

Emergent intraspecific multiple predator effects shape estuarine trophic dynamics across a gradient of habitat complexity

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ABSTRACT

Estuarine ecosystems are characterized by heterogeneity in species assemblages and habitat complexity, so prey in these systems are often threatened by multiple predators across a mosaic of habitats. When several predator species or conspecifics co-occur, behavioral interactions between them can introduce non-independence to their predator-prey dynamics, which can alter the fate of shared prey. Habitat complexity may regulate these interactions and further impact prey survivorship. We conducted an experiment that manipulated the densities and identities of two ecologically similar estuarine-dependent predators, Red Drum (*Sciaenops ocellatus*) and Spotted Seatrout (*Cynoscion nebulosus*), within three habitat complexities. The resulting survivorships of two shared prey types were utilized to determine whether these predators are substitutable, to examine their inter- and intra-specific trophic interactions, and to investigate the impact of habitat structural complexity on their trophic dynamics. The predators differed in their prey preferences and predation pressures, indicating they are generally not substitutable. Interactions between conspecific predators were particularly important in shaping trophic dynamics, and often resulted in combined predation impacts that either enhanced or reduced the survivorship of shared prey as compared to expected values based on foraging rates of individuals. Minimal evidence of interspecific trophic interactions was detected. The observed emergent multiple predator effects differed among the habitats, and were generally intense in intermediate habitat complexity but were either intense or weak in high habitat complexity, depending on the predator combination. These results collectively indicate that prey regulation in this estuarine trophic system is highly dependent on the predator-prey assemblage and habitat context, and enhances our understanding of how multiple predators interactively shape their shared ecosystem.

1. Introduction

Prey populations are often threatened by multiple predator species, and must respond to a variety of different predator densities and identities. In some instances, multiple predators can be functionally identical if their foraging strategies, prey preferences, and the pressures they place on shared prey are similar and independent (Hixon and Carr, 1997; Sokol-Hessner and Schmitz, 2002). However, the co-occurrence of multiple predators can lead to non-independent (i.e., non-additive, non-multiplicative) predation impacts compared to single-predator scenarios, which are referred to as emergent multiple predator effects (MPEs, Sih et al., 1998). The predator-prey and predator-predator interactions leading to MPEs can be influenced by habitat structural complexity (e.g., Grabowski et al., 2008), which further complicates trophic interactions in heterogeneous ecosystems. Habitat-mediated

MPEs are thus likely to be important factors in the distribution of predation pressure.

When emergent MPEs are observed, they result in two potential outcomes, risk reduction and risk enhancement. Risk reduction can occur when antagonistic behaviors or interference interactions among multiple predators result in greater prey survivorship than would be expected based on single predators foraging alone. Previously observed interference interactions have included both direct and indirect mechanisms, such as intraguild predation, physical contact between predators, prey-switching, and alterations of prey behavior (Crowder et al., 1997; Siddon and Witman, 2004; Vance-Chalcraft and Soluk, 2005; Grabowski et al., 2008; Booth et al., 2018). Alternatively, facilitation between predators can occur, which results in predation risk enhancement and decreased survivorship of prey. Although facilitation is less commonly observed than interference, it can occur when prey behavior

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is affected by the presence of one predator in a way that increases its vulnerability to the other predator (Soluk, 1993; Fodrie et al., 2008). MPEs can emerge in both inter- and intra-specific scenarios, either when two predator species co-occur or when multiple conspecifics are present (Griffen, 2006). High predator species diversity can increase the amount of prey that are consumed in an ecosystem through resource complementarity, while resource use is often limited by intraspecific competition at high conspecific predator densities (Northfield et al., 2010). The specific mechanisms driving emergent MPEs differ with the species involved, but the resulting impacts are critical for understanding trophic dynamics in ecosystems where multiple predators interact with shared prey populations.

In addition to their diverse predator-prey assemblages, estuarine ecosystems typically exhibit spatial and temporal heterogeneity in habitat structural complexity. Mud and sandflats are common and lack conspicuous biogenic structure, while a variety of structured habitats interrupt the dominant mud/sand matrix. Biogenic habitats such as submerged aquatic vegetation (SAV), oyster reefs, and emergent marsh edges often occur alongside artificial man-made structures such as docks, pilings, and artificial reefs. Many estuarine-dependent species of predators and prey rely on some or all of these structured habitats for foraging grounds or refuge space, with biogenic habitats in particular often supporting dense and speciose communities (Summerson and Peterson, 1984; Heck et al., 2003).

