Oecologia (2017) 185:107–117
DOI 10.1007/s00442-017-3928-y

POPULATION ECOLOGY – ORIGINAL RESEARCH

Contrasting complexity of adjacent habitats influences the strength of cascading predatory effects

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Received: 30 January 2017 / Accepted: 4 August 2017 / Published online: 12 August 2017
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Abstract Although cascading effects of top predators can help structure communities, their influence may vary across habitats that differentially protect prey. Therefore, to understand how and to what degree habitat complexity can affect trophic interactions in adjacent habitats, we used a combination of a broad regional-scale survey, manipulative field trials, and an outdoor mesocosm experiment to quantify predator–prey interaction strengths across four trophic levels. Within estuaries of the southeastern USA, bonnethead sharks (*Sphyrna tiburo*) hunt blue crabs on mudflats and adjacent oyster reefs, two habitats with vastly different aboveground structure. Using 12-h tethering trials of blue crabs we quantified habitat-dependent loss rates of 37% on reefs and 78% on mudflats. We hypothesized that the sharks' predatory effects on blue crabs would cascade down to release a lower-level mud crab predator, which subsequently would increase juvenile oyster mortality, but that the cascade strength would be habitat-dependent. We experimentally manipulated predator combinations in split-plot mesocosms containing reef and mudflat habitats, and quantified oyster mortality. Bonnetheads exerted strong consumptive and non-consumptive effects on blue crabs, which ceased eating oysters in the sharks' presence. However, mud crabs, regardless of shark and blue crab presence, continued to consume oysters, especially within the structural refuge of the reef where they kept oyster mortality high. Thus, bonnetheads indirectly boosted oyster survival, but only on the mudflat where mud crabs were less active. Our work demonstrates how structural differences in adjacent habitats can moderate trophic cascades, particularly when mesopredators exhibit differential use of structure and different sensitivities to top predators.

Keywords Ecotones · Edge effects · Intraguild predation · Trait-mediated indirect effects · Trophic interactions

Introduction

Species interaction strengths, especially between predators and their prey, have provided a powerful organizing principle for understanding mechanisms of food web and community structure (e.g., Carpenter et al. 1987; Schmitz et al. 1997; Pace et al. 1999). These interaction strengths can vary temporally and spatially, and several reviews have consequently emphasized their context dependency (Strong 1992; Estes and Duggins 1995; Shurin et al. 2002; Borer et al. 2005). An important aspect of context dependency is variation in predator and prey diversity, both at the species and functional level. This variation can influence interaction strength because diversity affects predator foraging choices and the potential for the presence of additional interactions like intraguild predation (Finke and Denno 2006). For example, along latitudinal scales researchers have documented that diversity contributes to heterogeneity in the magnitude of trophic cascades (McClanahan and Muthiga 2016). Furthermore, over these large scales, environmental gradients and changes in population densities, community assembly, and metabolic rates can influence the strength and consistency

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Communicated by Jeremy Long.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-017-3928-y) contains supplementary material, which is available to authorized users.

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of species' interactions (Peckarsky et al. 2013; Kimbro et al. 2014; Wellnitz 2014; Wu et al. 2014).

However, some of the most influential heterogeneity may arise over much smaller spatial scales (Heithaus et al. 2009). Structural properties of habitat are a major factor modulating species interactions like predation (e.g., Finke and Denno 2002; Heck et al. 2003; Griffen and Byers 2006; Grabowski et al. 2008). Often, structural differences between adjacent habitats are pronounced and may influence the trophic effects of mobile predators that move between them, reflecting small-scale gradients in prey refuge values and predator foraging efficiencies (Micheli 1997; Ferner et al. 2009; Burkholder et al. 2013; Carr and Boyer 2014; Catano et al. 2016). When structured areas abut more exposed habitats, the strength and even direction of indirect, non-consumptive predator effects can switch between the adjacent refuge and risky habitats even over small scales (Micheli 1997; Trussell et al. 2006).

Alternatively, trophic responses in neighboring systems could be similar since predators and prey often spill over across ecotones into adjacent habitats (Polis and Hurd 1996; Nakao et al. 1999; Tscharntke et al. 2005). Even if the species themselves do not spill over, their non-consumptive effects still may. In one well-documented study, cues from a toadfish predator permeated structurally complex refuge habitat causing their crab prey to reduce feeding even though the crabs had strong physical protection in the refuge (Grabowski and Kimbro 2005). To understand whether species' interaction strengths in adjacent habitats will be similar or different, it is important to know how structural differences between habitats affect these interactions. Furthermore, because organisms interact with structure on a size-dependent basis (e.g., Farji-Brener et al. 2004) and the body size of predators generally increases as trophic level increases (Cohen et al. 1993), increased size differences between predator and prey in longer food chains may accentuate the influence of habitat-associated structure on interaction strengths (DeLong et al. 2015).

Throughout estuaries of the southeastern US Atlantic coast, the Eastern oyster (*Crassostrea virginica*) is a reef-building ecosystem engineer that provides the majority of aboveground hard structure with interstitial living space. Settling oyster larvae are gregarious, attaching themselves to adult oysters, thereby building multi-generational reefs. This reef habitat contrasts starkly with the adjoining mudflat, which is largely devoid of aboveground structure. However, the mudflat, especially adjacent to oyster reefs, often contains scattered oyster shell which can also serve as a settling substrate for oyster larvae. Over time, oysters that settle on these sparse shells can potentially grow into a new reef or coalesce with an existing reef, fostering its expansion. Many estuarine organisms can be found in both the reef and mudflat habitats but at different densities. Blue crabs (*Callinectes sapidus*) move readily between reef and mudflat habitats (e.g., Lenihan and Peterson 1998; Harding and Mann 2010); mud crabs (*Panopeus herbstii*) are less common in unstructured habitat preferring the refuge of oyster reefs (Grabowski and Kimbro 2005; Grabowski and Powers 2004; Grabowski et al. 2008; Bishop and Byers 2015).

