

Environmental gradients influence biogeographic patterns of nonconsumptive predator effects on oysters

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Abstract. When prey alter behavioral or morphological traits to reduce predation risk, they often incur fitness costs through reduced growth and reproduction as well as increased mortality that are known as nonconsumptive effects (NCEs). Environmental context and trophic structure can individually alter the strength of NCEs, yet the interactive influence of these contexts in natural settings is less understood. At six sites across 1000 km of the Southeastern Atlantic Bight (SAB), we constructed oyster reefs with one, two, or three trophic levels and evaluated the traits of focal juvenile oysters exposed to predation risk cues. We monitored environmental variables (water flow velocity, microalgal resources, and oyster larval recruitment) that may have altered how oysters respond to risk, and we also assessed the cost of trait changes to oyster mortality and growth when they were protected from direct predatory loss. Regardless of trophic structure, we found that oyster shell strength and natural oyster recruitment peaked at the center of the region. This high recruitment negated the potential for NCEs by smothering and killing the focal oysters. Also independent of trophic structure, focal oysters grew the most at the northernmost site. In contrast to, and perhaps because of, these strong environmental effects, the oyster traits of condition index and larval recruitment were only suppressed by the trophic treatment with a full complement of risk cues from intermediate and top predators at just the southernmost site. But at this same site, statistically significant NCEs on oyster growth and mortality were not detected. More strikingly, our study demonstrated environmental gradients that differentially influence oysters throughout the SAB. In particular, the results of our trophic manipulation experiment across these gradients suggest that in the absence of predation, environmental differences among sites may overwhelm the influence of NCEs on prey traits and population dynamics.

Key words: anti-predator response; biogeography; context dependent; density-mediated effect; environmental gradient; intraspecific competition; larval recruitment; predation risk; predator cue; resource abundance; trait-mediated effect.

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INTRODUCTION

When prey perceive the risk of being consumed by predators, they often display plastic anti-predator traits such as seeking refuge or thickening protective shell armor (Werner and Hall 1988, Trussell 1996). While these anti-predator responses can reduce the probability of being consumed, they are often associated with fitness costs to the prey through reduced growth and reproduction as well as increased mortality (non-predation-related); these costs have been labeled nonconsumptive effects (NCE) of predators (Peckarsky et al. 2008). Because the NCEs and consumptive effects (CEs) of predators can lead to different outcomes for prey, their resources, and ecosystem functioning (Schmitz 2008), the frequency of research devoted to detecting NCEs and identifying when, where, and why they are important continues to grow (Preisser et al. 2005, Peacor et al. 2020).

While NCEs have the potential to structure ecological communities (Lima 1998), their occurrence and magnitude are context dependent (Werner and Peacor 2003, Chamberlain et al. 2014). One essential component of this context dependency is the trophic structure of food webs, such as lengthening a food chain by adding a higher-order predator, which can alter interactions between predator and prey on lower trophic levels. For example, on rocky shorelines, carnivorous snails (*Nucella lapillus*) induce herbivorous snails (*Littorina littorea*) to thicken their shell without a cost in growth because predation of the omnivorous *Nucella* on barnacles (*Semibalanus balanoides*) also opens limited substrate for algal resources of the herbivorous *Littorina* (Trussell et al. 2017). However, this balance between the negative and positive influences of *Nucella* on *Littorina* growth is disrupted by a higher-order omnivorous predator, the European green crab (*Carcinus maenas*). Although the combined risk of the crab and *Nucella* does not cause *Littorina* to thicken their shell more than in the presence of *Nucella* alone, the enhanced risk does cause the initial thickening of shell to incur a fitness cost of reduced growth (Trussell et al. 2017).

Regardless of food chain length, NCEs also likely depend on environmental factors such as whether prey perceive a risk signal above ambient background noise (Smee and Weissburg

2006, Weissburg et al. 2014). For instance, birds are less responsive to predator movements in windy environments that increase the movement of non-threatening objects (Carr and Lima 2010). Even when prey respond to risk cues that exceed background noise, the demographic costs of these responses can be influenced by other environmental contexts, such as resource availability. In a meta-analysis of experiments from a diversity of systems and taxa, low resource conditions generally inhibited prey from compensating for the energetic costs of anti-predator responses, leading to proportionally larger costs on growth than in high resource conditions (Preisser and Bolnick 2008, Preisser et al. 2009; but see Werner and Peacor 2006). Over time, this combination of risk and limited resources is predicted to cause stronger NCEs on prey fecundity and/or mortality (Nelson et al. 2004, McCauley et al. 2011). Thus, accurately predicting NCEs may first require identifying the relevant resource gradients in a particular food web.

Resource availability as an influential context of NCEs could be counterbalanced by variation in prey recruitment provided that predation risk does not inhibit the colonization of prey, as demonstrated by barnacle larvae avoiding shorelines with predatory snails during settlement (Ellrich et al. 2015) and tree frogs evading ponds with predatory salamanders during oviposition (Resetarits and Wilbur 1989). For the predator-prey dynamic between predatory dragonfly larvae (*Anax junius*) and tadpoles of the wood frog (*Rayna salvatica*), increasing tadpole recruitment counterbalances a pre-existing NCE on tadpole growth by increasing intraspecific competition among tadpoles for limited resources (Relyea 2004). However, a large influx of prey recruits could also swamp any NCEs on growth if the ensuing interference competition among prey leads to mortality as observed within populations of isopods (Grosholz 1992), larval amphibians (Pfenning and Collins 1993), and barnacles (Bertness 1989). Thus, the effects of predators on the traits and fitness of prey in natural settings are most likely influenced by the interplay of variability in resources and prey recruitment (Peacor and Werner 2003).

Three decades of research on predation risk has overwhelmingly demonstrated how individual environmental contexts and trophic structure

can dictate the outcome of NCEs. Therefore, it is imperative to build on this work by evaluating if environmental and trophic contexts interactively influence the strength and direction of NCEs in natural settings. Here, we ask: How do the environmental contexts of flow, resource availability, and prey recruitment vary biogeographically, and how do they interact with trophic structure to influence the direction and magnitude of NCEs on the eastern oyster (*Crassostrea virginica*)? To address these questions, we manipulated trophic structure on experimental oyster reefs across 1000 km of the Southeastern Atlantic Bight (SAB; Fig. 1). At each site, we created replicate experimental oyster reefs with one, two, or three trophic levels. We then evaluated the trait responses of juvenile oysters exposed to risk cues—but protected from predation—and measured whether these responses were associated with a fitness cost (NCE) in oyster mortality and growth. We also measured microalgal resources available to the oysters, oyster recruitment, and water flow velocity at each site, because they might alter how oysters perceive and respond to risk (Fig. 1). Overall, we sought to determine the amount of variation in trait and fitness responses of prey explained by trophic structure and environment (site).