Habitat structural complexity can influence predator-prey and predator-predator interactions through a variety of mechanisms. Predation intensity can be reduced in high-complexity habitats when the ability of a predator to locate or capture prey is diminished by structural elements (Crowder and Cooper, 1982; Peterson, 1982; Diehl, 1992; Grabowski, 2004). Alternatively, since both predator and prey density often increase with increasing habitat structure, predation pressure can remain constant or become greater in high-complexity habitats (Mattila et al., 2008). Despite the general value of high-complexity habitats as refuge for prey, habitat edges are often sites of increased predation rates as compared to structured habitat interiors in many marine systems (Bologna and Heck, 1999; Smith et al., 2011). Additionally, high habitat complexity can reduce encounter rates between predators, which can alter behaviors and predation intensities (Swisher et al., 1998; Grabowski and Powers, 2004; Warfe and Barmuta, 2004). Therefore, increasing habitat structural complexity can both strengthen and dampen MPEs, or can shift the direction of the effect, depending on the observed ecological and behavioral interactions (Swisher et al., 1998; Finke and Denno, 2002; Grabowski et al., 2008).

When two predators share similarities in diet and habitat use patterns, they might exhibit frequent or intense competitive interactions. Two estuarine-dependent mesopredator teleost fish species, Red Drum (RDM, *Sciaenops ocellatus*) and Spotted Seatrout (SPT, *Cynoscion nebulosus*), have overlapping ranges in the northern Gulf of Mexico and U.S. Atlantic coasts. Both species are demersal predators, and spend most or all of their life history within estuarine and coastal ecosystems (Pearson, 1929). Subadult RDM (age 1–3) associate with shallow seagrass beds and habitat edges (but see Mahoney et al., 2018), as well as oyster reefs, saltmarshes, and sand flats (Bacheler et al., 2009; Dance and Rooker, 2015; Fodrie et al., 2015; Moulton et al., 2017). Subadult and early adult SPT (age 0–2, of similar size to age 1–3 RDM) associate with seagrass beds to some degree, but they appear to mainly utilize unvegetated substrate and loose shell bottom (MacRae and Cowan, 2010; Moulton et al., 2017).

The diets of both species at their subadult to early adult life stages include penaeid shrimp and small finfish. RDM are considered generalist predators, but are known to prey heavily on crustaceans such as shrimp and crabs in the genus *Callinectes* (Overstreet and Heard, 1978; Scharf and Schlicht, 2000). SPT exhibit a distinct dietary ontogenetic shift, becoming more piscivorous as they transition to adulthood (Darnell, 1958; Wenner and Archambault, 1996). Few studies have explicitly compared the amount of dietary overlap between RDM and

SPT. Shaw et al. (2016) determined with stable isotope analysis that within a community of predators in a South Carolina estuary, subadult and adult RDM and SPT exhibited no isotopic niche overlap. However, their result is likely an overestimate of the degree of trophic partitioning between these species, considering separate investigations of their dietary habits have found some of the same prey species being consumed by both RDM and SPT (e.g., Overstreet and Heard, 1978; Darnell, 1958). Though most of the prey that these species consume is common and should not be limiting, competition over evolutionary timescales (and/or during periods of prey limitation) may have been influential in determining their present trophic niches.

Considering the distinct similarities and differences in diet and habitat use of these two predators, both ecological interactions and resource partitioning are likely to occur, making them interesting candidates for investigations of MPEs and trophic dynamics. This is especially true at their subadult to early adult life stages, when they are both estuarine-dependent and likely to co-occur and consume similar prey. The purpose of this study was to examine predator-prey and predator-predator interactions among subadult RDM, SPT, and two shared prey items in the context of differing assemblages (varying density and identity of predators) and habitat complexities. A mesocosm experiment was conducted to determine the following: (1) are these predators substitutable in their predation rates and prey preferences, (2) do they exhibit antagonistic or facilitative inter- or intra-specific behavioral interactions that influence their combined predation impacts, and (3) does habitat structural complexity alter their interactions and ultimately impact the survivorship of their shared prey?