Large mobile top predators also readily move between these habitat types, potentially influencing prey distributions and food web dynamics (e.g., Lenihan et al. 2001; Humphries et al. 2011). In particular, bonnethead sharks (*Sphyrna tiburo*) are a very abundant high-level predator in these systems (Ulrich et al. 2007; Belcher and Jennings 2009; Driggers et al. 2014), yet their preference of foraging habitat and their influence on estuarine communities remain largely unknown. Bonnetheads could shape trophic interactions in oyster reef and adjacent communities through both consumptive and non-consumptive effects on lower trophic level organisms. Bonnetheads are specialist predators of blue crabs (Cortes et al. 1996), and aside from humans are ostensibly the primary predator of adult blue crabs. Blue crabs in turn are omnivorous and prey heavily on reef-dwelling invertebrates, especially mud crabs and juvenile oysters (e.g., Eggleston 1990; Hill and Weissburg 2013). Because blue crabs both consume mud crabs and potentially compete with them for some food resources, blue crabs may be viewed as an intraguild predator of mud crabs. Several fish species also consume mud crabs, especially when the crabs are small. Mud crabs themselves feed heavily on lower invertebrates including small oysters (e.g., Grabowski 2004). Thus, bonnetheads are a top predator of a four trophic level food chain (Fig. 1) embedded within a larger estuarine food web. Although lower parts of this food chain have been examined in isolation, by including four trophic levels and examining the food chain across a large predator size spectrum, we can gain a fuller picture of how structure mediates community dynamics. Importantly, the habitat use, foraging preferences and predation efficiencies of bonnetheads and the crab mesopredators may be context dependent, leading to differential, habitat-dependent cascading effects.

In this study, we compared the community-wide influence of a top predator within adjacent habitats that differ greatly in structural complexity. To accomplish this we used a broad regional-scale survey, manipulative field trials and an outdoor mesocosm experiment to quantify how structural differences between adjacent habitats can affect species interactions, potentially mitigating a predatory cascade across

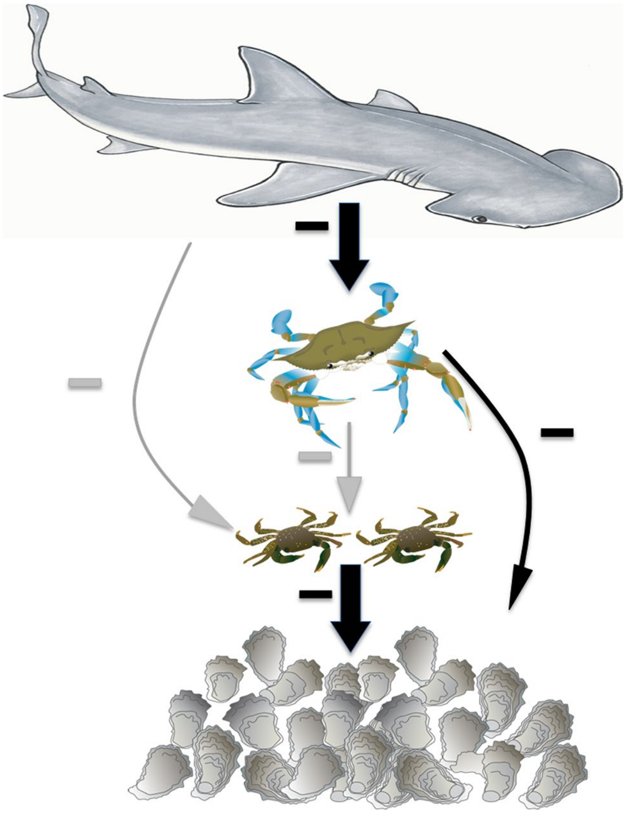

Fig. 1 The trophic interactions studied in Georgia and South Carolina estuaries. Highly abundant bonnethead sharks (*Sphyrna tiburo*) feed heavily on blue crabs (*Callinectes sapidus*). Blue crabs are omnivorous with two of their main prey species being the mud crab *Panopeus herbstii*, and the reef-building eastern oyster *Crassostrea virginica*. *Panopeus herbstii* forages heavily on oysters, particularly of smaller sizes. *C. virginica* are an important ecosystem engineer that provides the majority of hard structure and habitat in southeastern estuaries. For simplicity, only direct effects are depicted. The *lighter* (*gray*) *colored arrow* linking blue crabs to mud crabs indicates the interaction is not as strong and helps explain why predatory effects do not readily cascade downward in all habitats. The *light gray arrow* from bonnetheads to mud crabs denotes the rarity of a consumptive effect. The paucity of mud crabs in the diet of bonnetheads could reflect that mud crabs are a non-preferred prey or that a non-consumptive influence of the shark on mud crabs reinforces the crab's strong and effective association with the structured refuge habitat of the oyster reef. Crab and oyster images courtesy of the Integration & Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/). Bonnethead shark image courtesy of Jen Richards, marine life artist (http://www.jenrichardsart.com)

four trophic levels. Specifically, we asked the following: (1) How abundant are bonnethead sharks on reefs and adjacent mudflats in southeastern estuaries? (2) How does relative predation pressure on blue crabs differ between structured and unstructured habitats? and (3) Do structural differences between the two adjacent habitats affect the strength of cascading predatory effects from bonnetheads through to basal prey oysters?