The eastern oyster is an ideal system for this work. A well-studied trophic cascade operates in this habitat in estuaries of the U.S. Atlantic and Gulf coasts: Toadfish (*Opsanus tau*) and blue crab (*Callinectes sapidus*) predators on the third trophic level alter the foraging behavior of small xanthid crabs (*Panopeus herbstii*, hereafter mud crab) on the second trophic level, leading to lower oyster mortality at the basal trophic level (Grabowski 2004, Grabowski and Kimbro 2005, Kimbro et al. 2014, 2017). Meanwhile, chemical cues from mud crabs cause juvenile oysters to produce thicker shell that helps reduce predation (CE) at a cost (NCE) to the oyster in reduced soft-tissue somatic growth (Johnson and Smee 2012, Robinson et al. 2013, Scherer et al. 2016). But predictions about the degree to which NCEs of mud crabs on oysters are influenced by trophic structure (i.e., presence/absence of toadfish and blue crabs) remain much less developed. In particular, blue crabs also consume juvenile oysters (Grabowski et al. 2008, Byers et al. 2017) and cause oysters to produce thicker shell at a cost in

reduced growth (Scherer et al. 2016). Consequently, while blue crabs may indirectly benefit a population of oysters by reducing mud crab foraging (Grabowski et al. 2008), the co-occurrence of blue crab and mud crab cues may actually increase the risk perceived by individual oysters. Quantification of this context dependency is particularly relevant, because oyster food webs in the SAB likely occur across strong environmental gradients that may dictate risk perception, availability of microalgal resources, and/or recruitment of oyster larvae (Kimbrow et al. 2014, Byers et al. 2015; Fig. 1).

METHODS

This biogeographic experiment has been described in a previous publication (Kimbrow et al. 2014) that focused on how the density and behavior of intermediate predators (mud crabs and oyster drills) were influenced by top predators (toadfish and blue crab). We also previously evaluated how the effects of top predators on mud crab foraging rate indirectly cascaded to influence oyster mortality (i.e., mud crab CE on oysters; Kimbro et al. 2014). Here, we present unpublished results from the same experiment examining the degree to which trophic structure and environmental gradients (microalgal resource supply, oyster recruitment, and water flow velocity) interactively determine how strictly non-lethal predation risk affects oyster traits and fitness.

Trophic manipulation

In June 2011, we selected six intertidal locations at least 50 km apart across 1000 km of the SAB (Fig. 1). At each site, we established nine circular plots (2.5 m diameter) separated by 3 m of mudflat. A mesh cage (6 × 6 mm openings) was buried 0.5 m into the sediment around each plot and covered with a mesh top (19 × 19 mm). The cage was anchored in place by six 1.5-m rebar poles hammered to a depth of 1.0 m around the cage's perimeter. Within each cage, we created circular oyster reefs (1.5 m diameter) consisting of a layer of dead oyster shell (one bushel, volume = 35.2 L) covered by three bushels of living oyster clusters (cluster biomass = 200–400 g).

Within each site, the nine reefs were randomly assigned among three trophic structure

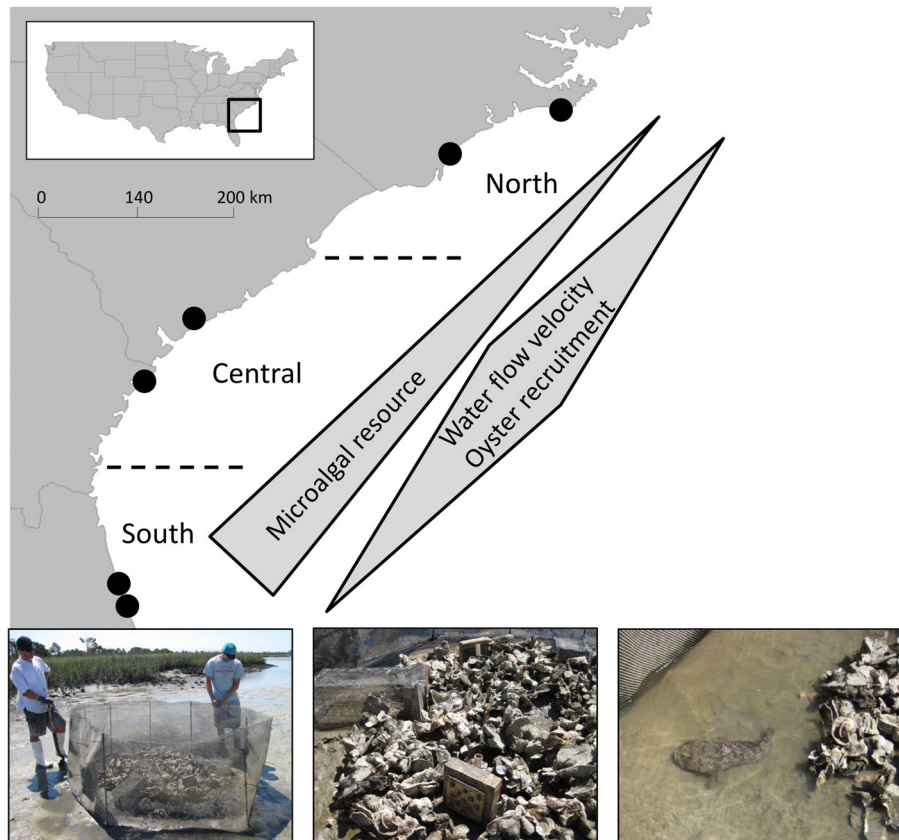


Fig. 1. Map of study locations throughout the Southeastern Atlantic Bight illustrating experimental sites (circles) and gradients in microalgal resource supply, water flow velocity, and oyster recruitment. Bottom images illustrate experimental oyster reefs within field enclosures at each site.

treatments: (1) basal bivalve prey (oysters and clams; one trophic level); (2) intermediate predators (oyster drills and mud crabs) and basal prey (two trophic levels); (3) top (toadfish and blue crab) and intermediate predators with basal prey (three trophic levels). All organisms were collected at each site. Reefs assigned to two trophic levels were stocked with 35 adult mud crabs (carapace width >20 mm) and 12 adult oyster drills (length >25 mm). Reefs assigned to three trophic levels were stocked with one toadfish (length >150 mm) and one male blue crab (carapace width >100 mm), as well as with the 35 mud crabs and 12 oyster drills. These organismal densities reflect mean values observed on natural reefs throughout the SAB (Kimbrow et al. 2014, Grabowski et al. 2020). Because of the anticipated arrival of Hurricane Irene, we ended this experiment after 90 d.

