across the seasons were small relative to the broad salinity tolerances of this species (Pusack et al., 2019). Perhaps variation in salinity-induced stress emerges between these two locations during years with repeated strong summer or winter storms (Bible et al. 2016). While Hurricane Dorian (landfall August 28, 2019) appears to have created spatial variation in salinity during the first month of this experiment, this difference was not sustained nor did it lower salinity below the tolerance threshold for the Eastern oyster. During non-storm years, the more important stress for juvenile survival may be aerial exposure during summer low tides. Because our study began at the end of summer when seasonality of tides results in infrequent aerial exposure of short duration in the daytime, this environmental variable did not vary substantially between our two experimental locations.

Like oysters, predatory mud crabs are euryhaline. Consequently, the observed variation in salinity between sites would be unlikely to generate variation in predation pressure and risk for juvenile crabs between our two locations (sensu Menge and Sutherland, 1987). However, the observed environmental variation does maintain a spatial difference in the abundance of a voracious predator of adult oysters, the crown conch (Garland and Kimbro, 2015); this follows the prediction by Hays et al., 2021 regarding the interaction between abiotic and biotic selection factors. As a result, we predicted that the excessive predation pressure by crown conchs on adult oysters at the Upper deme and the previously observed trait and fitness (growth) response by juvenile oysters to cues produced by the conch (Kimbro et al., 2020) may result in a post-settlement local adaptation signal. This prediction was met in part, though contrary to our expectation, the signal of local adaptation (thicker shell) was only expressed in the presence of predators and their cues, indicating that even this potentially locally adapted trait has plastic expression. It is also unclear why an adaptive response to potential predation by crown conch (the presence of which being the main difference between the Upper and Lower sites) would be elicited by the presence of mud crab predators, but not conchs, in the Lower outplants. Cues from both species produced similar changes in juvenile oyster growth in a prior study (Kimbro et al., 2020) so perhaps oysters cannot differentiate them and/or they are responding more to preyed-upon conspecifics. In other study systems, such as metamorphosing amphibians, cues from predators on different prey life stages can elicit complex and interacting effects on trait expression in early life stages (Vonesh and Warkentin, 2006), so perhaps there is more to be learned about the effects of different predator cues on juvenile oyster trait expression.

We intended this study to be an initial test of local adaptation in a marine invertebrate with high dispersal potential within an estuarine system, so it could be improved and extended in several ways. First, because the experimental oysters were collected from natural oyster beds, the study had to focus on the juvenile stage (5 mm – 15 mm) rather than on a smaller post-settlement stage (0–5 mm). In laboratory experiments, the smaller size classes demonstrated stronger responses to predation risk (Robinson et al., 2014). In addition, results from the natural cohort of oysters we collected for transplant may already have been influenced by environmental conditions they experienced during the pre-settlement pelagic stage and post-settlement benthic stage (just prior to collection). Specifically, the shell thickening response by Upper deme oysters at the Lower location may have been a continued plastic response by the oysters to predatory conch cues at their natal location prior to our collection. However, in a prior study, naive, hatchery-reared juvenile oysters exhibited approximately equal trait responses to mud crab cues and conch cues in the same study locations (Kimbro et al., 2020). That finding leads us to believe that the differentially greater trait response of transplanted Upper deme juvenile oysters (relative to Lower deme oysters) in the present study reflects local adaptation to a higher-predation environment and response to the local crab cue, rather than a carryover response to the conch cue. Any future study should also evaluate how predation risk influences the actual strength of oyster shell (as measured by crushing force), which may be a more reliable metric than shell thickness for the benefit oysters gain when allocating resources to defense versus growth and reproduction (Scherer et al., 2018). Our reciprocal transplant experiment also only promoted a home-away comparison between two demes, which does not account for variability among demes in the general area close to freshwater input and the general area far from freshwater input. Consequently, a future effort should work with oysters spawned and settled in the laboratory in order to diminish the influence of previous environmental conditions and to produce sufficient numbers of small, post-settlement oysters (0–5 mm length). Additionally, it would be preferable to sample replicated demes within each set of environmental conditions (Blanquart et al., 2013). Finally, what additional treatments would be needed to discern abiotic from predation effects and then disentangle the different effects of predators (e.g. consumptive versus non-consumptive effects), across multiple replicate demes? One key element would be to include treatments that include simulated consumptive effects in both the presence and absence of predator cues, as proposed by (Abrams, 2008); see Kimbro et al. (2020) for an example of this design. Additionally, coupling this work with efforts to document the scale of gene flow (e.g., estimating migration rates using neutral alleles, and/or via simulations of larval transport in circulation models; e.g., Baums et al., 2005) would allow us to compare the strength of selection to the rate of gene flow (Kawecki and Ebert, 2004). The complexity of that type of study is daunting, but given the growing number of examples of local adaptation in marine species with putatively high dispersal potential (e.g., De Wit and Palumbi, 2013; Pespeni and Palumbi, 2013), it would be a worthwhile test of our understanding of the evolutionary ecology of these systems.