2. Methods

2.1. Experimental design

The experiment included two predators, RDM and SPT, and two prey types, an invertebrate (White and Brown Shrimp, *Litopenaeus setiferus* and *Farfantepenaeus aztecus*) and a small finfish (Gulf Killifish, *Fundulus grandis*). These prey types provided options for the predators, with the possibility that prey selection could be affected by predator density or identity. Both prey types are common and abundant in estuaries throughout the Gulf of Mexico, and are representative of the variety of organisms that are consumed by these predators (finfish and invertebrates). Subadult RDM (324.19 ± 5.17 mm SL, 0.57 ± 0.03 kg weight, mean ± 1 SE) and SPT (313.35 ± 4.58 mm SL, 0.43 ± 0.02 kg weight) were collected using hook and line in Mobile Bay and Mississippi Sound, AL, between February and October 2017. Fishes were transported in aerated coolers to the Dauphin Island Sea Lab, where they were held with conspecifics in 2.5 m diameter outdoor, shaded tanks. Each tank received flow-through water from the Gulf of Mexico, and was aerated with one large airstone. Fish were acclimated to holding tanks for at least 3 days prior to use in experimental trials, and were fed cut squid (~3 oz. per individual) every 48 h. Killifish (61.81 ± 0.32 mm SL) were obtained using minnow traps in Mobile Bay, while Brown and White Shrimp (103.20 ± 0.45 mm TL) were obtained from local live bait shops. Prey were held in indoor tanks, with recirculating water at a salinity of approximately 20 psu, and were provided pelleted feed daily.

Multiple predator effects were examined using both an additive and substitutive design to capture both inter- and intra-specific interactions (Sih et al., 1998; Griffen, 2006; Byrnes and Stachowicz, 2009). The additive design included three predator treatment levels, 1 RDM, 1 SPT, and 1 RDM + 1 SPT, plus a predator-free control. The substitutive design held total predator density constant at 2, including a treatment with 2 RDM and another with 2 SPT (plus the 1 RDM + 1 SPT treatment and the predator-free control). Thus, our overall design included 6 unique predator treatment combinations (1 RDM, 1 SPT, 2 RDM, 2 SPT, 1 RDM + 1 SPT, Control). Each treatment received 8 individuals of each prey type (16 prey individuals total).

Treatment levels were randomly assigned to 6 experimental tanks using a random number generator, with one of each predator level running concurrently. Experimental tanks (2.5 m diameter, 0.75 m water depth) contained a 1-cm deep layer of clean sand, and a 0.25 m tall PVC standpipe in the center with mesh netting to secure the drain. Tanks received ambient flow-through water and aeration, and were monitored during each trial for temperature, salinity, and dissolved oxygen once during the day (after the start of the trial) and once at night (approximately 1 h. after sunset) using a YSI, Inc. Pro2030 handheld sonde. Predators were acclimated in experimental tanks for 24 h prior to trials, during which they were starved to standardize hunger. Prey were haphazardly assigned to tanks, and all individuals were measured (standard length for killifish, total length for shrimp) and acclimated in the experimental tank in a floating cage for at least 15 min. Trials began around midday, and predators were allowed to forage for 24 h. All predators were then quickly removed after draining each experimental tank. Surviving prey were recovered by thorough examination of the sediments, and any structures in the tanks were removed to ensure complete recovery of prey. The number of each prey type remaining in the tank was recorded. Lengths, weights, and stomach contents of each predator were then extracted and recorded. Neither predators nor prey were reused, and no within-species differences in size (length or weight) were observed among trials.

To investigate the role of habitat complexity, this experiment was repeated three times. The first round of trials was conducted without structure in April and May, and included 6 replicates of each predator combination (hereby referred to as “no structure”). A second round was conducted in June and July (6 replicates), with tanks containing the same amount of sand and two square 0.25 m² patches of artificial seagrass (similar in leaf height and width to turtlegrass, *Thalassia testudinum*) at a density of 650 shoots per 0.5 m², and two leaves per shoot (c.f. [Byron and Heck, 2006](#), hereby referred to as “SAV”). The patches were weighted down with three flat weights (total of 7 oz.), and buried within the sediment on opposite sides of each tank in a North-South orientation. The addition of seagrass was intended to provide structural refuge for the prey ([Heck et al., 2003](#)). A third round of trials was conducted in October and November, during which greater habitat complexity was included (6 replicates) and 2 extra replicates of each of the two other habitats were interspersed. Since the no structure and SAV habitat trials were conducted earlier in the year, these two additional replicates were intended to account for differences in seasonality among the habitats. High structural complexity tanks contained the same amount of sand and artificial seagrass as previous trials, with the addition of two small artificial reefs to mimic structures with vertical relief spanning the height of the water column (hereby referred to as “SAV + Reef”). Reefs consisted of two cinderblocks, with one placed horizontally on the sand and the other stacked vertically on top of the horizontal cinderblock. The two reefs were placed on opposite sides of each tank between artificial seagrass patches, in an East-West orientation. These reefs were intended to provide additional refuge for prey or foraging space for predators.