To examine how the risk cues associated with trophic structure and environment influenced oyster traits and fitness, we purchased juvenile oysters (mean size = 8 mm) from a hatchery in Florida, USA. Using marine epoxy, we attached 12 juvenile oysters to ceramic tiles (10 × 10 cm) and then used aquarium-safe silicone to attach tiles on concrete pavers (12 × 12 cm). Three tiles were deployed equidistantly around the perimeter of each reef, and each tile was enclosed by a small mesh cage (6 × 6 mm openings) to expose oysters to predator cues and tiles to recruitment of natural oysters without allowing predators to consume the oysters. Meanwhile, outside of these secondary protective cages, full interactions (CEs + NCEs) among organisms from the three trophic levels were possible (when top and intermediate predators were present) on the remaining portion of the experimental reef.

Trait responses to risk

After 90 d, we recovered the tiles from each reef and assessed two trait responses of the juvenile oysters (hereafter, focal oysters). First, in the laboratory, the focal oysters were removed from the tile and haphazardly assigned to either be processed for condition index or shell strength. Due to the destructive methods associated with each measurement, the same oyster could not simultaneously be evaluated for both responses. Shell strength was measured using the intact right valve of each focal oyster, which was disarticulated and cleaned of fouling and soft tissue prior to measurement. The strength of each shell was approximated by using a force probe (Kistler 5995 and 9222) to apply increasing pressure to the center of the articulated right valve until structural failure occurred, at which point the maximum force exerted was recorded. Based on preliminary data, oysters with diameter <1.3 cm were measured using a Kistler 9203 force probe, which has greater sensitivity (47.8 picocolumb per mechanical unit, pC/MU) but smaller range (50 pC/MU). Oysters with diameter ≥ 1.3 cm were measured using the Kistler 9222 force probe with sensitivity 19 and a greater range of 2000 (pC/MU). Crushing force was measured with amplifier (5995), which converts pC/MU of crushing force to Newtons (N) of crushing force (sensu Robinson et al. 2013). To standardize this response for differences in final oyster size among sites and trophic structure treatments, we divided the crushing force (N) by shell diameter (cm).

Second, we quantified the condition index of the remaining focal oysters, which illustrates the degree to which an oyster allocates energy to the production of tissue vs. shell mass (grams). Oysters typically allocate a higher proportion of their energy to shell production when exposed to predation risk. The calculation of a condition index followed the protocol of Johnson and Smee (2012), with the entire oyster being placed in a drying oven at 70°C for 24 h and then weighed to estimate the combined weight of tissue and shell mass. Next, oysters were placed in a muffle furnace at 525°C for two hours and re-weighed to estimate shell mass. By subtracting an oyster's shell mass (second weight) from the combined mass of its tissue and shell (first weight), we estimated oyster tissue mass. Condition index of an

oyster was calculated as its ratio of tissue to shell mass.

Additionally, to determine how trophic structure and environment influenced local and biogeographic-scale patterns of oyster larval recruitment, we quantified the density of natural oysters that recruited to each experimental tile at the conclusion of the experiment, generating a trait response of a cohort of juvenile prey to risk. To account for differences in study duration among sites, we divided the number of recruits at each site by the associated duration of the experiment ($\text{no.} \times 0.01 \text{ m}^{-2} \times \text{day}^{-1}$).

Fitness consequences (NCEs) of risk

To quantify whether trait responses of oysters to risk are associated with fitness costs (NCEs), we quantified the number of focal oysters that died and growth (initial length subtracted from final length) of the surviving focal oysters at the end of the experiment. Although we expected most of the results to occur on growth, it is possible for chronic risk-induced stress to increase prey mortality. Therefore, we also quantified prey mortality as evidence for a NCE on prey. Because these focal oysters were protected by cages from being consumed, differences in mortality and/or growth among treatments were inferred to represent NCEs of predators. To account for slight differences in the study duration among sites, we divided the mortality and growth results at each site by the duration of the associated experiment ($\text{no. deceased oysters} \times \text{days}^{-1}$ and $\text{cm} \times \text{days}^{-1}$, respectively).

Environmental gradients in water flow rate, oyster recruitment, and resource supply

Based on previously published and unpublished research, we expected that the six experimental sites would span three important environmental contexts (Fig. 1). First, we expected the sites to span a unimodal abiotic gradient in water flow across latitude with a peak in velocity at the central SAB sites. As explained in Byers et al. (2015), tidal amplitudes at the central sites exceed the tidal amplitudes at the northern and southern sites due to the geomorphology of the coastline. For a given inundation period, higher tidal amplitude implies more water volume delivery per unit time (i.e., higher flow and energy; Byers et al. 2015). Because velocity is

inversely proportional to cross-sectional area, the translation of tidal amplitude to water flow assumes equal cross-sectional areas across the biogeographic range. We tested this assumption by quantifying the cross-sectional area of the water column seaward of five oyster reefs within ten estuaries across the SAB (50 total reef locations). These sites were part of a biogeographic assessment of oyster reef communities described in Byers et al. (2015) and Grabowski et al. (2020). ArcGIS Pro v. 2.5 software by Esri was used to analyze tidal channel width by measuring the distance (meters) perpendicular from reef edge to the nearest intertidal polygon feature in the National Wetlands Inventory (<https://www.fws.gov/wetlands/Data/State-Downloads.html>). Also moving at a perpendicular angle away from the reef edge, we determined the maximum depth (meters) of water intersecting the tidal channel using data from Navionics SonarChart by Garmin (<https://www.navionics.com/usa/charts/features/sonarchart>). Next, the cross-sectional area estimate (maximum depth \times channel width) associated with each reef was multiplied by the predicted tidal amplitude (meters) for that estuary (Egbert and Erofeeva 2002) to generate an estimate of volumetric flow rate (volume $\text{m}^3 \times \text{time}^{-1}$) for a six-hour tidal cycle.

