Regional environmental variation and local species interactions influence biogeographic structure on oyster reefs

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Regional environmental variation and local species interactions influence biogeographic structure on oyster reefs

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Abstract. Although species interactions are often assumed to be strongest at small spatial scales, they can interact with regional environmental factors to modify food web dynamics across biogeographic scales. The eastern oyster (*Crassostrea virginica*) is a widespread foundational species of both ecological and economic importance. The oyster and its associated assemblage of fish and macroinvertebrates is an ideal system to investigate how regional differences in environmental variables influence trophic interactions and food web structure. We quantified multiple environmental factors, oyster reef properties, associated species, and trophic guilds on intertidal oyster reefs within 10 estuaries along 900 km of the southeastern United States. Geographical gradients in fall water temperature and mean water depth likely influenced regional (i.e., the northern, central and southern sections of the SAB) variation in oyster reef food web structure. Variation in the biomass of mud crabs, an intermediate predator, was mostly (84.1%) explained by reefs within each site, and did not differ substantially among regions; however, regional variation in the biomass of top predators and of juvenile oysters also contributed to biogeographic variation in food web structure. In particular, region explained almost half (40.2%) of the variation in biomass of predators of blue crab, a top predator that was prevalent only in the central region where water depth was greater. Field experiments revealed that oyster mortality due to predation was greatest in the central region, suggesting spatial variation in the importance of trophic cascades. However, high oyster recruitment in the middle region probably compensates for this enhanced predation, potentially explaining why relatively less variation (17.9%) in oyster cluster biomass was explained by region. Region also explained over half of the variation in biomass of mud crab predators (55.2%), with the southern region containing almost an order of magnitude more biomass than the other two regions. In this region, higher water temperatures in the fall corresponded with higher biomass of fish that consume mud crabs and of fish that consume juvenile and forage fish, whereas biomass of their prey (mud crabs and juvenile and forage fish, respectively) was generally low in the southern region. Collectively, these results show how environmental gradients interact with trophic cascades to structure food webs associated with foundation species across biogeographic regions.

Key words: biogeography; food web dynamics; foundation species; oyster reef; physical and biological coupling; predation; South Atlantic Bight; top-down forcing.

Introduction

Food webs are often influenced by multiple environmental drivers that can operate at different temporal and spatial scales, and these drivers can vary in how they interact with local species to affect the trophic distribution of biomass in food webs. For example, Sanford (1999) demonstrated that a slight decrease in water temperature greatly reduced sea star (*Pisaster ochraceus*) predation on its primary prey in coastal Oregon, such that *Pisaster's* per capita impact may decrease in regions where the sea stars experience colder water temperatures. But within the same food web, mussels are not fully released from predation due to a carnivorous snail (*Nucella caniculata*). *Nucella* is locally adapted to oceanographic regions with differences in prey recruitment, such that it regularly consumes larger mussels (*Mytilus californianus*) in the south where prey diversity is low, but not in the north where it preferentially forages on barnacles and smaller mussels (Sanford and Worth 2009, 2010). Meanwhile, in salt marsh systems in the Gulf of Mexico, tidal inundation controls predator risk

perception, and consequently, snail grazing on marsh plants (Kimbro 2012). As a result, snail grazing increases and reduces plant biomass on shorelines with diurnal tides relative to plants on shorelines with mixed semidiurnal tides (Kimbro 2012). Thus, environmental drivers fundamentally influence ecological processes across biogeographic gradients, with consequences for species interactions and the composition of biomass within lower trophic levels. These examples also suggest that advancing our understanding of biogeography requires the identification of environmental drivers and species interactions that mediate regional variation in food web structure.

When predator effects cascade across multiple trophic levels and alter the survival or traits of basal species, they can have profound effects on food web structure (Carpenter et al. 1985, Power et al. 1985, Chase 1996). Yet, generalizing the effects of predators across biogeographic regions requires an understanding of how environmental gradients potentially modify biotic processes and food web structure. For example, increasing temperature tends to strengthen the cascading effects of top predators in pond and grassland systems (Kratina et al. 2012, Shurin et al. 2012). Thus, the physical and biological processes that structure food webs are often fundamentally coupled. In this study, we examined how physical drivers influence food web structure and the cascading effects of predators within oyster reef communities in the southeastern United States.

Given the disproportionately large role of foundation species in driving food web structure, determining the factors that modify spatial and temporal patterns in their dynamics and persistence is important and often also has critical conservation implications. For instance, in areas of western Alaska, killer whale consumption of sea otters increased in the 1990s, thereby releasing their prey, urchins, to deforest kelp beds, with potential negative impacts on the communities that associate with this foundation species (Estes et al. 1998). Although such trophic cascades that influence the survival of foundation species can indirectly alter food web structure, foundation species often also directly dampen the strength of trophic cascades by sheltering prey from predators (Werner et al. 1983, Beckerman et al. 1997, Trussell et al. 2006, Bishop and Byers 2015). Experimental manipulation of foundation species coupled with field observations of their properties and associated communities have advanced our understanding of their impacts on food web structure (Shurin et al. 2002, Grabowski et al. 2005, Orrock et al. 2013). However, it is less clear how environmental conditions influence the degree to which foundation species modify the strength of ecological processes and the structure of food webs across spatial scales.