2.2. Data analysis

Data were analyzed using generalized linear models with a binomial error distribution. Survivorship was modeled as a proportion of successes (survived) to failures (killed), which accurately represents the survivorship response, since it assumes the data exists between 0 and 1. Since all killifish were recovered from all controls, resulting in a mean survivorship of 1 with no variance, the control killifish survivorship value of the first replicate of each habitat was changed from 1.0 to 0.875 (a false killing of one individual, which is a conservative manipulation relative to Type I statistical error in comparing different treatments) to allow the models to operate. Separate generalized linear models (binomial distribution) were constructed for each prey type in each habitat, and included only the effect of predator treatment on prey

Table 1
Average abiotic parameter values across habitat complexities (mean ± 1 SE).

Habitat	Temperature (°C)	Salinity (psu)	Dissolved oxygen (mg/L)
No structure	22.4 ± 0.3	19.4 ± 0.5	7.6 ± 0.1
SAV	25.6 ± 0.6	17.8 ± 0.9	6.8 ± 0.1
SAV + Reef	22.1 ± 0.3	22.9 ± 0.6	7.2 ± 0.1

survivorship. Significance of pairwise differences across predator treatment levels was tested using profile-likelihood 95% confidence intervals based on the chi-square distributed likelihood ratio test ([Venzon and Moolgavkar, 1988](#)). Direct, quantitative comparisons of habitats were avoided, since differences in abiotic conditions among the habitats were observed (see Results, [Table 1](#)).

Emergent multiple predator effects were examined by comparing the observed results to a null expectation that the predators produce independent effects on prey. Since prey depletion was not prevented, the expected combined effect of the two predators cannot be assumed to be additive. Expected prey survivorship was thus calculated using a multiplicative risk model (MRM), which predicts the independent combined effects of two predators based on single-predator trials, while accounting for the issue of prey depletion by not allowing prey to be consumed twice ([Sih et al., 1998](#)). Under the additive design, the expected proportional survival of prey when exposed to both predator species together was calculated as:

$$E_{1,2} = (P_1 \times P_2)/P_c$$

where P_1 is the proportion surviving one individual of predator 1, P_2 is the proportion surviving one individual of predator 2, and P_c is the proportion surviving in the no-predator control. This version of the MRM includes background mortality not imposed by predators, which did occur in some control trials, and is thus preferable over other MRM equations ([Griffen, 2006](#)). Similarly, the expected proportional survival of prey when exposed to two conspecifics can be calculated as:

$$E_{i,i} = (P_i \times P_i)/P_c$$

where P_i is the proportion of prey surviving one individual of predator i , and P_c is the proportion surviving in the no-predator control. In this case, the results from the additive model can be used to predict intraspecific interactions in the substitutive model. An expected survivorship value was calculated for each individual replicate, resulting in an expected value to correspond with all observed survivorship values for multiple-predator trials.

Although emergent multiple predator effects have generally been investigated by comparing data from the additive experimental design to the null MRM using two-way ANOVAs ([Sih et al., 1998](#)), assumptions of ANOVA (normality in particular) were consistently violated using this method in our study. Both inter- and intra-specific interactions were instead examined by directly comparing the calculated expected survivorship values under the null MRM to the corresponding observed survivorship value using paired t -tests. A significant paired t -test indicates non-independence in the combined effects of the predators (observed is greater or less than expected). All t -tests correspond with previously described linear models, and fit Shapiro-Wilk normality parameters. This methodology allowed for the same statistical test to be conducted for both inter- and intra-specific scenarios, and required no transformations of the data.

To determine whether these predators exhibited preferences for either prey type, killifish survivorship was subtracted from shrimp survivorship for each trial. With all habitats pooled, a one-way ANOVA was performed on the difference between shrimp and killifish survivorship as the response, and predator treatment as the explanatory factor. A negative difference would indicate that killifish survivorship was greater than shrimp survivorship, suggesting that shrimp was the preferred prey type, while a positive difference would indicate killifish

was preferred. The control level, which exhibited very low variance and was not important for determining prey preference, was not included in this analysis. Model residuals were confirmed for the assumption of normality with a Shapiro-Wilk test, and homogeneity of variances among groups was confirmed with a Levene's Test. Post-hoc pairwise comparisons were conducted with a Bonferroni correction to control the Type I error rate during multiple comparisons.

Differences between the stomach contents of predators in trials with 2 individuals were analyzed to determine whether single individuals dominated the observed predation. For trials with 2 conspecifics, the number of prey (shrimp or killifish separately) in one individual's stomach was subtracted from the number of that prey type in the other predator's stomach, then converted to its absolute value. For interspecific trials, SPT stomach contents were subtracted from RDM stomach contents for each prey type separately; positive values indicated RDM dominance, while negative values indicated SPT dominance. One-sample *t*-tests were then performed for each prey type in each habitat. For conspecific predator combinations, data were compared to a null mean of 1 to allow for a slight baseline level of deviation between individuals. Since the data were allowed to remain positive or negative for interspecific trials, differences were compared to a null mean of 0, indicating no dominance by either species. Data were confirmed for the assumption of normality with Shapiro-Wilk tests.