The second expected environmental context concerned a unimodal gradient in oyster recruitment across latitude, with highest recruitment at the central sites (Byers et al. 2015). In this study, we quantified oyster recruitment to the experimental tiles to contemporaneously measure the pattern in recruitment. Lastly, unpublished results from the Grabowski et al. (2020) biogeographic assessment of oyster reef communities revealed a north–south gradient in benthic chlorophyll *a* (Fig. 1; see Appendix S1 for full methods and results), which was used as a proxy for benthic microalgae, an important contributor to oyster diet (Fukumoria et al. 2008, Abeels et al. 2012). To test further whether microalgal resources increase with decreasing latitude, we collected triplicate 250 mL water samples at each site at the beginning and end of the experiment. Samples were collected 0.5 m below the water surface at each site, transported in the dark on ice to the laboratory, filtered on GFF filters, stored frozen, and extracted and analyzed according to Welschmeyer (1994). To supplement

these point samples, we obtained similar chlorophyll *a* grab samples from National Estuarine Research Reserves (NOAA NERRS System Wide Monitoring Program) and The St. John's Water Management District. These additional data collection sites were in close proximity to five of the monitoring sites in Grabowski et al. (2020). In addition, these samples were collected during the same time frame as this experiment (1 June–15 August 2011). See Appendix S2 for full details on number of samples collected per site as well as distance of sample collection from the monitoring sites used in the companion studies by Byers et al. (2015) and Grabowski et al. (2020). For each data set, we calculated monthly averages and combined these monthly averages into one data set to evaluate how water chlorophyll *a* concentration ($\mu\text{g/L}$) varied across latitude during this experiment.

Statistical analysis

We used ANOVA to test whether the trait responses of individual oysters (shell strength, CI) and a cohort of oysters (larval recruitment) as well as fitness measures (mortality and growth) of individual oysters depended on site, trophic structure treatment, and the interaction between site and trophic structure treatment. For these analyses, the multiple experimental tiles per cage were treated as subsamples and were averaged to create one value for each cage. ANOVA models were fit in R 3.4.1 (R Core Team 2019). When we detected effects of site, trophic structure, or an interaction between these main effects ($P < 0.05$), we conducted Tukey's post hoc test to compare means among treatments. Post hoc tests were conducted with the stats package in R 3.4.1, and for trophic structure, were restricted to testing for relative differences among trophic treatments within each site, not among sites, leading to a maximum of three possible mean comparisons for each site. Within a site, significant mean comparisons between responses on reefs with only one trophic level and responses on reefs with two and three trophic levels were considered as a measure of NCE strength. Because the highest mortality of the focal oysters occurred at the sites with the highest recruitment of oyster larvae, we used linear regression to evaluate the relationship between the mortality of the focal oysters and the recruitment of a natural oyster

cohort. In this regression approach, we also evaluated a model that included linear and quadratic predictors, with the F -ratio test of the latter evaluating whether the addition of the quadratic term significantly contributes to explained variation between oyster mortality and recruitment (Quinn and Keough 2002). Finally, for all statistically significant main and interaction effects, we calculated Eta^2 (η^2), which is the proportion of the total variance explained by each factor in the ANOVA model (Pierce et al. 2004).

To determine whether the environmental contexts of volumetric water flow, oyster recruitment, and water column chlorophyll a concentration varied with latitude, we evaluated separate regression models with latitude as a linear and quadratic predictor. In each regression, the F -ratio test of the latter evaluated whether the addition of the quadratic term significantly contributed to the explained variation between each response variable and latitude (Quinn and Keough 2002).

All data were checked for parametric assumptions of normality and homogeneous variances by conducting diagnostic plots as well as conducting Shapiro–Wilks test for normality of residuals and Levene’s test for homogeneous variances. To meet parametric assumptions, we log-transformed the oyster recruitment, volumetric water flow, and chlorophyll a concentration data. Meanwhile, a squared transformation was applied to the mortality data. Due to one outlier, we were unable to transform the data on shell strength to meet the assumption of normally distributed residuals. But when we excluded the one outlier, the statistical results remained consistent while also passing the assumption of normally distributed residuals. We present the results of the analysis excluding this point.

RESULTS

Trait responses to risk

At the end of the experiment, the force required to cause structural failure of focal oyster shells (i.e., shell strength) varied significantly with site ($F_{5,33} = 21.49$, $P < 0.001$; $\eta^2 = 0.72$), but not trophic structure ($F_{2,33} = 1.04$, $P = 0.37$, $\eta^2 = 0.01$) or an interaction between site and trophic structure ($F_{10,32} = 0.69$, $P = 0.73$, $\eta^2 = 0.05$; Fig. 2A). Across the SAB, there was a unimodal pattern between shell strength and

latitude, with significantly stronger shells at sites North 2, Central 1-2, and South 1 when compared to shell strength at the northernmost (North 1) and southernmost sites (South 2; Tukey HSD, $P < 0.01$). See Appendix S3 for results of all ANOVAs and associated post hoc comparisons of means.

The condition index of the focal oysters also differed among sites ($F_{5,35} = 12.79$, $P < 0.001$, $\eta^2 = 0.54$), and there was a trend ($P < 0.10$) suggesting an interaction between site and trophic structure ($F_{10,35} = 1.95$, $P = 0.07$, $\eta^2 = 0.16$; Fig. 2B). Meanwhile, the main effect of trophic structure was not significant ($F_{2,35} = 0.26$, $P = 0.77$, $\eta^2 = 0.004$). Across the SAB, the condition indices of focal oysters were significantly higher at the North 2 and South 2 sites than all other sites (Tukey HSD, $P < 0.05$). Within the South 2 site, the condition index of oysters on reefs with three trophic levels trended strongly lower than the condition index of focal oysters on reefs with only one trophic level (Tukey HSD, $P = 0.06$; Fig. 2B).

The recruitment of natural oyster larvae (log-transformed) to experimental tiles differed significantly among sites ($F_{5,36} = 97.67$, $P < 0.001$, $\eta^2 = 0.86$; Fig. 2C), and there was a significant interaction between site and trophic structure ($F_{10,36} = 3.96$, $P < 0.001$, $\eta^2 = 0.07$; Fig. 2C). The main effect of trophic structure was not significant ($F_{2,36} = 2.86$, $P = 0.07$, $\eta^2 = 0.01$). Across the SAB, recruitment at the Central 1 and Central 2 sites was significantly greater than at all other sites (Tukey HSD, $P < 0.005$). Within the northern sites, recruitment to experimental tiles at the North 1 site was significantly greater than recruitment to tiles at the North 2 site (Tukey HSD, $P < 0.0001$). Within the southern sites, recruitment to experimental tiles at the South 2 site was significantly higher than recruitment at the South 1 site (Tukey HSD, $P < 0.005$). At the South 2 site, the presence of three trophic levels significantly decreased the recruitment of oysters to tiles when compared to recruitment on reefs with one and two trophic levels (Tukey HSD, $P < 0.0001$).