The balance between selection for fine-scaled local adaptations and broadly plastic traits is expected to vary in marine systems depending on the strength of selection and dispersal potential (Sotka and Palumbi, 2006). In our study system, abiotic stress (varying salinity) did not directly produce selection gradients on oysters, but indirectly affects the abundance of a key oyster predator. That spatial difference in predation risk appears to have, in turn, selected for increased expression of an antipredator trait at the riskier site. However, we did not detect either an energetic cost of expressing that trait or positive benefits for the fitness components of those oysters. Thus, despite evidence that some oyster traits are structured by adaptation to a fixed gradient of predation risk, this system appears to be largely driven by plastic responses to predation risk that vary over the ontogeny of oysters and with a greater overall effect of predator consumption than non-consumptive trait effects (Kimbro et al., 2020). Finally, it is important to consider that the selective forces operating during any short-term experiment may not reflect the varied history of past selection, including extreme events or shifts in estuarine circulation; the dynamic nature of many estuaries may further drive selection towards plastic trait responses.

Author contributions

D.L.K conceived idea of study; D.L.K, A.B-P, A.N., and N·P designed the experiment; D.L.K. and J.W.W. analyzed the data; A.B-P, C.C., N.P., A.N, and D.L.K collected the data; D.L.K and J.W.W. co-wrote the manuscript with contributions from all other co-authors.

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2022.151784.

References

Abrams, P.A., 2008. Measuring the impact of dynamic antipredator traits on predator–prey–resource interactions. Ecology 89, 1640–1649. https://doi.org/10.1890/07-0764.1.

Adrian, A.J., Lack, C.E., Kamel, S.J., 2017. Kin aggregations occur in eastern oyster Crassostrea virginica reefs despite limited regional genetic differentiation. Mar. Ecol. Prog. Ser. 584, 79–90. https://doi.org/10.3354/meps12361.

Almany, G.R., Hamilton, R.J., Bode, M., Matawai, M., Potuku, T., Saenz-Agudelo, P., Planes, S., Berumen, M.L., Rhodes, K.L., Thorrold, S.R., Russ, G.R., Jones, G.P., 2013. Dispersal of grouper larvae drives local resource sharing in a coral reef fishery. Curr. Biol. 23, 626–630. https://doi.org/10.1016/j.cub.2013.03.006.

Baums, I.B., Miller, M.W., Hellberg, M.E., 2005. Regionally isolated populations of an imperiled Caribbean coral, Acropora palmata. Mol. Ecol. 14, 1377–1390. https://doi.org/10.1111/j.1365-294X.2005.02489.x.

Becker, B.J., Fodrie, F.J., McMillan, P.A., Levin, L.A., 2005. Spatial and temporal variation in trace elemental fingerprints of mytilid mussel shells: a precursor to invertebrate larval tracking. Limnol. Oceanogr. 50, 48–61. https://doi.org/10.4319/lo.2005.50.1.0048.

Bible, J.M., Sanford, E., 2016. Local adaptation in an estuarine foundation species: implications for restoration. Biol. Conserv. 193, 95–102. https://doi.org/10.1016/j.biocon.2015.11.015.

Blanquart, F., Kaltz, O., Nuismer, S.L., Gandon, S., 2013. A practical guide to measuring local adaptation. Ecol. Lett. 16, 1195–1205. https://doi.org/10.1111/ele.12150.

Bohonak, A.J., 1999. Dispersal, gene flow, and population structure. Q. Rev. Biol. 74, 21–45. https://doi.org/10.1086/392950.