Oyster reefs are a model system to explore how physical and biological processes are coupled and mediate variation in food web dynamics over both local and regional scales. Previous investigations of oyster reef communities throughout the southeastern United States suggest that top predators can exert top-down forcing within oyster reefs (Grabowski 2004, Grabowski et al. 2008). Yet, the degree to which they release oysters and other basal prey from intermediate consumers such as crabs and predatory drills depends on environmental factors and biotic processes that vary across biogeographic regions (Kimbro et al. 2017). For instance, manipulation of top and intermediate predators on experimental oyster reefs resulted in stronger trophic cascades at the northern edge of the South Atlantic Bight (SAB) in North Carolina than in central to southern regions from South Carolina through Florida (Kimbro et al. 2017). Meanwhile, bottom-up effects (juvenile oyster recruitment) in the central portion of the SAB and sedimentation in the southern region reduced the degree to which top predators indirectly benefitted oysters. Therefore, spatial variation in physical and biotic processes that influence species interactions can explain why predation is important locally in North Carolina but may not be easily generalized throughout this biogeographic region (Kimbro et al. 2017). Furthermore, biogeographic variation in oyster reef properties (e.g., Byers et al. 2015) likely influences the delivery of important ecosystem services.

As a foundation species, oysters grow in reefs or bars and provide biogenic structure, explaining why a diverse array of fish and invertebrates utilize this habitat for refuge and foraging grounds (Wells 1961, Bahr and Lanier 1981, Coen et al. 1999, Peterson et al. 2003, Zu Ermgassen et al. 2016). Oyster reef food webs consist of multiple groups of predators and prey, thereby setting up several pathways by which top predators might indirectly influence juvenile oyster survival. For instance, predators such as bonnethead shark (*Sphyrna tiburo*), adult black drum (*Pogonias cromis*), and sail catfish (*Bagre marinus*) primarily consume blue crabs (*Callinectes sapidus*), thereby potentially releasing juvenile oysters from blue crab predation. Meanwhile, smaller predators such as toadfish (*Opsans tau*), juvenile red drum (*Scianeops ocellatus*) and black drum, and hardhead catfish (*Arius felis*) consume mud crabs (Family Xanthidae), one of the most abundant intermediate predators of oysters that resides on reefs. Because blue crabs also consume mud crabs, these two food web compartments are linked through intraguild predation (Grabowski et al. 2008). A third food web compartment on oyster reefs consists of piscivorous fish including bluefish (*Pomatomus saltatrix*), Atlantic sharpnose (*Rhizoprionodon terraenovae*) and blacktip (*Carcharhinus limbatus*) sharks, and longnose gar (*Lepisosteus osseus*) that consume juvenile and forage fish. All three of these pathways could affect oyster survival, and each is potentially influenced by different environmental drivers and biotic processes (e.g., temperature, oyster recruitment, and tidal inundation as per Byers et al. 2015) that vary across the biogeographic regions of the southeastern United States, so that generalizing this web across biogeographic regions is challenging.

Here, we used simultaneous observations of, and experiments on, oyster reef communities spanning 900 km to determine whether food web structure of intertidal oyster reefs differs predictably along multiple environmental gradients throughout the southeastern United States, the South Atlantic Bight (SAB). First, we examined spatial variation in the distribution of biomass across trophic guilds within oyster reef communities. Next, we examined field observations of oyster reef communities and reef properties using multivariate analyses to identify any subregional spatial structure as well as potential environmental drivers. In addition, we examined the amount of variation in the biomass of each trophic guild explained at each spatial scale to indicate the relative importance of regional vs. local processes in structuring oyster reef food webs. Finally, we experimentally examined oyster survival by deploying caged and predator-exposed juvenile oysters to quantify whether spatial variation in food web structure corresponds with differences in predation pressure, and thus potential trophic cascades, among our sites. We asked whether variability in physical processes such as tidal inundation mediates food web structure at regional scales. Given that the morphology and the depth of inundation of intertidal oyster reefs differ greatly throughout the southeastern U.S. (Byers et al. 2015), we set out to address whether variation in water depth influences larger predators' access to intertidal oyster reefs, thereby potentially cascading to lower trophic levels. In addition, we were interested in examining if oyster recruitment and oyster reef biomass are linked and can help predict regional variation in food web structure on reefs.

METHODS

Study area

Our study examined how food web dynamics vary on oyster reefs throughout the SAB, which extends from Cape Hatteras, North Carolina to Cape Canaveral, Florida (Fig. 1). We surveyed oyster reefs within 10 estuaries that were intermittently dispersed along a 900-km range. The 10 estuaries were divided into the following three regions: Northern (Middle Marsh, Virginia Creek, Masonboro Island, and Lockwoods Folly, North Carolina), central (North Inlet and Ace Basin South Carolina; Skidaway Island and Sapelo Island, Georgia) and southern (Jacksonville, and St. Augustine, Florida). Selecting estuaries within the SAB permitted examination of regional variation in food web structure while maintaining a relatively consistent species pool (Briggs 1974, Spalding et al. 2007, Pappalardo et al. 2015), so that differences among regions do not merely reflect idiosyncratic species distributions.