Methods

Bonnethead abundance and diet

To quantify bonnethead utilization of estuarine habitat across a regional scale, we surveyed four estuaries within the southeastern US that were approximately evenly interspersed along a 300-km domain (Fig. 2). Within each of the four estuaries we selected five intertidal oyster reefs with adjacent mudflat. Reefs were chosen to standardize certain influential environmental variables; thus all reefs were located on tidal creek banks near the mouth of an estuary, had summertime salinity greater than 25 ppt, and had cordgrass (*Spartina alterniflora*) habitat located immediately behind. Reefs had an oyster density of at least 30 adults/m^2 that were at least 5 cm in shell length. The reefs were separated by a minimum of 100 m and located in at least two different tidal creeks. On each reef we marked a permanent 5 m × 5 m sampling area on the creek bank adjacent to the salt marsh habitat behind it.

Beginning in July 2010 we deployed monofilament gill nets (10 m long, 1.22 m high, 7.6 cm mesh size) quarterly for 1 year on each reef during a nocturnal high tide. We set each net to be flush with the substrate and to make a right angle encompassing the reef, with 5 m running along the bottom edge of the reef parallel to the water line, and 5 m running perpendicularly up the tidal creek bank toward the abutting marsh at the high end of the intertidal zone. The interior of this right angle net was oriented to face the incoming tide. We set the nets during a late afternoon low tide so that the nets would fish during the nocturnal high tide and be retrieved as soon as they were exposed on the falling tide. During each quarterly sampling period, all five reefs at a site were sampled on one night, and all four sites were sampled on four successive dates. We counted and measured the length of all bonnetheads and obtained and analyzed the gut contents of a subsample of sharks (41% of all sharks caught) that died in the capture process. We averaged the number of bonnetheads caught per reef within each estuary ($n = 5$ reefs per estuary) in each season and calculated their overall size across all sites.

In-situ blue crab mortality as a function of structure

Because our surveys revealed a high abundance of bonnetheads and a large quantity of blue crabs in their diet, we sought to quantify the susceptibility of blue crabs to predation and how it might be mitigated by the structural refuge of oyster reefs. In summer 2012 at SKIO, one of our surveyed estuaries (Fig. 2), we deployed adult blue crabs on 50 cm tethers, both on oyster reefs and on adjacent mudflat habitat in the low intertidal zone (~0.3 m above MLW). Although there can be artifacts of tethering (e.g., Peterson and Black

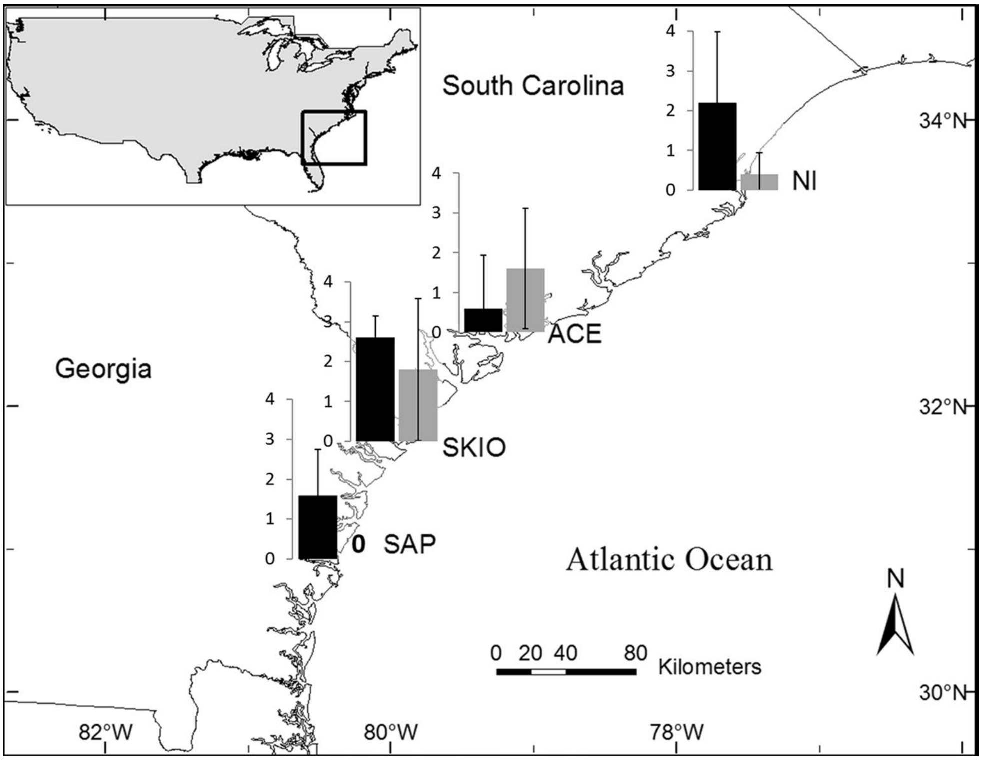

Fig. 2 The South Atlantic Bight USA showing the four estuaries surveyed for predatory fish. *SAP* Sapelo Island, Georgia, *SKIO* Skidaway Island, Georgia, *ACE* Ace Basin, South Carolina, *NI* North Inlet, South Carolina. The *bars* depict the average number of bonnetheads captured on each oyster reef ($n = 5$) during one nocturnal high tide (±SD) for July (*black*) and October 2010 (*gray*). No bonnetheads were captured in January or April 2011, except 0.80 sharks per reef ± 0.84 were captured in April at SKIO