Booth, H.S., Pusack, T.J., White, J.W., Stallings, C.D., Kimbro, D.L., 2018. Intraspecific predator inhibition, not a prey size refuge, enables oyster population persistence during predator outbreaks. Mar. Ecol. Prog. Ser. 602, 155–167. https://doi.org/10.3354/meps12704.

Briscoe Runquist, R.D., Gorton, A.J., Yoder, J.B., Deacon, N.J., Grossman, J.J., Kothari, S., Lyons, M.P., Sheth, S.N., Tiffin, P., Moeller, D.A., 2020. Context dependence of local adaptation to abiotic and biotic environments: a quantitative and qualitative synthesis. Am. Nat. 195, 412–431. https://doi.org/10.1086/707322.

Burford, M.O., Scarpa, J., Cook, B.J., Hare, M.P., 2014. Local adaptation of a marine invertebrate with a high dispersal potential: evidence from a reciprocal transplant experiment of the eastern oyster Crassostrea virginica. Mar. Ecol. Prog. Ser. 505, 161–175. https://doi.org/10.3354/meps10796.

Connell, J.H., 1961. The influence of interspecific competition and other factors on the distribution of the barnacle Chthamalus Stellatus. Ecology 42, 710–723. https://doi.org/10.2307/1933500.

D'Aloia, C.C., Bogdanowicz, S.M., Francis, R.K., Majoris, J.E., Harrison, R.G., Buston, P.M., 2015. Patterns, causes, and consequences of marine larval dispersal. Proc. Natl. Acad. Sci. 112, 13940–13945. https://doi.org/10.1073/pnas.1513754112.

De Wit, P., Palumbi, S.R., 2013. Transcriptome-wide polymorphisms of red abalone (Haliotis rufescens) reveal patterns of gene flow and local adaptation. Mol. Ecol. 22, 2884–2897. https://doi.org/10.1111/mec.12081.

Fodrie, F.J., Rodriguez, A.B., Baillie, C.J., Brodeur, M.C., Coleman, S.E., Gittman, R.K., Keller, D.A., Kenworthy, M.D., Poray, A.K., Ridge, J.T., Theuerkauf, E.J., Lindquist, Niels L., 2014. Classic paradigms in a novel environment: inserting food web and productivity lessons from rocky shores and saltmarshes into biogenic reef restoration. J. Appl. Ecol. 51, 1314–1325. https://doi.org/10.1111/1365-2664.12276.

Garland, H.G., Kimbro, D.L., 2015. Drought increases consumer pressure on oyster reefs in Florida, USA. PLoS One 10, e0125095. https://doi.org/10.1371/journal.pone.0125095.

Gosnell, J.S., Spurgin, K., Levine, E.A., 2017. Caged oysters still get scared: predator presence and density influence growth in oysters, but only at very close ranges. Mar. Ecol. Prog. Ser. 568, 111–122. https://doi.org/10.3354/meps12070.

Grabowski, J.H., Gouhier, T.C., Byers, J.E., Dodd, L.F., Hughes, A.R., Piehler, M.F., Kimbro, D.L., 2020. Regional environmental variation and local species interactions influence biogeographic structure on oyster reefs. Ecology 101, e02921. https://doi.org/10.1002/ecy.2921.

Hanley, T.C., Hughes, A.R., Williams, B., Garland, H., Kimbro, D.L., 2016. Effects of intraspecific diversity on survivorship, growth, and recruitment of the eastern oyster across sites. Ecology 97, 1518–1529. https://doi.org/10.1890/15-1710.1.

Hays, C.G., Hanley, T.C., Hughes, A.R., Truskey, S.B., Zerebecki, R.A., Sotka, E.E., 2021. Local adaptation in marine foundation species at microgeographic scales. Biol. Bull. 241, 16–29. https://doi.org/10.1086/714821.

Hellberg, M.E., Burton, R.S., Neigel, J.E., Palumbi, S.R., 2002. Genetic assessment of connectivity among marine populations. Bull. Mar. Sci. 70, 273–290.

Hughes, A.R., Hanley, T.C., Byers, J.E., Grabowski, J.H., Malek, J.C., Piehler, M.F., Kimbro, D.L., 2017. Genetic by environmental variation but no local adaptation in oysters (*Crassostrea virginica*). Ecol. Evol. 7, 697–709. https://doi.org/10.1002/ece3.2614.

Johnson, K.D., Smee, D.L., 2012. Size matters for risk assessment and resource allocation in bivalves. Mar. Ecol. Prog. Ser. 462, 103–110.