Abiotic parameters (temperature (°C), salinity, and dissolved oxygen (mg/L)) were compared across habitats and predator treatment levels using Kruskal-Wallis rank sum tests, since each parameter was non-normally distributed. Additionally, to determine whether differences in abiotic predictors across the habitats influenced prey consumption, generalized linear models (quasi-poisson error distribution) were utilized to determine whether individual abiotic parameters impacted the number of surviving individuals for each prey type. The quasi-poisson distribution was chosen since the response consisted of overdispersed count data. For each abiotic parameter (temperature, salinity, or DO), a model was run that included the number of surviving prey (0 to 8) as the response, and one of the abiotic parameters, habitat complexity, and the interaction between them as the explanatory variables. All analyses were conducted in the open-source statistical software R (R Development Core Team, 2008, version 1.1.383) using the R packages MASS, stats, and ggplot2.

3. Results

3.1. Multiple predator effects

No emergent multiple predator effects, either intra- or inter-specific, were detected for shrimp or killifish in the no structure habitat (Fig. 1A,B). However, species interactions appeared to be more complicated in trials with higher habitat complexity. In SAV, both shrimp and killifish experienced risk enhancement when exposed to 2 RDM (Fig. 2A,B, shrimp $t(7) = 2.70$, $p = 0.03$, mean difference = 0.06, killifish $t(7) = 3.24$, $p = 0.01$, mean difference = 0.39). Killifish also experienced risk reduction when exposed to 2 SPT (Fig. 2B, $t(7) = -3.13$, $p = 0.02$, mean difference = -0.34). In SAV + Reef, no emergent effects were observed for killifish (Fig. 3B), but shrimp experienced risk enhancement when exposed to 2 RDM (Fig. 3A, $t(5) = 2.70$, $p = 0.04$, mean difference = 0.37). The only interspecific emergent multiple predator effect within the habitats was observed for shrimp in SAV + Reef, when they experienced marginally significant risk enhancement (Fig. 3A, $t(5) = 2.402$, $p = 0.06$, mean difference = 0.27).

3.2. Predator impacts

In the no structure habitat, shrimp survivorship decreased with the addition of a second RDM (model predicted value: 0.11) as compared to 1 RDM alone (0.31), while the impact of 2 SPT (0.59) did not differ

significantly from 1 SPT (0.73, Fig. 1A). For killifish in no structure (Fig. 1B), adding a second RDM decreased survivorship (0.41) as compared to 1 RDM (0.73), but survivorship did not differ between 1 SPT (0.63) or 2 SPT (0.63). For shrimp in SAV (Fig. 2A), adding a second conspecific decreased survivorship as compared to the single predator alone (1 RDM (0.22) vs. 2 RDM (0.02), 1 SPT (0.61) vs. 2 SPT (0.39)). Killifish in SAV (Fig. 2B) experienced lower survivorship when exposed to 2 RDM (0.17) as compared to 1 RDM (0.70), but no difference in survivorship was detected between 1 SPT (0.56) or 2 SPT (0.70). For shrimp in SAV + Reef (Fig. 3A), adding a second conspecific consistently decreased survivorship as compared to the single predator alone (1 RDM (0.56) vs. 2 RDM (0.02), 1 SPT (0.73) vs. 2 SPT (0.52)). Survivorship of killifish in SAV + Reef (Fig. 3B) was also lower when exposed to 2 conspecifics as compared to 1 (1 RDM (0.67) vs. 2 RDM (0.40), 1 SPT (0.69) vs. 2 SPT (0.42)). Survivorship did not differ between 1 RDM (0.67) and 1 SPT (0.69), or between 2 RDM (0.40), 2 SPT (0.42), and the combination of both species (0.27).

3.3. Prey preference and stomach contents

Single RDM exhibited a distinct preference for shrimp (mean of shrimp – killifish survivorship = -0.36), while single SPT exhibited a lack of preference that fell slightly towards killifish (mean = 0.07, Fig. 4, $p < 0.01$ between 1 RDM and 1 SPT). Preference did not differ between 1 RDM (-0.36), 2 RDM (-0.27) or the interspecific combination (-0.21), indicating that the preference of RDM for shrimp was not affected by the identity of a predator when one was added to the system. However, while the lack in prey preference of SPT was not significantly affected by the addition of a conspecific (1 SPT mean = 0.07, 2 SPT mean = -0.09), the addition of a RDM significantly shifted the prey preference of the overall predator field towards shrimp (mean 1 RDM / 1 SPT = -0.21, Fig. 4, $p = 0.02$ between 1 SPT and 1 RDM / 1 SPT).