1994), we designed our experiment to minimize these and limited our inferences to only the relative predation risk between treatments. A tether length of 50 cm was chosen to minimize tethering artifacts by allowing crabs to exercise their normal anti-predator behaviors like hiding and fighting, and only eliminating their ability to completely run away or switch habitats. On each deployment, we tethered eight crabs (carapace width = 13.2 ± 1.2 cm; mean ± SD) in each habitat at a chosen location, left them for one tidal cycle (typically overnight), and recorded their survival. Crabs were deployed in this paired manner eight times across different locations throughout the estuary spanning from the Skidaway River (31.99°N, 81.02°W) to Romerly Creek (31.92°N, 80.99°W).

We fitted crabs with a monofilament harness (50 lb-test) created by tying a strangle-snare knot in the monofilament, securing the resulting noose around the two lateral spines of the carapace, and fusing the knot with cyanoacrylate glue. The remaining loose end of the monofilament extended 50 cm to be used as a tether. To assess the integrity of each harness and the well-being of each crab, we held the harnessed crabs in individual containers with flow-through seawater in the lab ~12 h before field deployment. In the field we attached the tethers to 30 cm rebar stakes that had been securely sunk into the sediment. The stakes were set a minimum of 1.5 m apart, such that no crab could come within 50 cm of another crab or another habitat type. To minimize exposure to terrestrial predators and desiccation stress, the stakes were placed at a consistent, low tidal height (~0.3 m above MLW). Also, we deployed crabs within 1 h of low tide and collected them on the subsequent low tide approximately 12 h later.

To examine the mortality of blue crabs as a function of habitat, we used a generalized linear mixed effects model (GLMM) to analyze individual crab survival with a binary distribution and a logit link function as influenced by the fixed effect of habitat (i.e., reef or mudflat). Because the response of crabs within a given deployment batch may not be fully independent, deployment replicate (i.e., date) was used as a random effect. Statistical analyses were performed in SAS v9.4.

Mesocosm experiment to examine predation strengths as function of structure

To examine the strengths of interactions within the four-trophic level food web and how they might be moderated by physical structure, we conducted a mesocosm experiment at SKIO in the summer of 2012. We covered the bottoms of 3 m diameter, 86 cm deep, flow-through tanks with a 2-cm layer of store-bought sand and added four bushels (~90 kg) of dead, sun-bleached oyster clusters to half of each tank to mimic the structure found on living oyster reefs. Though sediment in and around oyster reefs in the field is a mixture of mud and sand, the use of store-bought sand allowed us to maintain similar grain size while avoiding the introduction of any alternative food sources into the tanks. Tanks were outdoors and received full sunlight.

Because survival of basal oyster prey was our primary response variable, each tank contained 14–16 mm juvenile *C. virginica* (also known as spat) adhered to the exterior surface of individual sun-bleached oyster shells (4 spat/shell) using underwater epoxy (Splash Zone A-788). We added 16 such shells (64 spat) to each tank with the attached spat facing up, eight shells within the reef structure and eight in the unstructured sandflat. We arranged the shells in two rows of four in each half of the tank, separating them by a minimum of 50 cm from the tank edge and the reef border. See Appendix S1 for additional details.

We ran six successive randomized blocks (replicates) of five treatments of increasing trophic complexity to quantify the effects of various predator combinations on oyster spat survival. The treatments, which were assigned to tanks randomly during each temporal block, included: oysters only (as a control for non-predation mortality), mud crabs (MC), blue crabs (BC), mud crabs and blue crabs (MC + BC), and the full food chain including a bonnethead (MC + BC + BH). At the end of the blocked trials we also conducted two replicates of a sixth treatment to isolate possible non-consumptive effects of bonnetheads on blue crabs (BC + non-lethal BH). For this treatment, poultry netting (2.5 cm mesh) was installed in the tank just above reef height to prevent physical contact between the bonnethead above it and the blue crabs and oysters below it. To diminish artifacts from intense predator exposure that can occur in enclosure experiments, we kept the trails short at 24 h, at which point the tanks were drained and fully surveyed for oyster and crab survival.

For treatments containing mud crabs, 15 crabs (20–40 mm carapace width) were added to the center of the tank. For treatments containing blue crabs, we added one small crab (8–10 cm carapace width) and one large crab (12–14 cm carapace width). To reduce potential agonistic interactions, two male blue crabs were never included in the same trial. We examined the experimental results to confirm that no cannibalism occurred among the blue crabs (see "Results"). We added blue crabs to the center of the tank 10 min after the mud crabs to allow the mud crabs acclimation time. The MC + BC + BH and BC + non-lethal BH treatments both contained a bonnethead shark. The same shark was used in all replicates (female, 1 m total length). For a full week before the first trial, and for a minimum of 2 days in between every trial, the shark was held in a separate, identical tank with only flow-through seawater. Within this holding tank, we fed her a combination of frozen squid and live small blue crabs, but ceased feeding 24 h prior to any trial. We added the shark to the tank ~3 min after the addition of the blue crabs. One replicate of the oyster-only treatment and one replicate of the MC treatment were discarded because of crab contamination from a previous trial. Also, one replicate of the BC treatment was not run due to a tank malfunction.