Johnson, K.D., Smee, D.L., 2014. Predators influence the tidal distribution of oysters (Crassostrea virginica). Mar. Biol. 161, 1557–1564. https://doi.org/10.1007/s00227-014-2440-8.

Jones, G.P., Almany, G.R., Russ, G.R., Sale, P.F., Steneck, R.S., van Oppen, M.J.H., Willis, B.L., 2009. Larval retention and connectivity among populations of corals and reef fishes: history, advances and challenges. Coral Reefs 28, 307–325. https://doi.org/10.1007/s00338-009-0469-9.

Kawecki, T.J., Ebert, D., 2004. Conceptual issues in local adaptation. Ecol. Lett. 7, 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x.

Kimbro, D.L., Grabowski, J.H., Hughes, A.R., Piehler, M.F., White, J.W., 2017a. Nonconsumptive effects of a predator weaken then rebound over time. Ecology 98, 656–667. https://doi.org/10.1002/ecy.1702.

Kimbro, D.L., White, J.W., Tillotson, H., Cox, N., Christopher, M., Stokes-Cawley, O., Yuan, S., Pusack, T.J., Stallings, C.D., 2017b. Local and regional stressors interact to drive a salinization-induced outbreak of predators on oyster reefs. Ecosphere 8, e01992. https://doi.org/10.1002/ecs2.1992.

Kimbro, D.L., Tillotson, H.G., White, J.W., 2020. Environmental forcing and predator consumption outweigh the nonconsumptive effects of multiple predators on oyster reefs. Ecology 101, e03041. https://doi.org/10.1002/ecy.3041.

Livingston, R.J., Lewis, F.G., Woodsum, G.C., Niu, X.-F., Galperin, B., Huang, W., Christensen, J.D., Monaco, M.E., Battista, T.A., Klein, C.J., Howell, R.L., Ray, G.L., 2000. Modelling oyster population response to variation in freshwater input. Estuar. Coast. Shelf Sci. 50, 655–672. https://doi.org/10.1006/ecss.1999.0597.

Long, J.D., Hay, M.E., 2012. The impact of trait-mediated indirect interactions in marine communities. In: Ohgushi, T., Schmitz, O., Holt, R.D. (Eds.), Trait-Mediated Indirect Interactions: Ecological and Evolutionary Perspectives. Cambridge University Press, Cambridge UK, pp. 47–68.

Maynard, A., Bible, J.M., Pespeni, M.H., Sanford, E., Evans, T.G., 2018. Transcriptomic responses to extreme low salinity among locally adapted populations of Olympia oyster (Ostrea lurida). Mol. Ecol. 27, 4225–4240. https://doi.org/10.1111/mec.14863.

Menge, B.A., Sutherland, J.P., 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. Am. Nat. 130, 730–757. https://doi.org/10.1086/284741.

Morgan, S.G., Fisher, J.L., Miller, S.H., McAfee, S.T., Largier, J.L., 2009. Nearshore larval retention in a region of strong upwelling and recruitment limitation. Ecology 90, 3489–3502. https://doi.org/10.1890/08-1550.1.

Munday, P.L., Eyre, P.J., Jones, G.P., 2003. Ecological mechanisms for coexistence of colour polymorphism in a coral-reef fish: an experimental evaluation. Oecologia 137, 519–526. https://doi.org/10.1007/s00442-003-1356-7.

Paris, C.B., Cowen, R.K., 2004. Direct evidence of a biophysical retention mechanism for coral reef fish larvae. Limnol. Oceanogr. 49, 1964–1979. https://doi.org/10.4319/lo.2004.49.6.1964.

Peacor, S.D., Barton, B.T., Kimbro, D.L., Sih, A., Sheriff, M.J., 2020. A framework and standardized terminology to facilitate the study of predation-risk effects. Ecology 101, e03152. https://doi.org/10.1002/ecy.3152.

Pespeni, M.H., Palumbi, S.R., 2013. Signals of selection in outlier loci in a widely dispersing species across an environmental mosaic. Mol. Ecol. 22, 3580–3597. https://doi.org/10.1111/mec.12337.

Ponce, M., Belgrad, B.A., Walton, W., Smee, D.L., 2020. Hatchery exposure of oyster spat to different predators strengthens oyster shells. Gulf Caribbean Res. 31. SC36 – SC40.