Based on the analysis of the predators' stomachs, there was little evidence of single individuals dominating feeding between conspecifics, and no evidence of either predator dominating in interspecific trials for either prey type in any of the habitats. The only exceptions are as follows: The difference in the number of prey items between 2 RDM stomachs deviated slightly from 1 for shrimp in SAV + Reef (mean = 2.83, $p = 0.07$), and for killifish in SAV (mean = 2.38, $p = .07$).

3.4. Impact of abiotic parameters

Since trials were conducted over multiple seasons, with unaltered water and exposure to weather, abiotic parameters differed between at least one of the habitats ($p < .01$ for each parameter). Temperature was highest in SAV trials, salinity was highest in SAV + Reef trials, and DO was lowest in SAV trials (Table 1). The number of surviving shrimp or killifish was generally not affected by any of the abiotic parameters. The only exception was the number of surviving killifish being marginally affected by the interaction between salinity and habitat complexity ($p = 0.06$). This indicates that the differences in abiotic conditions across the habitat complexities did not strongly influence survivorship, but it is possible that the interactive effect of habitat complexity and abiotic conditions on prey consumption are partially confounded. We do not suspect, however, that differences in predation pressure across the habitats influenced our conclusions regarding patterns of emergent multiple predator effects.

4. Discussion

The results of this experiment indicate that although RDM and SPT co-occur and share similarities in their diets and habitat use patterns, they are largely not substitutable predators. They exhibited distinct differences in prey preference, most notably when they foraged alone.