We selected the experimental duration and densities of the study organisms mentioned above to be realistic and also to ensure oyster mortality did not commonly approach the boundaries of 0 and 100%. To keep the experiment tractable, the live oyster densities we used were small compared to field values (Byers et al. 2015). Because oysters were at low densities, mud crabs and blue crabs were also scaled back to ~60% of their measured field densities at this site (Gehman et al. 2017; Byers unpublished data).

We used a GLMM to analyze individual oyster spat survival with a binary distribution and logit link function as affected by the fixed factors of trophic treatment, habitat (i.e., reef or sandflat), and trophic treatment × habitat. We also included two nested random variables of block(treatment) and block(treatment) × habitat. The nesting of block in these two variables reflects the split-plot design, accounting for the paired nature of the reef/sandflat data at the level of tank, and thus the grouping structure of oysters (Quinn and Keough 2002). The oyster-only control and BC + nonlethal BH treatments were excluded from this formal analysis because they had 100% survival of all oysters, and thus no variation to analyze. We used two post hoc analyses to contrast how predator effects were influenced by habitat type. First, we tested for significant differences in oyster survival between the two habitats within each trophic treatment. These tests also used block and block(habitat) as random factors. Second, we used two separate GLMM analyses to examine oyster survival across all trophic treatments within each of the two habitats (i.e., reef and sandflat), followed by post hoc Tukey tests to identify significant groupings. These tests used block(treatment) as a random factor. BC + nonlethal BH and oyster-only control treatments were included in this second set of post hoc comparisons, after coding one oyster in each of these two treatments as dead in order to provide a non-zero variability that could allow statistical calculations. Statistical analyses were performed in SAS v9.4 (proc glimmix).

Finally, we sought to determine if structural differences of the two habitats affected interactions among the crab mesopredators (e.g., interference or intraguild predation), resulting in risk reduction (reduced mortality risk) for oysters (Vance-Chalcraft and Soluk 2005). Thus, in the MC + BC treatment we compared observed oyster mortality when the two mesopredators foraged together (i.e., when predator interference potentially occurred) to predicted prey consumption if predators had purely additive effects. Predicted prey consumption when the predators were combined was derived from predation by each species separately using a multiplicative risk model (Soluk 1993). Specifically, we calculated predicted prey mortality rates for each habitat as: $P_{\mathrm{MC}} + P_{\mathrm{BC}} - P_{\mathrm{MC}}P_{\mathrm{BC}}$, where P_{MC} and P_{BC} are the probabilities of prey consumption by mud crabs and blue crabs alone, respectively. We computed probability values using averages of the empirically measured values for each of the relevant treatments. The $P_{MC}P_{BC}$ term accounts for the fact that as prey are consumed they are no longer available to other predators. Predicted mortality probabilities were converted to survival (1-mortality) to enable direct comparison to the format of the experimental results.

Results

Bonnethead abundance and diet

Bonnetheads were abundant on oyster reefs during nocturnal high tides in summer and fall. It was the only fish captured capable of eating adult blue crabs. A total of 58 bonnetheads were caught, 93% of which were in July and October. None were caught in January, and only four in April, all of which were at the SKIO site (Fig. 1). We captured an average of 1.75 bonnetheads per reef ±1.41 (SD) in July and 0.95 ± 1.36 in October. The average total length of the bonnetheads was 1.06 ± 0.15 m (SD) and ranged from 0.63 to 1.44 m. Of the 24 sharks surveyed for gut contents, blue crabs were the predominant prey, with 96% of the sharks having blue crab in their guts. The average number of blue crabs in each shark's gut was 1.38 ± 0.71 (SD); two sharks had three blue crabs in their guts, one had none, and all the others had one or two. In most instances the blue crabs had been swallowed whole, enabling measurement of their size (spine to spine carapace width). The average size of intact crabs was 10.7 ± 2.5 cm (SD). Other diet items complementing the blue crabs were not common: shrimp were found in four sharks, and mud crabs (*P. herbstii*) and a mud snail (*Ilyanassa obsoleta*) in one.

In-situ blue crab mortality as a function of structure

Survival of blue crabs was significantly higher on reefs than on mudflat (Habitat $F_{1,116} = 19.76$, $P < 0.0001$; Fig. 3). The overall survival rate of tethered crabs over one tidal cycle was 63% on-reef compared to 22% on mudflat. Based on forensics of occasional remaining crab parts and the strength required to break tethers, as well as the absence of other candidate large mobile predators from our summer gill net sampling, up to 85% of the crab mortality was estimated to be due to bonnethead predation (mudflat: 92%; on-reef: 70%).