Within this region of the United States, oyster reefs are commonly found in estuaries on the edges of salt marsh, on mudflats, and within tidal creeks (Bahr and Lanier 1981, Grabowski et al. 2005). Here we focused on the transient and resident fauna that inhabit intertidal fringing marsh reefs. We selected intertidal fringing marshes because they were present throughout the SAB and were relatively intact in comparison to mud flat, and especially subtidal, oyster reefs that have formed the basis of oyster fisheries in the region. In each of the 10 estuaries, five oyster reefs were selected that were adjacent to marsh cordgrass (Spartina alterniflora) on tidal creek banks near the mouth of the estuary. Reefs within an estuary were spatially separated by at least 100 m, and we marked out a 3×3 m intertidal sampling area on each reef adjacent to the saltmarsh. At each reef location, ambient water salinity was >25 ppt in summer, and large oyster (>5 cm shell height [SH]) densities were >30 oysters/m^2 (Byers et al. 2015). Furthermore, none of the reefs selected had visible signs of active or recent oyster harvesting pressure prior to or during the study.

Oyster reef community sampling

In August 2010, three teams of researchers (1 per region) sampled the bottom two trophic levels of the oyster community (i.e., xanthid crabs, oysters, and associated reef infauna) using 0.5×0.5 m quadrats along the upper edge of our sampling area directly adjacent to the marsh. Within each quadrat, all oysters and fauna were excavated to 10 cm and returned to the lab where they were rinsed of mud using a 1-mm sieve. Living oysters were weighed using a spring scale (Byers et al. 2015). All clusters of living oysters with two or more oysters >5 cm SH were combined to generate a total cluster shell mass for each quadrat sample as a proxy for reef biomass and habitat complexity (per methods in Grabowski et al. 2005). We also quantified the density of recently settled (<2.5 cm SH; hereafter referred to as "juvenile") oysters in each quadrat. In addition, all mud crabs (family Xanthidae) were measured (carapace width [CW]), counted, and weighed.

Because juvenile oyster counts per 0.25 m^2 ranged up to 3012, a subset of 100 oysters were randomly selected from each reef and SH was measured. The density of juvenile oysters per reef was then calculated by multiplying the proportion of the 100 measured oysters that were <2.5 cm SH on each reef by the overall oyster density. Juvenile oysters recruit and attach on to oysters, so that quantifying the individual weight of each one is challenging. To convert juvenile oyster densities to biomass estimates, we first created a shell height to mass equation for juvenile oysters using individual shell height and weight measurements for ~500 oysters collected in northwestern Florida. An exponential model was used to fit the relationship between oyster SH and mass (W [g] $= 0.0008 \times$ SH [mm]$^{2.2224}$), and this equation was used to convert the length of each measured juvenile oyster to an oyster weight. We then calculated a mean juvenile oyster mass for the subset of oysters on each reef that were juveniles by summing the mass of these oysters and dividing it by the number of juvenile oysters measured in each reef subset. For each reef, this estimate was
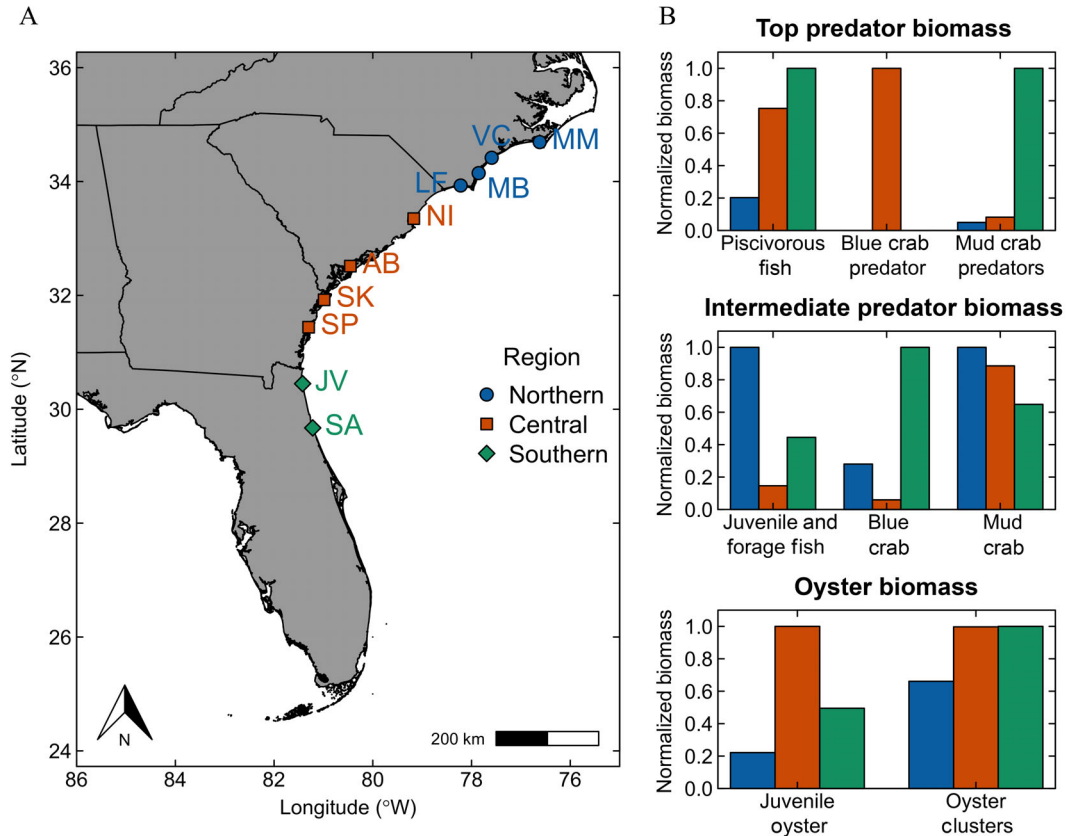
Fig. 1. (A) Oyster reefs were sampled from sites located throughout the South Atlantic Bight (SAB) ranging from North Carolina to Florida, USA. MM, Middle Marsh, North Carolina; VC, Virginia Creek, North Carolina; MB, Masonboro Island, North Carolina; LF, Lockwoods Folly, North Carolina; NI, North Inlet, South Carolina; AB, Ace Basin, South Carolina; SK, Skidaway Island, Georgia; SP, Sapelo Island, Georgia; JV, Jacksonville, Florida; SA, St. Augustine, Florida. Sites are separated by region (northern, blue circles; central, orange squares; southern, green diamonds). (B) Normalized biomass for predators (piscivorous fish, blue crab predators, and mud crab predators), Intermediate predators (juvenile and forage fish, blue crabs, and mud crabs), and oysters (juvenile and oyster clusters) within each region of the SAB. Within each trophic guild, data were normalized relative to the region with the highest biomass.