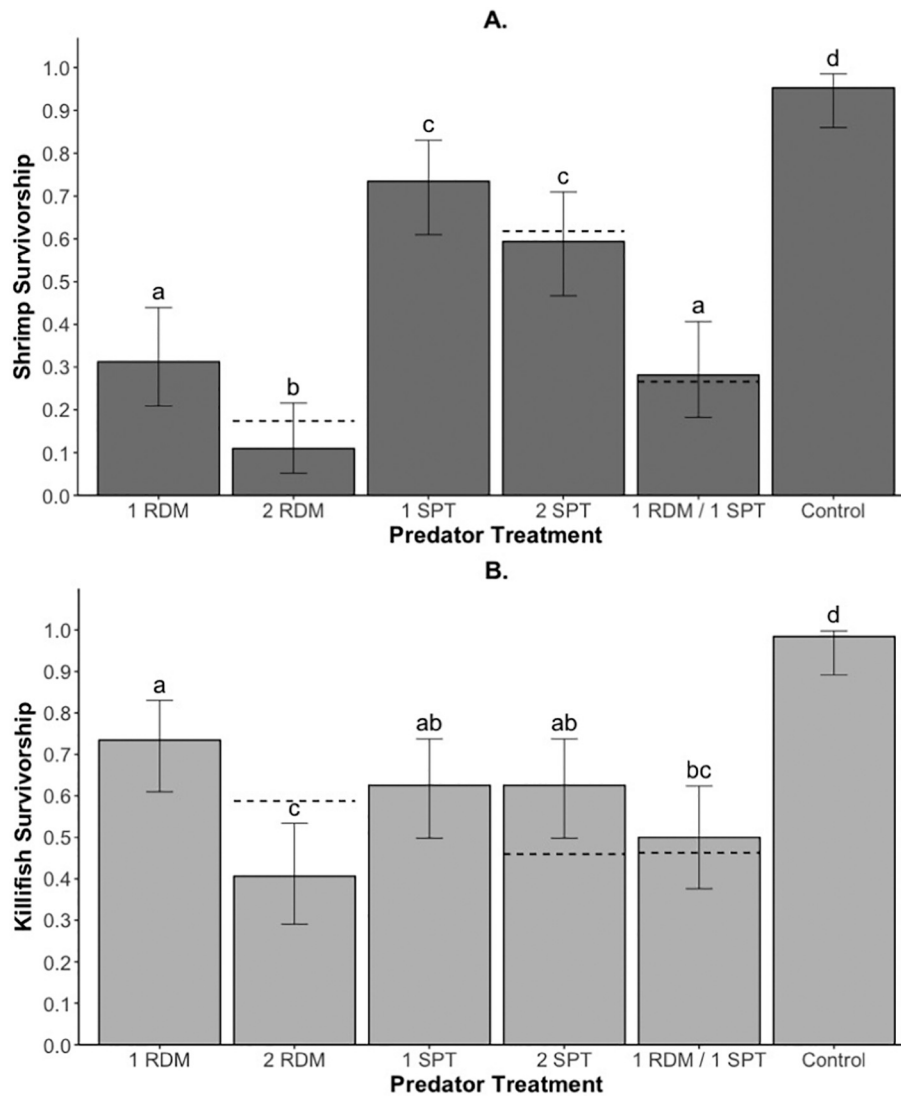


Fig. 1. Predicted values and 95% confidence intervals from binomial models describing the no structure habitat. Letters in (A) and (B) represent significance among predator treatment levels for shrimp and killifish, respectively, and dashed lines denote average expected survivorship from the multiplicative risk model.

Additionally, RDM enacted greater predation pressure than SPT in most cases, especially for their preferred prey (shrimp). Intraspecific interactions were particularly important in shaping prey survivorship, and often led to non-independent predation impacts when two conspecifics were present. Although statistical comparisons among habitats were not conducted, qualitative observations revealed complex patterns of emergent MPEs that differed across the habitats. Non-independent impacts were generally intense in intermediate habitat complexity, and either intense or weak in high habitat complexity. Our results indicate that prey regulation in this estuarine trophic system likely varies spatially and temporally, depending on the predator-prey assemblage and habitat.

Risk enhancement has generally been considered uncommon in most systems, because prey can usually increase or alter their anti-predator behaviors when multiple predator species are present (Sih et al., 1998). Additionally, when multiple conspecific predators co-occur, their use of similar or identical foraging strategies usually results in risk reduction due to intense or frequent negative interference interactions (Clark et al., 1999; Abrams and Ginzberg, 2000). However, the detection of risk enhancement can differ across experimental designs, with substitutive experiments in particular frequently resulting in positive diversity effects (Griffin et al., 2013). This study utilized both additive and substitutive methods, so it should not be biased by

experimental design choice. We observed unexpected risk enhancement for both penaeid shrimp and Gulf Killifish when two RDM foraged together, suggesting a lack of negative predator-predator interactions and an inability of the prey to adjust their anti-predator defenses at this predator density. We caution that this risk enhancement resulted in extreme depletion of prey in some trials, which can potentially bias estimates of MPEs, even when the multiplicative risk model is utilized (McCoy et al., 2012). Further investigations of the functional responses of RDM and SPT would enhance the conclusions of this study.

Since the experimental trials were not able to be visually monitored or recorded due to consistently high natural turbidity, it is difficult to determine the cause of the observed risk enhancement. One possibility is that chemical cues released via tissue damage during a predation event by one RDM may have alerted the other predator to the availability of foraging opportunities. Chemical cues that result from bodily damage can attract additional predators or increase predator activity levels, which could result in enhanced predation when interference behaviors are not observed (Mathis et al., 1995; Moore and Lepper, 1997; McCarthy and Dickey, 2002). In trials with two RDM, there was an especially high occurrence of abandoned portions of shrimp found in the tanks, indicative of incomplete and chaotic predation events which could have created cues that enhanced collective foraging success.