Mesocosm experiment to examine predation strengths as function of structure

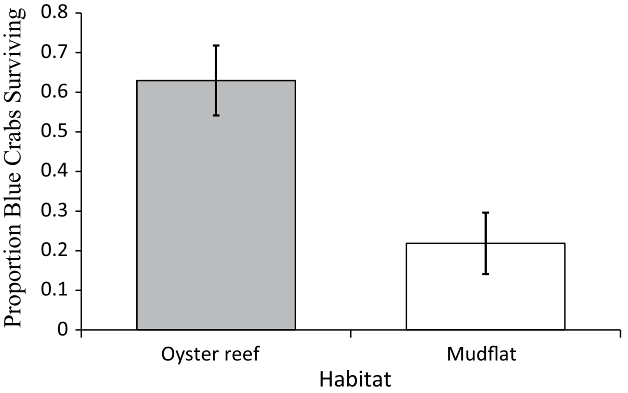

Fig. 3 Survival of blue crabs tethered on oyster reefs ($n = 62$) and mudflats ($n = 63$) within the Wassaw Sound estuary that surrounds the SKIO site. Values depict the mean survivorship (±SE) calculated over the eight replicated paired deployment sets

In the oyster-only treatment, i.e., in the absence of all predators, oysters had complete survival (Fig. 4), underscoring that all observed spat mortality in other treatments was due to predation. The interaction of trophic treatment and habitat had a significant effect on oyster spat survival ($F_{3,18} = 8.74$, $P = 0.0009$; Table 1). In post hoc analyses that isolated the influence of habitat type within a trophic treatment [and included random nested factors of block and block(habitat)], in the mud crab (MC) treatment the spat survival rate was six times higher on the sandflat than on-reef ($t = -4.76$, $P = 0.009$). With blue crabs only (BC), the pattern was reversed, with spat survival rates almost twice as high on-reef compared to the sandflat ($t = 2.6$, $P = 0.06$). When both mud crabs and blue crabs were present (MC + BC), oyster survival was essentially equivalent on-reef and off ($t = -1.12$, $P = 0.31$). When a shark was added to the two crab species (MC + BC + BH), the pattern of oyster survival converged on what it had been when only mud crabs were present (MC), i.e., with significantly high oyster survival on the sandflat and low survival on-reef ($t = -3.16$, $P = 0.025$). An average of 1.2 blue crabs ±0.75 (SD) out of two was eaten when a shark was present; no blue crab mortality occurred in the absence of a bonnethead. Finally, with blue crabs in the presence of a non-lethal bonnethead (BC + non-lethal BH), oysters had complete survival in both habitats (Fig. 4).

Comparing across just the oysters in the sandflat habitat, there was a significant effect of predator treatment ($F_{5,23} = 3.62$, $P = 0.015$). Treatments that contained blue crabs, but not a shark (i.e., BC and MC + BC), had significantly lower oyster survivorship than all the other treatments (Fig. 4; Table S1). Across the on-reef oysters, the overall effect of treatment was also significant ($F_{5,23} = 7.79$, $P = 0.0002$). Treatments for on-reef oysters fell into three groups: BC + non-lethal BH and oysters-only had the highest (complete) oyster survival, BC had intermediate survival, and all treatments with mud crabs had the lowest

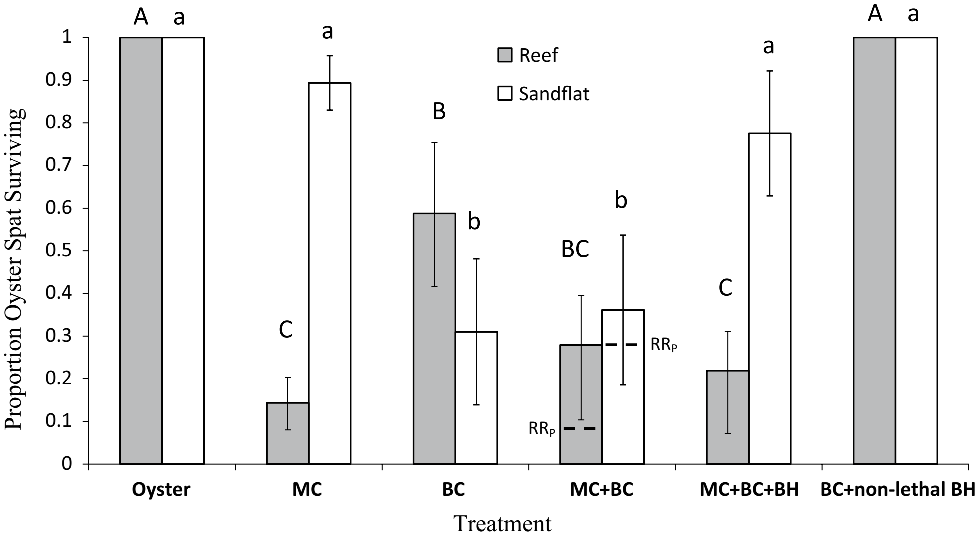

Fig. 4 Survival of oyster spat (±SE) ($n = 1844$) on reef and sandflat within the six trophic treatments used in the mesocosm experiment. Treatment abbreviations are *MC* mud crab, *BC* blue crab, and *BH* bonnethead shark. Although data were analyzed at the level of individual oysters in a GLMM, for easier visualization, means and SE of oyster spat surviving are depicted for each habitat at the level of tank [accounted for in the statistical model as block(trophic treatment)]. No *error bars* are visible on the oyster-only and the BC + non-lethal BH treatments because every oyster in these treatments survived. *Capital letters* above *bars* denote significantly different treatment effects among on-reef oysters; *lower case letters* signify significantly different groupings among sandflat oysters as identified from post hoc comparisons. Oyster survival between each habitat pair within a given trophic treatment was significantly different for MC and MC + BC + BH treatments and marginally significant for BC ($P = 0.06$), when including the random effects of block and block(habitat). The *dashed horizontal lines* on the MC + BC *bars* (labeled RR_P) represent the predicted risk reduction values for each habitat in this treatment. See text for more details