then multiplied by the density of juvenile oysters to quantify juvenile oyster biomass. We also quantified the density of large adult (>75 mm SH) oysters and biomass of oyster clusters (i.e., two or more attached living or dead oysters) as a proxy for reef biomass.

In July and August 2010, the same three research teams sampled juvenile fish abundance with the following unbaited traps on each reef: two minnow traps (44.5 cm long × 24.3 cm diameter with 5-mm mesh screen and 2.5-cm openings on two opposing sides), two crab traps (60 cm long × 24 cm high × 60 cm wide with 3.8 cm mesh and openings centered on each side that are 16 cm long × 5 cm high), and two fish traps (81 cm long × 51 cm wide × 30 cm high with 1.3 cm mesh and a 12 cm diameter opening centered at each end). We also conducted gill net (10 m long × 1.5 m tall; 7.5 cm maximum slit opening) sampling to quantify piscivorous fish, fish that eat crustaceans, and blue crabs that were opportunistically caught on reefs. Gill nets were wrapped from the corner of the reef along the lower, seaward edge

parallel to the marsh edge and then continuing up to the marsh along the downstream edge of the reef during flood tide (sensu Grabowski et al. 2005). Gill nets and traps were deployed at mid-flood tide in the evening and retrieved ~6 h later, midway on the ebb tide, and sampling at 9 of 10 sites was conducted between 25 and 30 July to maintain consistent tides. Reefs in Jacksonville, Florida, were sampled between 25 and 26 August because this site was added to the study in August 2010. All captured fish and crabs were identified and measured (total length and carapace width, respectively).

All fish and invertebrates captured during quadrat, trap, and gillnet sampling efforts were grouped into the following trophic guilds: biomass of (1) piscivorous fish (i.e., fish that consume juvenile and forage fish), (2) fish that consume mud crabs, (3) fish that consume blue crabs, (4) juvenile and forage fish, (5) adult (>12 mm carapace width) mud crabs, (6) blue crabs, (7) juvenile oysters, and (8) adult oyster clusters. Fish were assigned to trophic guilds using stomach content analyses from our previous

and current efforts (J. H. Grabowski, *unpublished data*), as well as information in the literature on the foraging habits of each predator species, and insights from a fish ecologist (D. Grubbs, *personal communication*). Trophic guilds containing fish were nonoverlapping except for black drum because we captured both small and large adults; therefore, we assigned guilds for these different size classes based on what they most commonly eat (see Appendix S1 for more details). For each fish species, individual lengths were converted to masses (g) using species specific length–mass relationship equations (Appendix S1: Table S1). Blue crab carapace widths were also converted to weights using a carapace-width–mass relationship equation (Appendix S1). Estimates of biomass were then summed within each trophic guild.

Physical properties

Average water temperature, salinity, and the duration and depth of water inundation of reefs were quantified at each site (see details in Byers et al. 2015). Specifically, water depth was standardized by dividing the summer of 2010 into 2-week intervals that matched the spring-neap tidal cycle and the median was calculated for each 2-week period. At each site, the average of medians for each 2-week period was calculated and is referred to as "water depth" in this study. Fall water temperature was calculated using the same approach after excluding all temperature recordings when reefs were exposed during low tide. Fall water temperature was used in this study even though fish and reef sampling occurred in summer, because fall temperature best captured the seasonal biogeographic variation in water temperature that occurs throughout the SAB (Byers et al. 2015). Thus, fall temperature should not be considered as a driver affecting summer fish and crustacean abundances, but rather as a metric that captures quantitative environmental differences among sites. Salinity was recorded every six weeks from summer 2010 to summer 2011 at all sites during the study, and again reflects general environmental differences among sites.