Powell, E., Klinck, J., Hofmann, E., 1996. Modeling diseased oyster populations. II. Triggering mechanisms for Perkinsus marinus epizootics. J. Shellfish Res. 15.

Pusack, T.J., Kimbro, D.L., White, J.W., Stallings, C.D., 2019. Predation on oysters is inhibited by intense or chronically mild, low salinity events. Limnol. Oceanogr. 64, 81–92. https://doi.org/10.1002/lno.11020.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.

Richardson, J.L., Urban, M.C., Bolnick, D.I., Skelly, D.K., 2014. Microgeographic adaptation and the spatial scale of evolution. Trends Ecol. Evol. 29, 165–176. https://doi.org/10.1016/j.tree.2014.01.002.

Robinson, E.M., Lunt, J., Marshall, C.D., Smee, D.L., 2014. Eastern oysters Crassostrea virginica deter crab predators by altering their morphology in response to crab cues. Aquat. Biol. 20, 111–118. https://doi.org/10.3354/ab00549.

Sanford, E., Roth, M.S., Johns, G.C., Wares, J.P., Somero, G.N., 2003. Local selection and latitudinal variation in a marine predator-prey interaction. Science 300, 1135–1137. https://doi.org/10.1126/science.1083437.

Scherer, A.E., Smee, D.L., 2017. Eastern oysters *Crassostrea virginica* may produce plastic morphological defenses in response to crab predators despite resource limitation. Biol. Bull. 233, 144–150. https://doi.org/10.1086/695470.

Scherer, A.E., Bird, C., McCutcheon, M., Hu, X., Smee, D.L., 2018. Two-tiered defense strategy may compensate for predator avoidance costs of an ecosystem engineer. Mar. Biol. 165, 131. https://doi.org/10.1007/s00227-018-3391-2.

Shanks, A.L., 2009. Pelagic larval duration and dispersal distance revisited. Biol. Bull. 216, 373–385. https://doi.org/10.1086/BBLv216n3p373.

Sheng, Y.P., Tutak, B., Davis, J.R., Paramygin, V., 2008. Circulation and Flushing in the Lagoonal system of the Guana Tolomato Matanzas National Estuarine Research Reserve (GTMNERR), Florida. J. Coast. Res. 9–25. https://doi.org/10.2112/SI55-002.1.

Smee, D.L., Weissburg, M.J., 2006. Hard clams (Mercenaria mercenaria) evaluate predation risk using chemical signals from predators and injured conspecifics. J. Chem. Ecol. 32, 605–619. https://doi.org/10.1007/s10886-005-9021-8.

Sotka, E.E., Palumbi, S.R., 2006. The use of genetic clines to estimate dispersal distances of marine larvae. Ecology 87, 1094–1103. https://doi.org/10.1890/0012-9658(2006)87[1094:TUOGCT]2.0.CO;2.

Swearer, S.E., Caselle, J.E., Lea, D.W., Warner, R.R., 1999. Larval retention and recruitment in an island population of a coral-reef fish. Nature 402, 799–802. https://doi.org/10.1038/45533.

Urban, M.C., Strauss, S.Y., Pelletier, F., Palkovacs, E.P., Leibold, M.A., Hendry, A.P., De Meester, L., Carlson, S.M., Angert, A.L., Giery, S.T., 2020. Evolutionary origins for ecological patterns in space. Proceed. National Acad. Sci., USA 117, 17482–17490.

Vonesh, J.R., Warkentin, K.M., 2006. Opposite shifts in size at metamorphosis in response to larval and Metamorph predators. Ecology 87, 556–562. https://doi.org/10.1890/05-0930.

Waples, R., 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J. Hered. 89, 438–450. https://doi.org/10.1093/jhered/89.5.438.

Warner, R.R., 1997. Evolutionary ecology: how to reconcile pelagic dispersal with local adaptation. Coral Reefs 16, S115–S120. https://doi.org/10.1007/s003380050247.

White, J.W., Carr, M.H., Caselle, J.E., Washburn, L., Woodson, C.B., Palumbi, S.R., Carlson, P.M., Warner, R.R., Menge, B.A., Barth, J.A., Blanchette, C.A., Raimondi, P. T., Milligan, K., 2019. Connectivity, dispersal, and recruitment: connecting benthic communities and the coastal ocean. Oceanography 32, 50–59.