Table 1 Oyster spat survival as affected by the fixed factors of trophic treatment (i.e., mud crabs, blue crabs, mud crabs and blue crabs, or both crab species plus a bonnethead), habitat (i.e., reef or sandflat), and treatment × habitat

Fit statistics				
−2 Res log pseudo-likelihood				7391.7
Generalized χ^2				1065.4
Generalized χ^2/DF				0.77
Random factors		Estimate		Standard error
Block(treatment)		2.43		1.37
Block(treatment) × habitat		2.31		1.02
Type II tests of fixed effects				
Effect	Num DF	Den DF	*F*	*P*
Trophic treatment	3	18	0.83	0.50
Habitat	1	18	18.42	0.0004
Trophic treatment × habitat	3	18	8.74	0.0009

Block(treatment) and block(treatment) × habitat were nested random factors also included in this split-plot generalized linear mixed effects model. Survival was modeled with a binary distribution and logit link function. The variable block(treatment) accounts for the paired nature of the reef/sandflat data at the level of tank and thus provides a statistical grouping structure for the oysters. The oyster-only control and BC + non-lethal shark treatments were excluded from this formal analysis because they had 100% survival of all oysters, and thus no variation to analyze oyster survival (MC, MC + BC, MC + BC + BH) (Fig. 4; Table S1).

Risk reduction for oysters occurred in the presence of both crab predators and was accentuated by structure. With both crab species present (MC + BC) oyster mortality was less than additive, especially on-reef, meaning that the overall mortality rate was lower than predicted based on predation by each crab species separately. Specifically, oyster mortality on-reef was predicted to be 0.92, but was observed to be 0.72 (i.e., survival = 0.28); sandflat mortality was predicted to be 0.72, but was observed to be 0.64 (i.e., survival = 0.36) (Fig. 4). Some dead mud crabs were found in the MC + BC treatment supporting that interference and intraguild predation among the predators improved oyster survival, particularly on-reef where mud crabs' predatory influence on oysters was most apparent.

Discussion

Our manipulations of predators and the isolated structural complexity provided by oyster reefs demonstrated that even in adjacent habitats that overlap in species composition, differences in physical complexity alter the strength of cascading predatory effects. Habitat boundaries commonly mark a sharp transition in environmental context that affects the strength of biotic interactions (Fagan et al. 1999; Burkholder et al. 2013). In the mesocosm experiment, the inclusion of the shark predator more than doubled oyster spat survival on the sandflat (from 36% in MC + BC to 78% in MC + BC + BH); however, in the structured reef habitat, oyster survivorship was low in these treatments regardless of shark presence (Fig. 4).

A large reason for the persistently low oyster survivorship on-reef is that mud crabs seemingly prefer to forage within the oyster reef structure, even in the absence of threat from crab and shark predators, perhaps because of an evolved or learned behavior in response to more concentrated resources or better predator refuge on reefs. When mud crabs foraged alone (MC), spat were approximately six times more likely to die on the reef compared to the sandflat. In contrast, blue crabs foraged everywhere, but with apparent preference for sandflat (BC) (Fig. 4). When blue crabs were added in with mud crabs (MC + BC), predation on oysters was high everywhere, which ostensibly resulted from mud crabs and blue crabs continuing to forage in their preferred areas, with low predator interference. Of the interference that did occur (as inferred from additivity comparisons), there was more on-reef than off, presumably because blue crabs forage on-reef more than mud crabs forage off-reef.

The contrast in sensitivity of the two crab species to the top predator was dramatic. The BC + non-lethal shark treatment had 100% oyster survival, reflecting that blue crabs completely ceased feeding in the presence of a shark (Fig. 4). In fact, the cessation of predation by blue crabs, coupled with our observation that oyster survival in the full predator treatment (MC + BC + BH) was nearly identical to the mud crab only treatment (MC), suggests an indifference or invulnerability of mud crabs to bonnethead presence (Fig. 4). Thus, the strong influence of habitat on cascade strength seemingly arose due to one mesopredator (mud crabs) favoring the more structured reef habitat and being indifferent to the top predator, at least when within the structure of the reef. As a result, there is little cascade on-reef where mud crabs' influence persists regardless of shark or blue crab presence, while the trophic cascade is strong on the sandflat just a meter away where predator-sensitive blue crabs concentrated. Furthermore, because the strong inhibitory effect on blue crab feeding was elicited even when the shark was non-lethal, purely behavioral (i.e., non-consumptive) effects are capable of driving the cascading, indirect benefit on oyster survival.

Because our mesocosms exposed prey to a more sustained bonnethead presence than might be typical in the field, this may have intensified the measured cascade responses. However, even under intensified conditions of the mesocosm, mud crab foraging appeared largely unaffected by all predator treatments, suggesting their effect of short-circuiting the cascade should be robust. In contrast, the cascade intensity we measured off-reef could lessen in the wild if blue crabs do not sustain the same extreme investment in anti-predator behavior under naturally lower, but chronic exposure to sharks. Also, it would be informative to see if the presence of other oyster reef-dwelling MC predators like toadfish (*Opsanus tau*) contributes to the effect on oysters measured here. We predict that in the field, toadfish might depress MC consumption of oysters on reefs through strong consumptive and non-consumptive effects (Grabowski 2004) and that toadfish might show a relative indifference to BH (since we never observed toadfish in bonnethead gut content analyses). A critical future investigation will be to examine whether in the field the natural cascading predatory effects off-reef protect oysters to the same high degree as measured here and if they are sufficiently strong to promote reef expansion. In general, a strong top-down influence of sharks has been commonly seen throughout many marine ecosystems (Ferretti et al. 2010; Heithaus et al. 2012). Namely, sharks often exert consumptive and non-consumptive effects on mesopredators, sometimes driving trophic cascades, and shaping marine communities over large spatial and temporal scales.