Oyster mortality

At two sites each in the northern (North Carolina), central (South Carolina, Georgia), and southern (Florida) regions of the SAB, we conducted an oyster mortality experiment to assess predation rates. At each site, we collected oyster shell from the field and used a rotary tool to remove sections of dead shell with living juvenile (<2.5 cm) oysters. Twenty-five juvenile oysters were then attached to ceramic tiles (10×10 cm) using marine epoxy, and each tile was attached to a concrete paver (12×12 cm) using aquarium-safe silicone. At each of the five reefs within each site, two pavers with oysters were deployed 1 m from the marsh edge within the reef and recovered after 6 weeks to determine oyster survival rates. One of the two pavers (predator exclosure) in

each deployed pair had a plastic-coated wire cage (6 mm mesh) surrounding it, and the other paver was completely exposed. Pavers were oriented vertically with oysters facing in the seaward direction.

Statistical analysis

Examining oyster reef food web patterns and structure in the South Atlantic Bight.—First, we explored regional variation in the distribution of biomass among trophic guilds. To compare regional differences in each of the seven trophic guilds, biomass estimates were normalized by dividing the biomass for a particular trophic guild within each region by the amount in the region with the greatest biomass for that trophic guild. This resulted in a relative biomass estimate within each region for each trophic guild between 0 and 1.

Next, we used an unconstrained ordination method (nonmetric multidimensional scaling [nMDS]) to identify spatial structure in oyster food webs throughout the SAB by visualizing the relationship between food web structure (piscivores, fish that consume mud crabs, fish that consume blue crabs, juvenile and forage fish, adult mud crabs, blue crabs, and oyster clusters) and potential explanatory variables (spatial [region], environmental [mean fall water temperature, mean water inundation depth on reef, salinity, and duration of aerial exposure], and biological [juvenile oysters]). This method attempts to maintain the ranked distances between sampled communities, so that sites with less similar food web structure in the original N-dimensional space (where N = number of species guilds in the community = 7 here) are farther apart in two-dimensional (2D) space. The ability of nMDS to retain the rank order of the community dissimilarities was expressed by computing the 2D stress and R^2, where $R^2 = 1 - \text{stress}^2$. Prior to conducting the analysis, the data were Hellinger transformed to down-weight the effects of rare species on community dissimilarity (Legendre and Gallagher 2001).

To help interpret the ordination, we projected each environmental variable by finding the direction of the vectors that maximized their correlation with the nMDS axes. This procedure results in each vector pointing in the direction that (1) represents the most rapid change in the environmental variable and (2) maximizes its correlation with the ordination axes. A permutation test was then performed to assess the significance of the association between the ordination axes and each environmental vector by using the procedure described above to fit 1,000 randomized (shuffled) versions of each environmental variable and calculating the P value as the proportion of randomizations whose correlation (squared) was greater than or equal to that observed with the original data. The significant environmental variables ($P < 0.05$) were then plotted as vectors, with the length (direction) of each vector indicating the strength (sign) of the correlation between the axes and the variables.

We then examined the relative importance of the spatial (region and site nested within region), environmental (fall mean water temperature and water depth), and biological (juvenile oyster biomass) variables in explaining oyster reef food web structure. We primarily focused on variables that the nMDS suggested were important, and we removed environmental variables such as salinity and duration of aerial exposure that were not significant. PERMANOVA was used to relate the biomass of trophic guilds defined above (i.e., piscivorous fish, fish that consume mud crabs, fish that consume blue crabs, juvenile and forage fish, adult mud crabs, blue crabs, and oyster clusters) to these variables. Both the PERMANOVA and nMDS were performed using the vegan package for the R programming environment (Oksanen et al. 2013).

Exploring spatial variation in trophic guilds.—Next, we were interested in exploring the importance of local vs. regional processes in structuring oyster food webs. Thus, we examined the amount of variation explained by region, site nested within region, and reef within site using a series of analyses for each trophic guild. Specifically, we conducted separate ANOVAs with site nested within region for each of the following response variables and used the sum of squares to calculate the proportion of variance explained for each trophic level: (1) piscivorous fish, (2) fish that consume mud crabs, (3) fish that consume blue crabs, (4) juvenile and forage fish, (5) adult mud crabs, (6) blue crabs, (7) juvenile oysters, and (8) adult oyster clusters. For each analysis, a Shapiro-Wilk test was conducted to test whether the residuals were normally distributed. The residuals were then plotted and visually inspected for potential violations of the assumptions of homoscedasticity. Because we were primarily interested in quantifying the amount of variation explained by each spatial scale and a number of these analyses violated the assumption of normality, we chose not to interpret whether region or site nested within region were significant. All univariate analyses were conducted using R (R Development Core Team 2013).