Fitness consequences (NCEs) of risk

At the end of the experiment, the number of focal oysters that died per day (square transformed) depended on a significant effect of site

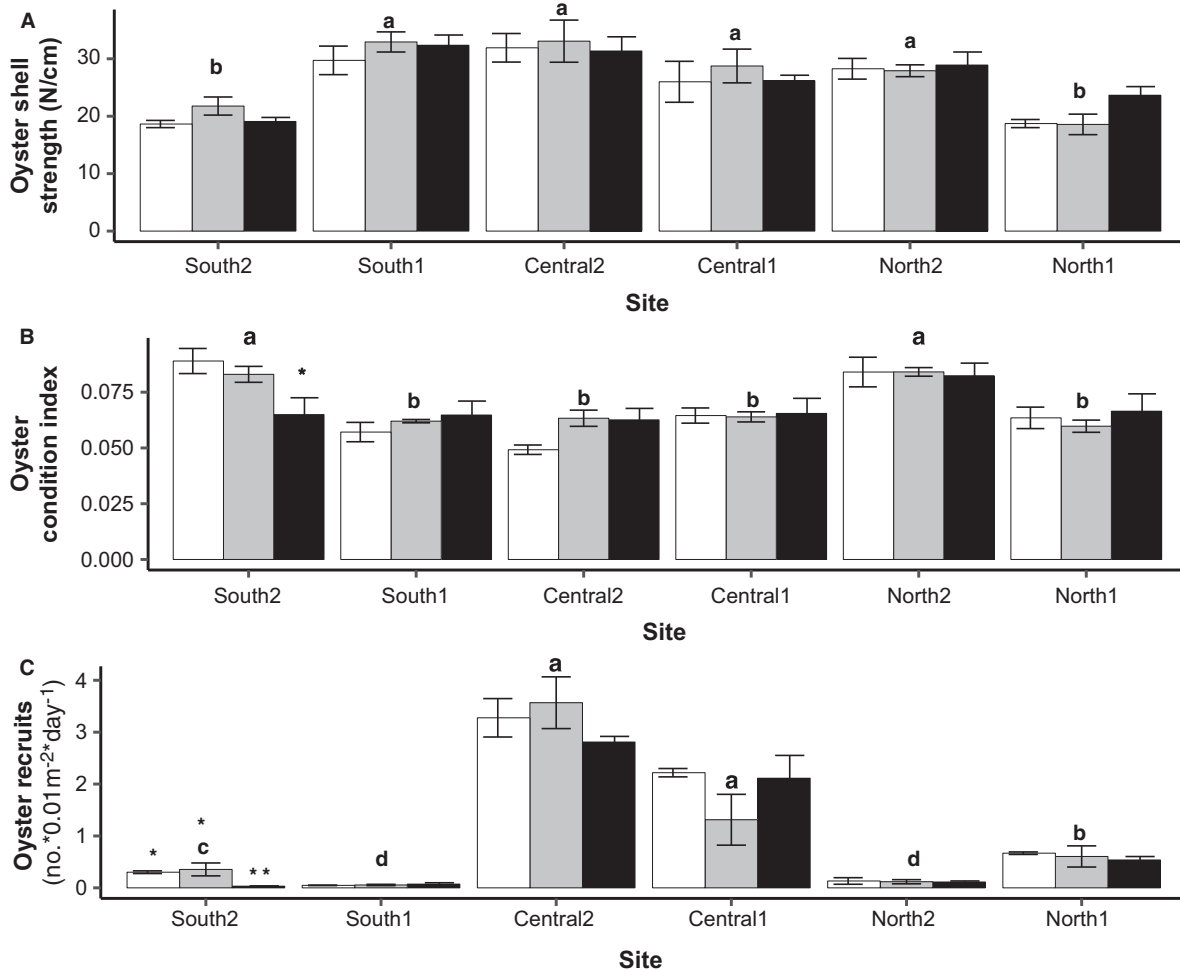


Fig. 2. (A) Mean (\pm standard error [SE]) shell strength of experimental oysters after 90 d as a function of trophic structure across all sites. (B) Mean (\pm SE) condition index (tissue mass/shell mass) of experimental oysters after 90 d as a function of trophic structure across all sites. (C) Mean (\pm SE) recruitment of natural oysters to reefs after 90 d as a function of trophic structure across all sites. In all panels, open bars refer to reefs with only bivalves (one trophic level), gray bars refer to reefs with consumers and bivalves (two trophic levels), and closed bars refer to reefs with predators, consumers, and bivalves (three trophic levels). Different letters indicate a significant mean comparison among sites (averaged across trophic structure). Within each site, single (*) vs. double asterisks (**) indicate significant mean comparison between trophic structure treatments.

($F_{5,36} = 65.83$, $P < 0.001$, $\eta^2 = 0.88$), but not on trophic structure ($F_{2,36} = 0.45$, $P = 0.64$, $\eta^2 = 0.02$), or an interaction between site and trophic structure ($F_{10,36} = 0.000007$, $P = 0.58$, $\eta^2 = 0.02$; Fig. 3A). Across the SAB, oyster mortality displayed a unimodal pattern, with the greatest amount of mortality at the Central 1 site (Tukey HSD, $P < 0.001$). Although mortality at the Central 1 site was significantly greater than

mortality at Central 2 (Tukey HSD, $P < 0.001$), mortality at Central 2 was significantly greater than mortality at all other sites (Tukey HSD, $P < 0.001$). Meanwhile, mortality did not differ significantly among the North 1, North 2, South 1, and South 2 sites (Tukey HSD, $P < 0.001$).