In addition to the large behavioral effects of bonnetheads on blue crabs, large consumptive effects were also very apparent, as observed in our mesocosm experiment, field surveys, and tethering trials. Over half the blue crabs exposed to the shark in the mesocosm experiment died and over half of tethered crabs died over 12 h in the field. Our gill net surveys found blue crabs to be the predominant food for bonnetheads, with an average of 1.4 adult blue crabs in every gut (see also Cortes et al. 1996; Lessa and Almeida 1998). The surveys, although a conservative measure of relative shark abundance (since each net likely captures only a fraction of all bonnetheads visiting a reef), still caught an average of 1.75 bonnetheads per reef in summer and these averaged over 1 m in length. Given what our surveys and related regional surveys reveal about the bonnetheads' high relative abundance, activity level, and large per capita biomass (Thorpe et al. 2004; Belcher and Jennings 2009; Driggers et al. 2014), the collective caloric demand of the sharks must be substantial.

The tethering experiments further highlight blue crab vulnerability to the consumptive effects of predators, but also show that blue crabs can benefit from the structure of the oyster reef as a predator refuge in a field setting. Over just one tidal cycle the overall survival rate of tethered crabs was threefold higher on-reef compared to the adjacent mudflat (Fig. 3). Variation in survival within each habitat type across deployment replicates that differed in space and time is likely attributable to at least two factors. First, shark presence and activity likely differed across deployment locations due to factors like distance from the ocean, proximity to human activity, and abiotic attributes. Second, the structure and quality of the oyster reef likely differed at the site of each deployment, presumably influencing its effectiveness as a refuge.

However, even though the oyster reef may provide blue crabs with protection from lethal effects of predators, our mesocosm experiments demonstrate that non-consumptive effects of bonnetheads can be sufficiently strong to limit the blue crab's trophic effects on the reef. That is, despite the lowered vulnerability of blue crabs on the reef, non-consumptive effects extend the shark's debilitating influence into the refuge habitat. The proximity and concentration of olfactory and other cues necessary to enact these non-consumptive effects in a field setting are crucial to consider if the shark is to retain its stark effect on blue crabs in the wild, both in the immediate study area, as well as in other oyster reef communities of the southeastern US. In the Georgia and South Carolina estuaries studied here, high tidal amplitude generates deep water over reefs that facilitate access by large-bodied predators like bonnetheads. Underscoring this point, no bonnetheads were captured on intertidal oyster reefs in a similar year-long companion survey in North Carolina and Florida (Grabowski et al. unpublished data) where high-tide inundation is roughly half as deep (Byers et al. 2015). The degree to which non-consumptive effects of predators extend into refuge habitat could differ based on properties of the physical environment and predator proximity (e.g., Weissburg and Zimmerfaust 1993; Smee et al. 2008; Wilson and Weissburg 2013). This could be another important factor contributing generally to large-scale spatial variation in interaction strengths and trophic cascades.

In summary, our mesocosms demonstrate that on unstructured sediment adjacent to oyster reefs bonnethead sharks can positively affect oysters. By readily consuming blue crabs and shutting down their predatory behavior, sharks ameliorate a large source of oyster mortality at the periphery of the reefs. Higher oyster survival in adjacent mudflats could be a mechanism that fosters expansion of the reef's footprint. However, on-reef, the net effect of sharks on oysters is minimal. Even though blue crabs may attempt to shelter within the relative safety of the reef structure and this would seemingly intensify their predatory effects there, non-consumptive effects of the sharks can stop their feeding, which has a direct positive effect on oysters. But this influence is muted by the fact that the majority of oyster mortality on-reef is caused by mud crabs that appear relatively indifferent to shark or blue crab presence. The net result is an interesting spatial juxtaposition of contrasting strengths of cascading predatory effects in adjacent habitats. Mechanistically the contrast arises because of the apparent invulnerability of a mesopredator (MC) that has a strong affinity for structured habitat. We suggest that ecotones and habitat transition zones are likely places to see such contrasts given the continuity of species composition across a rapidly changing structural and environmental context.

Acknowledgements We thank J. DeVore, R. Smith, M. Walker, H. Weiskel and C. Yeager for field assistance and. J. Grabowski, R. Hughes, D. Kimbro, and M. Piehler for consultation and input. We are grateful to the staff at the Skidaway Institute of Oceanography for their support. Our work was supported by NSF-OCE-0961853 (Directorate for Geosciences). The study and procedures were approved by the UGA Institutional Animal Care and Use Committee.

Data accessibility Data from this work is archived in Dryad Digital Repository. doi:10.5061/dryad.429t4.

Author contribution statement JB obtained funding and conceived the experiments. ZH and JM performed the experiments. JB analyzed the data. JB and ZH wrote the manuscript; JM provided editorial advice.

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