Assessing consumer foraging on oyster reefs.—To determine whether consumer pressure on oysters varied biogeographically, we used ANOVA to test whether region (fixed) and treatment (fixed) influenced oyster survival. Oyster survival data were arcsine-square-root transformed prior to conducting analyses to meet the assumptions of normality and homoscedasticity. We then quantified predation effect sizes for each site to normalize our transformed survival data and compare among regions. First, an effect size was calculated for each reef individually by subtracting survival on each exposed replicate tile from survival in the cage treatment, and dividing this estimate by survival in the cage. We then used an ANOVA to examine the effect of region on effect size and conducted post hoc analyses using Kramer's HSD ($\alpha = 0.05$).

RESULTS

Oyster reef food web structure in the South Atlantic Bight

The PERMANOVA indicated that biomass of the trophic guilds significantly differed as a function of several of the environmental (water depth and fall temperature) and spatial (region) variables (Table 1). Juvenile oyster biomass was also marginally significant ($P = 0.08$, Table 1). Meanwhile, the nMDS indicated that there were strong regional differences in food web structure that aligned with two different environmental gradients, water depth and fall water temperature (Fig. 2). The central region was characterized by greater water depth, with levels that were 26% and 196% greater than those in the southern and northern regions, respectively. Average fall water temperature was warmer in the southern (24.3°C) than the central (22.4°C) and northern (19.3°C) regions of the SAB.

The central region was also the only one with larger-bodied fish that consume blue crabs, and the biomass of blue crabs was 7% and 26% of those in the southern and northern regions, respectively (Fig. 1B). In addition, the central region contained two to four times more juvenile oyster biomass than in the other two regions (Fig. 1B). Biomass of piscivorous fish was 33% greater in the southern than the central region, and ~400% greater than that of the northern region (Fig. 1B). Biomass of fish that consume mud crabs was also greatest in the southern region, and it was ~95% greater than mud crab consumers in either of the other two regions. Meanwhile, the northern region contained 100–600% more juvenile and forage fish biomass than the other two regions, and slightly (13–54%) higher mud crab biomass, but one-third less oyster cluster biomass than the other two regions (Fig. 1B).

Spatial variation in oyster reef food webs

Fish that consume blue crabs (i.e., primarily bonnethead sharks) were not captured in the northern and

TABLE 1. Results from PERMANOVA examining the effects of region and environmental (water depth, fall water temperature) and biotic (juvenile oyster biomass) variables on the biomass of oyster reef trophic guilds (i.e., piscivorous fish, fish that consume mud crabs, fish that consume blue crabs, juvenile and forage fish, adult mud crabs, blue crabs, and oyster clusters).

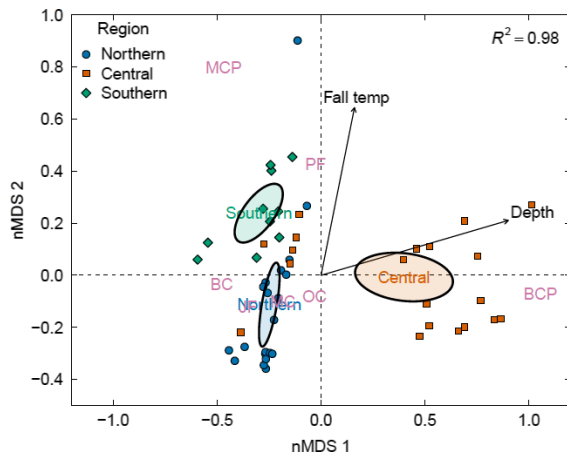| Factor	df	SS	MS	F	R^2	P
Water depth	1	1.62	1.62	25.65	0.25	0.001
Fall water temperature	1	0.32	0.32	5.00	0.05	0.01
Juvenile oyster biomass	1	0.14	0.14	2.23	0.02	0.08
Region	2	1.02	0.51	8.13	0.16	0.001
Site(Region)	5	0.98	0.20	3.11	0.15	0.001
Residuals	39	2.46	0.06		0.38	
Total	49	6.53			1.00	

Fig. 2. Results of the nonmetric multidimensional scaling (nMDS) with significant explanatory variables (mean fall water temperature and mean water inundation depth on reef) correlated to the axes and plotted. Food web guilds include piscivorous fish (PF), blue crab predators (BCP), mud crab predators (MCP), juvenile and forage fish (JF), blue crabs (BC), mud crabs (MC), and oyster clusters (OC). Ellipses include the 95% confidence interval for each region, and those that do not overlap indicate significant differences. $R^2 = 0.98$, 2D stress = 0.13.

southern regions, whereas they were common in the central region. In contrast, blue crab biomass was lowest in the central region, greatest in the south, and intermediate in the north (Fig. 1). Meanwhile, hardhead catfish was the most common predatory fish that consumed adult mud crabs, and was prevalent at the two most southern sites, whereas only one catfish was captured farther north (at Skidaway, Georgia). Other predators of mud crabs such as black drum, red drum, and gray snapper (*Lutjanus griseus*) also were more prevalent at the southern sites than at the central or northern regions. In the southern region, piscivorous fish consisted of bony fishes such as spotted sea trout (*Cynoscion nebulosis*), longnose gar, ladyfish (*Elops saurus*), and to a lesser extent, bluefish. In comparison, Atlantic sharpnose sharks were the most common fish species that consumed juvenile and forage fish in the central and northern regions. Finally, pinfish (*Lagodon rhomboides*) was the most common juvenile and forage fish species, with silver perch (*Bairdiella chrysoura*) and mummichogs (*Fundulus heteroclitus*) also prevalent throughout the study area.