Across the SAB, mortality of the focal oysters demonstrated a significant negative linear ($F_{1,52} = 29.92$, $P < 0.001$) relationship with the

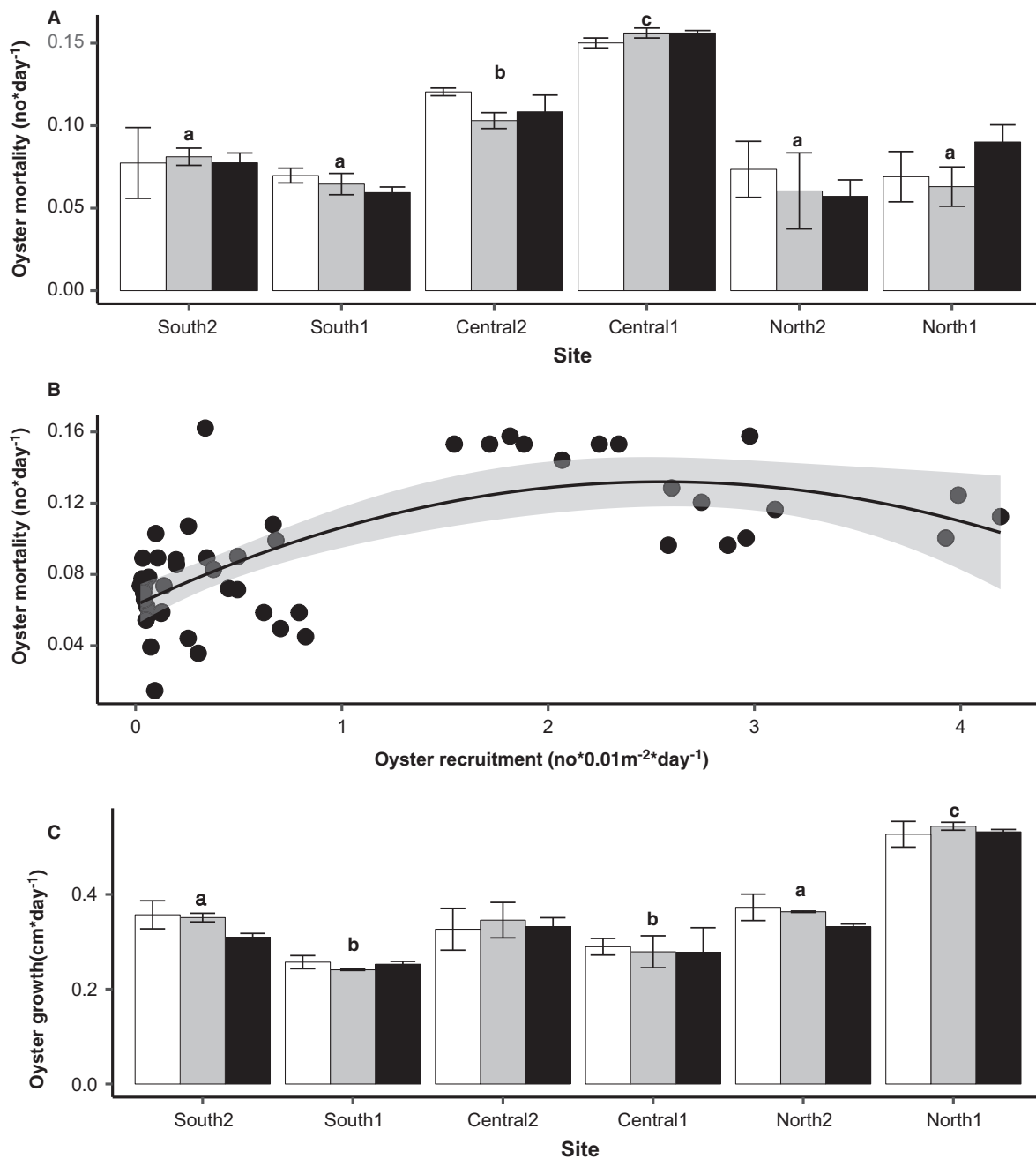


Fig. 3. (A) Mean (\pm standard error [SE]) mortality (deaths \times day⁻¹) of focal oysters after 90 d as a function of trophic structure across all sites. (B) Polynomial regression fit between mortality (deaths \times day⁻¹) of focal oysters and recruitment of natural oysters to reefs. (C) Mean (\pm SE) growth increment (day⁻¹) of focal oysters after 90 d as a function of trophic structure across all sites. In all panels, open bars refer to reefs with only bivalves (one trophic level), gray bars refer to reefs with consumers and bivalves (two trophic levels), and closed bars refer to reefs with predators, consumers, and bivalves (three trophic levels). Different letters indicate a significant mean comparison among sites (averaged across trophic structure). Within each site, the absence of single (*) and double asterisks (**) indicates the lack of significant mean comparisons between trophic structure treatments.

recruitment of natural oysters, and a quadratic ($F_{1,51} = 13.11$, $P < 0.01$, $R^2 = 0.50$) term significantly explaining additional variation in this relationship (Fig. 3B). Oyster mortality was lower at sites with lower recruitment (northern and southern sites).

Of the surviving oysters, growth depended on a significant effect of site ($F_{5,35} = 58.80$, $P < 0.001$, $\eta^2 = 0.87$; Fig. 3C), but not on trophic structure ($F_{2,35} = 0.84$, $P = 0.44$, $\eta^2 = 0.03$), or on the interaction between site and trophic structure ($F_{10,35} = 0.33$, $P = 0.97$, $\eta^2 = 0.01$). Across the SAB, the growth of oysters at site North 1 was significantly higher than growth at all other sites (Tukey HSD, $P < 0.0001$), while the growth of oysters at site South 1 was significantly lower than growth at all other sites except for Central 1 (Tukey HSD, $P < 0.01$). Growth at the Central 1 site was significantly less than growth at the North 1 and North 2 sites (Tukey HSD, $P < 0.01$).

Environmental gradients in water flow rate, food supply, and larval recruitment

Across the SAB, the estimated volumetric flow of water exhibited a significant linear ($F_{1,47} = 4.55$, $P < 0.05$) and quadratic ($F_{1,47} = 34.07$, $P < 0.001$) relationship with latitude ($R^2 = 0.45$; Fig. 4A). Similarly, the recruitment of natural oysters to experimental tiles exhibited a significant linear ($F_{1,48} = 10.14$, $P < 0.01$) and quadratic ($F_{1,48} = 50.52$, $P < 0.001$) relationship with latitude ($R^2 = 0.56$; Fig. 4B). In contrast, water microalgae (chl *a*) concentration decreased linearly with increasing latitude ($F_{1,25} = 4.72$, $P < 0.05$, $R^2 = 0.16$; Fig. 4C) and lacked a significant quadratic trend across latitude ($F_{1,25} = 0.17$, $P = 0.68$).