In exploring the amount of variation in each trophic guild explained by each scale spanning from the reef to the region, we examined how key components of oyster food webs are structured spatially. For both piscivorous fish as well as juvenile and forage fish biomass, most of the variation was explained by reefs, whereas less was explained by region or site nested within region (Table 2). Meanwhile, region explained approximately one-half of the variation in both blue crab predator and mud crab predator biomass, whereas site was less important. Conversely, region and site within region were equally

important for blue crab biomass, whereas most of the variation in mud crab biomass occurred among reefs. Finally, approximately one-third of the spatial variation in juvenile oyster biomass was explained by both region and site within region, whereas a larger proportion of the variation in oyster cluster biomass was explained by site-level variation than that of region.

Assessing consumer foraging on oyster reefs

The interaction between region and caging treatment on oyster survival was marginally significant ($F_{2,54} = 2.4$, $P = 0.10$), and both main effects were significant (region $F_{2,54} = 11.2$, $P < 0.001$; treatment $F_{1,54} = 40.7$, $P < 0.001$). Oyster survival was significantly lower on the uncaged exposed tiles (33.5%) than in predator exclusion cages (65.2%), and it was also lower in the central (39.8%) and southern (42.8%) regions than in the northern (65.4%) region. Predation effect sizes also varied marginally as a function of region ($F_{2,27} = 3.2$, $P = 0.056$; Fig. 3). In particular, the central region experienced significantly more predation than the southern region (Tukey multiple comparison of means, $P = 0.045$), but did not differ from the northern region ($P = 0.32$). There was no difference in predation effect sizes in the northern and southern regions ($P = 0.55$).

Discussion

Examining intertidal oyster reef communities throughout the southeastern United States revealed support for the coupling of physical and biological processes influencing oyster reef food web patterns. Specifically, physical drivers such as fall water temperature and water depth were associated with food web structure on oyster reefs. By fall, a north-south gradient in mean water temperature predictably develops, with warmer waters at the southern end and colder waters towards the northern edge (Byers et al. 2015). In our study, warmer fall water temperatures generally corresponded with greater piscivorous fish, mud crab predator, and oyster biomass, but was not strongly associated with intermediate predator (juvenile and forage fish, mud crab, blue crab) biomass. Although mud crabs consume juvenile oysters, they also use oyster reefs as refuge from predation (Grabowski 2004, Grabowski et al. 2008), potentially explaining why reefs with greater structure often contain higher densities of mud crabs (Grabowski et al. 2005, Ziegler et al. 2018). Thus, the negative top-down effects of greater amounts of predator biomass at warmer temperatures on intermediate predators in oyster reef food webs may be dampened by higher refuge quality as a result of greater oyster recruitment. Given that temperature is an important predictor of regional patterns in food web structure on oyster reefs, to the extent that temperature is causally related, our results could suggest that future changes in seawater temperature might modify components of these food webs.

TABLE 2. The amount of variation explained by multiple spatial scales for each oyster reef trophic guild.

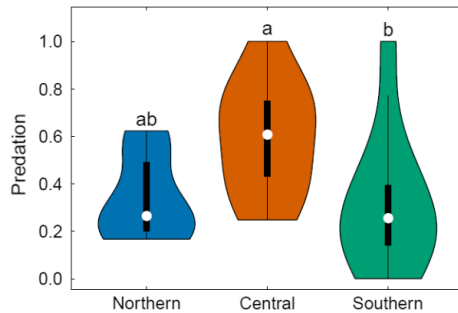| Factor	Piscivorous fish (%)	Juvenile and forage fish (%)	Blue crab predators (%)	Blue crabs (%)	Mud crab predators (%)	Mud crabs (%)	Oyster clusters (%)	Juvenile oysters (%)
Region	18.3	24.7	40.2	20.6	55.2	2.7	17.9	34.9
Site (Region)	18.0	26.7	14.1	26.2	2.8	13.2	40.3	38.3
Residual	63.8	48.6	45.7	53.2	42.0	84.1	41.8	26.8

FIG. 3. Predation effect sizes for juvenile oyster deployed at sites in each region. The median (white dot), interquartile range (thick line), and 95% confidence interval (thin line) are represented within each plot, and the shape of each plot represents the probability distribution of predation within each region.

Unlike the linear gradient in fall water temperature, water depth over reefs in the central region was greater than in either of the other two regions (Byers et al. 2015). In particular, reefs in Georgia and South Carolina experience greater tidal forcing and are more sloped, resulting in greater water depth over the reefs at high tide. The ability of large transient predators to access intertidal oyster reefs is likely mediated by water depth, which is influenced by both the slope of a reef and tidal amplitude (Byers et al. 2017). Large consumers such as bonnethead sharks average over 1 m total length as adults, are common throughout the SAB, and are specialists that predominately consume blue crabs (Cortes et al. 1996, Byers et al. 2017). They were only caught in our study on reefs in the central region where reef slope and the depth of water inundation are greatest (Byers et al. 2015). Thus, our results suggest that bonnethead sharks may be exerting top-down forcing on intertidal oyster reefs only in the central region, which could explain why oyster reef food web structure differs greatly between the middle and other two regions. Similar to our findings, Power et al. (2008) found strong evidence of physical-biological coupling in a coastal California river system. In particular, they revealed that fish mediated algal communities when summer is preceded by rainy winter conditions that result in large bed-scouring floods, whereas fish had no impacts on algal standing crops during drought years with lower river water levels. Collectively, these studies suggest that temporal and spatial variation in hydrologic setting can control predator access to aquatic habitats, thereby influencing benthic food web structure at local and biogeographic spatial scales. Furthermore, our study illustrates how large-scale variation in physical properties such as water depth on oyster reefs potentially alters the communities of predators and prey that utilize them as well as key ecological processes such as predation.

In our study, oyster mortality from predation was greatest in the central region. Mud crabs, a strong oyster predator, were prevalent in all three regions, with the vast majority (>80%) of variation in mud crab biomass explained by variation among reefs within a site. Yet regional variation in mud crab predator biomass was an important driver of food web structure in this system: fish that eat mud crabs only had high biomass at the southern end of the range. Thus, higher mud crab predation risk and subsequent reduced foraging may have contributed to low oyster mortality from predation in the southern region (Grabowski 2004, Grabowski et al. 2008). Meanwhile, biomass of blue crab predators was inversely related to blue crab biomass (Figs. 1, 2), with blue crab predator biomass only present on reefs in the central region, which have greater water depths. Thus, blue crabs were released from predation risk in the northern and southern regions to prey upon mud crabs and potentially induce them to forage less and consume fewer oysters in these regions (Grabowski et al. 2008). Meanwhile, oyster mortality from predation was high in Georgia where biomass of fish that consume mud crabs was largely nonexistent. Furthermore, high biomass of fish that consume blue crabs in the central region potentially suppressed blue crabs from pursuing and consuming mud crabs in this region. These results suggest that the influence of nonconsumptive effects, which have been found to be important in this and a wide diversity of other systems (Werner and Peacor 2003, Grabowski 2004, Preisser et al., 2005, Kimbro et al. 2017), likely varies on intertidal oyster reefs in the southeast Atlantic.

Spatial variation in the biomass of top predator and intermediate consumer levels aligned with our oyster predation results, but juvenile and reef oyster biomass did not. Mud crab biomass was positively correlated with oyster cluster weight, a proxy for habitat complexity, and oyster cluster mass and juvenile oyster mortality from predation were high in the central region. The fact that oyster cluster mass was high at sites with the greatest oyster mortality from predation is counterintuitive; however, oyster reef settlement and recruitment

dynamics could be contributing to this pattern. For instance, in areas with high recruitment, prey may swamp predation and potentially benefit predator survival and growth. We found large differences in oyster recruitment (Byers et al. 2015), and hence juvenile oyster biomass, which could have overwhelmed crab consumption of oysters at sites with high recruitment (predominately in the central region). Meanwhile, high biomass of mud crabs and few predators of mud crabs in the northern region could explain why we found intermediate levels of oyster mortality from predation and low oyster cluster biomass in this region. Thus, weak trophic cascades that are reinforced by low oyster recruitment may be important in driving food web structure in the northern region.

Several factors not explored in this study may have influenced the patterns of spatial variability that we observed. For instance, many of the species in this study are subjected to fishing pressure, including the oysters themselves, which likely could have influenced our estimates of oyster, fish, and blue crab biomass. Yet, we specifically focused on and selected intact fringing reefs that had no visible signs of oyster harvesting prior to or during the study, thereby hopefully minimizing any impacts from this potential confounding factor. Blue crabs are commercially harvested in all four states, yet they were still prevalent throughout the study range. Recreational fishing pressure is high throughout the SAB, and it is unclear if any of the fish species in this study are subjected to differences in harvesting rates that could explain the spatial patterns that we observed. Beyond fishing pressure, the patterns that we observed in the summer of 2010 could have been impacted by physical and biotic processes that preceded it, such as the extremely cold winter of 2009–2010 in the southeastern United States and the protracted anomalous high water levels experienced in fall 2009 in North Carolina. We did not observe any signs of abnormally high oyster mortality rates on oyster reefs just prior to the inception of our study, but these abiotic anomalies likely impacted the communities that we studied. Longer-term sampling efforts and experiments would help resolve the degree to which the intriguing differences that we observed in communities across the regions of the SAB are consistent or dynamic.

Foundation species, such as oyster and coral reefs, salt marshes, shrubs, and trees, commonly harbor both intermediate predators and their prey, and the densities of predators and prey likely scales positively with habitat complexity because it reduces predation risk (Diehl 1992, Beukers and Jones 1997). Crowder and Cooper (1982) noted that prey densities often increase with greater habitat complexity, and they predicted a unimodal relationship between habitat complexity and predator foraging efficiency. Yet, how environmental gradients, which often alter ecological processes, affect foundation species and their associated communities is less clear. Thus, attempts to generalize food web structure at regional scales will benefit from considering how foundation species and their communities are affected by coupled physical and biological processes. Given the profound and diverse ways in which foundation species can influence food webs, determining how their properties vary across environmental gradients is a critical step in investigating biogeographic patterns in food web structure.

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Supporting Information