DISCUSSION

In this study, the traits and fitness of juvenile oysters throughout the SAB were driven primarily by environmental and biological differences among sites, with marginally influential effects of risk associated with trophic structure treatments. Shell strength of focal oysters and recruitment of a natural oyster cohort both displayed unimodal patterns across the SAB, with the former peaking in the Central 2 and South 1 sites and the latter peaking in the two Central sites (Fig. 2A, C). In contrast, the condition

index of focal oysters did not exhibit a clear spatial pattern. Furthermore, oyster condition index was reduced by predation risk on reefs with three trophic levels at the southernmost site but this trait response did not incur a negative NCE on oyster mortality or growth (Figs. 2B, 3A, C). At this same site (South 2), predation risk on reefs with three trophic levels also suppressed the recruitment of natural oysters. Meanwhile, the high recruitment to reefs in the central SAB (Fig. 4B) negated the potential for NCEs by smothering and killing the focal oysters (Fig. 3B). Of the surviving focal oysters, growth did not respond to predation risk at any of the sites. Rather, oyster growth demonstrated a large-scale spatial pattern with the highest growth at the northernmost site (Fig. 3C). Collectively, these results demonstrate that oyster traits and fitness can respond to enhanced risk associated with trophic structure. But in the absence of consumptive predator effects, our results also suggest that variation in larger-scale environmental contexts (Fig. 4) may more often be the primary driver of prey traits and population dynamics.

Because previous studies have found that the plastic traits of animal prey such as bivalves (Scherer et al. 2016) and gastropods (Trussell et al. 2017) strongly respond to predation risk with a clear fitness cost, it is interesting to consider why our experimental oysters were much less responsive. Beginning with the only site to display significant trait responses to risk, focal oysters at a moderate growth site (South 2; Fig. 3C) responded to risk on reefs with three trophic levels by reallocating 50% of their tissue mass to shell mass (i.e., a lower CI, Fig. 2B), but without a statistically significant NCE cost in survival or growth (Fig. 3A). Although growth (shell length) on the same reefs with the full complement of risk cues from top and intermediate predators did trend downward by 15% compared to the controls (Fig. 3C), this was not a statistically significant NCE. Because Dodd et al. (2018) experimentally demonstrated in a laboratory setting that oysters suspension feeding was not altered in the presence of risk cues, we suggest that a cost to shell growth was avoided as oysters were able to re-allocate tissue to shell mass without altering their foraging behavior.

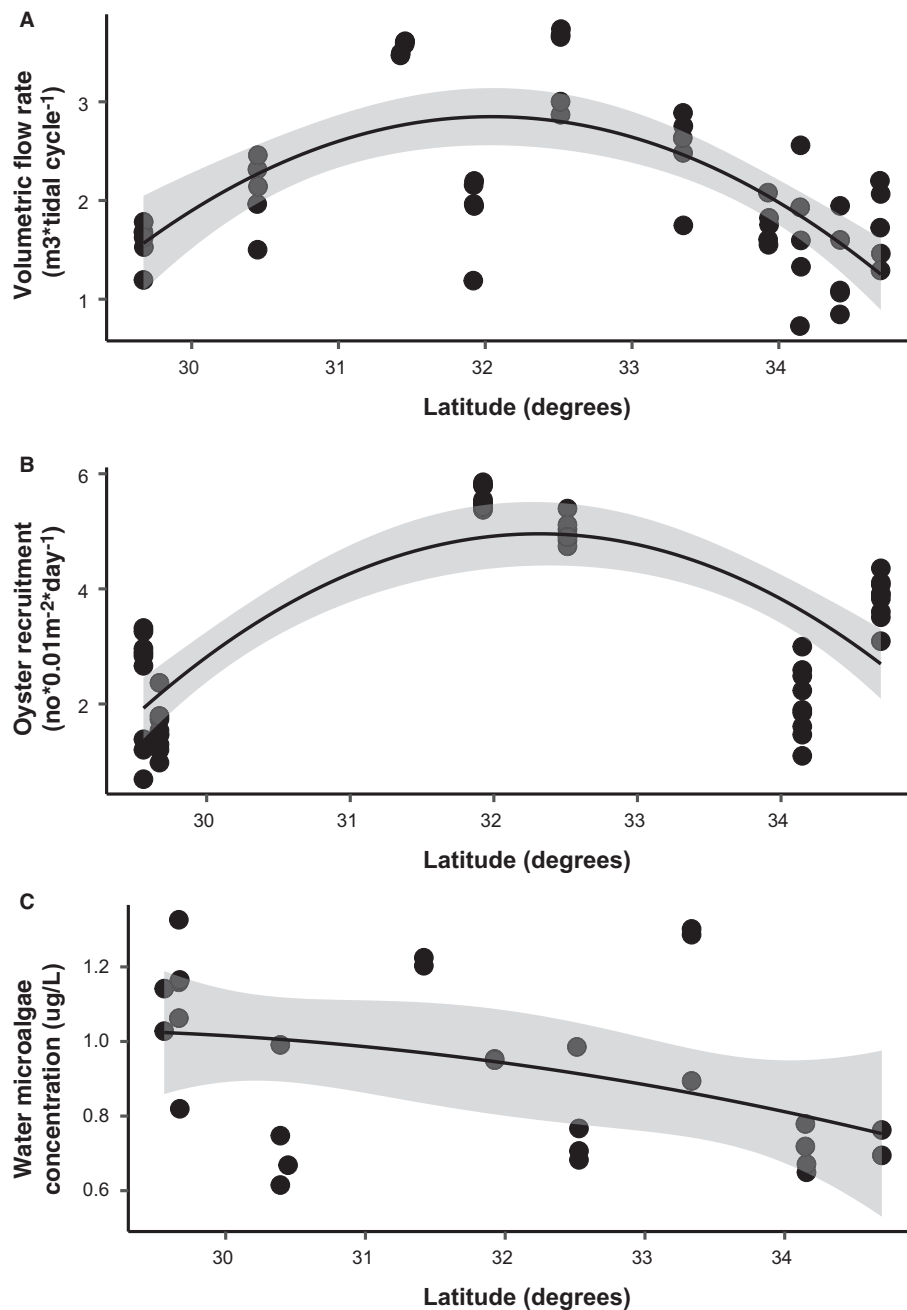


Fig. 4. Regression results of environmental contexts across the Southeastern Atlantic Bight (SAB). (A) Polynomial regression fit between estimated volumetric water flow rate ($\text{m}^3/\text{tidal cycle}$) and latitude of 50 oyster reefs throughout the SAB. These reefs are part of a biogeographic assessment of oyster reef communities conducted by Grabowski et al. (2020). (B) Polynomial regression fit between the recruitment of natural oysters to experimental reefs and the latitude of experimental sites in this study. (C) Linear regression fit between phytoplankton concentration (chlorophyll *a*) and latitude of monitoring locations (Grabowski et al. 2020) and experimental sites in this study.

That the focal oysters of this experiment grew the most at the northernmost site (Fig. 3C) suggests that microalgal resources, which were lowest at that site (Fig. 4C), did not independently limit growth potential and thus the potential for NCE emergence (*sensu* Peacor and Werner 2003, Preisser et al. 2009). Instead, low juvenile oyster recruitment may have set the stage for higher growth by limiting intraspecific competition, for space as well as food. For example, at the central sites, the environmental context of high oyster recruitment limited growth (Fig. 3C) and increased mortality of focal oysters by increasing intraspecific competition for space in the form of smothering (Fig. 3B, C). Although oyster recruitment to reefs was low at both the northern and southern sites, focal oysters at the northern sites responded more strongly to this open space and thus growth potential than did focal oyster growth at the southernmost site (Fig. 3C). One testable hypothesis for more oyster growth under lower microalgal resources in the northern SAB (Fig. 4C) may concern the quality of microalgal resources rather than the quantity of resources available to oysters: Lower microalgal resources at the northernmost site could have been more palatable and nutritious than the higher microalgal resources at the southern site (Fritz et al. 1984; Fig. 4C). Given that over 80% of the variation in growth was explained by site, identifying the underlying explanatory factors for such spatial variation in growth would be useful for future research on NCEs as well as the conservation and restoration of oysters.

Another trait response of prey that can interact with ambient resource levels to influence NCE emergence is prey colonization or recruitment. As observed in marine and freshwater systems (Resetarits and Wilbur 1989, Ellrich et al. 2015), increasing risk can alter local-scale patterns of prey recruitment. Although a recruitment response was detected statistically in our study, it only occurred on reefs with three trophic levels (Fig. 2C), which contained a greater amount and diversity of predator cues. This suggests that the settlement avoidance behavior of oyster recruits may occur only at very high levels of risk. Nevertheless, this trait response was only detected at one of our six sites, with substantially more variation (94%) in recruitment explained by a biogeographic gradient in larval supply (Fig. 4B; Byers

et al. 2014, Byers et al. 2015, Grabowski et al. 2020). Furthermore, this regional recruitment pattern appears to be a consistent feature of the SAB given that a similar unimodal pattern of oyster recruitment across latitude was also demonstrated by Byers et al. (2015) and Grabowski et al. (2020).

In contrast to the marginal responses of oysters to risk in this field experiment and in an associated laboratory experiment (Dodd et al. 2018), the response of this food web's intermediate consumer (mud crab) to risk cues of top predators has been demonstrated to be strong in the laboratory (Grabowski 2004) and field (Kimbrow et al. 2014, 2017). In both settings, these risk cues immediately suppress the foraging activity of mud crabs and indirectly decrease oyster mortality (trait-mediated indirect effect; TMIE). Although this strong TMIE on oyster reefs of the SAB can also be influenced by large-scale environmental gradients over time (Kimbrow et al. 2014), we suggest that the effects of risk primarily emanate from the interaction between the mobile top predators and mobile intermediate consumers, not between the mobile intermediate consumers and the sessile oyster resource.

Given that the variation of each response variable in our study was most strongly linked to the factor of site, future research on NCEs throughout the SAB would benefit from focusing on the environmental differences among sites that influence recruitment, traits, growth, and mortality. For instance, predictable spatial differences in tidal amplitude, channel width, and depth may lead to predictable differences among sites in volumetric rate of water flow (Fig. 4A; Byers et al. 2015). But it remains less clear exactly how water flow is linked to spatial variation in oyster recruitment (Fig. 4A, B). Furthermore, it remains complicated whether and how water flow velocity explains the presence/absence of NCEs across the SAB. On the one hand, and according to experimental results on how hydrodynamics influence prey perception of predation risk (Large et al. 2011), high and low flow velocity at the central and northern sites (respectively) could have inhibited prey detection of waterborne risk cues and NCEs at those sites, while moderate water flow may have promoted prey detection of cues and NCEs at the southern sites (Fig. 4A). But on the other hand, oyster trait responses to

risk and associated NCEs may have been detectable at the central SAB sites, if we had examined the traits and fitness costs of the naturally recruiting oysters that settled upon and smothered the focal oysters. Consequently, for prey with continuous recruitment dynamics and the potential for interference competition, future research on predation risk and NCEs should evaluate multiple prey cohorts.

One limitation should be considered when interpreting the results of this study. First, focal oyster size at the beginning of our field experiment ranged from 5 to 10 mm in shell length. In contrast, similar experiments that documented stronger oyster responses to risk in controlled settings involved smaller oysters with initial shell length at 1.0 mm (Johnson and Smee 2012). Consequently, the weaker oyster responses in our field experiment could be due to differences between studies in the ambient sensory background and/or in initial oyster size, with smaller oysters being more susceptible and more responsive to predation risk. In addition to a limitation, this study also contained a strength that addressed a frequent criticism of NCE experiments: A majority of these experiments compare risk cue treatments to control treatments even though predator cues never occur in nature without any predation (Abrams 2008, Kimbro et al. 2017, 2020). This experimental design likely misses important interactions and nonlinearities that may occur between the CEs and NCEs of predators (Peacor and Werner 2003). Our experiment addressed this criticism by exposing the protected (focal) juvenile oysters to the cues of predation on unprotected juvenile oysters in all of the experimental oyster reefs (see predation results in Kimbro et al. 2014).

In conclusion, ecologists recognize that the responses of prey to predation risk can lead to fitness consequences that more strongly drive prey population dynamics than do the consumptive effects of predators. However, this recognition is largely based on research that uses simplistic sensory backgrounds instead of natural gradients in resource supply, prey recruitment, and trophic structure. In fact, our research demonstrated that the trait responses of oysters to risk and the associated fitness consequences observed in controlled settings may occur less frequently or be less detectable as oysters age in

the varying environmental contexts of the South Atlantic Bight. Furthermore, when statistically significant responses to risk were detected, larger-scale environmental factors were still the primary driver of prey traits, growth, survival, and recruitment. These findings emphasize that the importance of NCEs vs. CEs to prey population dynamics should be re-evaluated across environmental and biological gradients relevant to prey demography.

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