Similar intraspecific facilitation and synergism has been observed

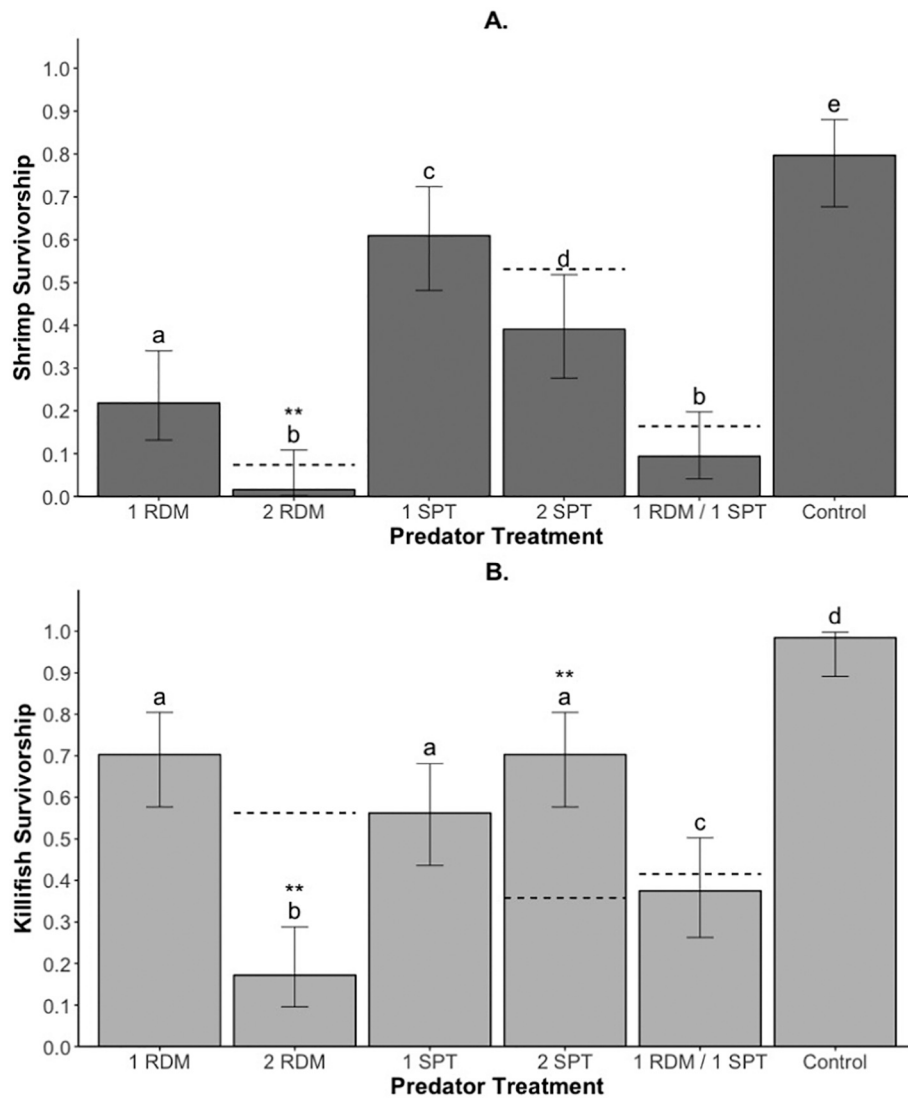


Fig. 2. Predicted values and 95% confidence intervals from binomial models describing the submerged aquatic vegetation (SAV) habitat. Letters in (A) and (B) represent significance among predator treatment levels for shrimp and killifish, respectively, dashed lines denote average expected survivorship from the multiplicative risk model, and asterisks represent significant differences between expected and observed survivorship.

for fish species that exhibit schooling behavior, which reduces the risk of predation and allows for increased feeding activity (Booth, 2004; Schrandt and Powers, 2015). RDM are known to school throughout their life history, especially as adults during spawning periods (Overstreet, 1983). At the subadult stage, shoaling behavior and aggregations of individuals have been observed (Dresser and Kneib, 2007), but it is unclear whether true schooling (i.e., synchronized behavior) occurs. Shoaling behavior in subadult RDM may be utilized as a defense against predators such as dolphins or coastal sharks, but may also be a simple response to high resource availability or favorable environmental conditions. When these groups of subadult RDM occur, prey populations may experience greater losses to predation than when the same number of solitary individuals are feeding separately, suggesting that prey regulation is likely unevenly distributed in space and time.

Risk enhancement in the presence of two RDM for both shrimp and killifish was most extreme when habitat complexity was moderate or high, in the form of patches of SAV or SAV with artificial reefs. Since subadult RDM associate with seagrasses and habitat edges extensively, the structural complexity of the SAV or artificial reefs may have provided favorable conditions for RDM foraging. Penaeid shrimp and Gulf Killifish are also both highly structure-associated, and likely

congregated within or near the SAV patches or artificial reefs (Minello and Rozas, 2002). Instead of acting as refuge space, these structures could have concentrated the prey in specific areas, reducing the amount of time or effort required for the RDM to locate and capture their prey, thus exacerbating predation risk. Finally, both the SAV patches and the artificial reefs provided an abundance of habitat edges. Many marine predators utilize habitat edges, and predation pressure can be much higher in those regions when compared to the internal portions of habitat patches (Smith et al., 2011). These edge effects may have elevated predation risk for shared prey in the presence of conspecific RDM.

In contrast with RDM, intraspecific interactions between SPT often resulted in risk reduction for killifish, and survivorship of shrimp was generally close to the null multiplicative risk. Risk reduction for shared prey in the presence of multiple conspecifics is not entirely unexpected, considering density-dependent competition for prey often regulates population sizes of predatory fish (Lorenzen and Enberg, 2002). That is, individuals of the same species will compete for the same prey resources, and will forage similarly, creating frequent opportunities for negative interactions. Stomach contents of SPT did not indicate dominance by single individuals, so it is likely that indirect interactions between the predators decreased the per-capita predation rate on killifish. Additionally, since individual SPT exhibited a weak preference

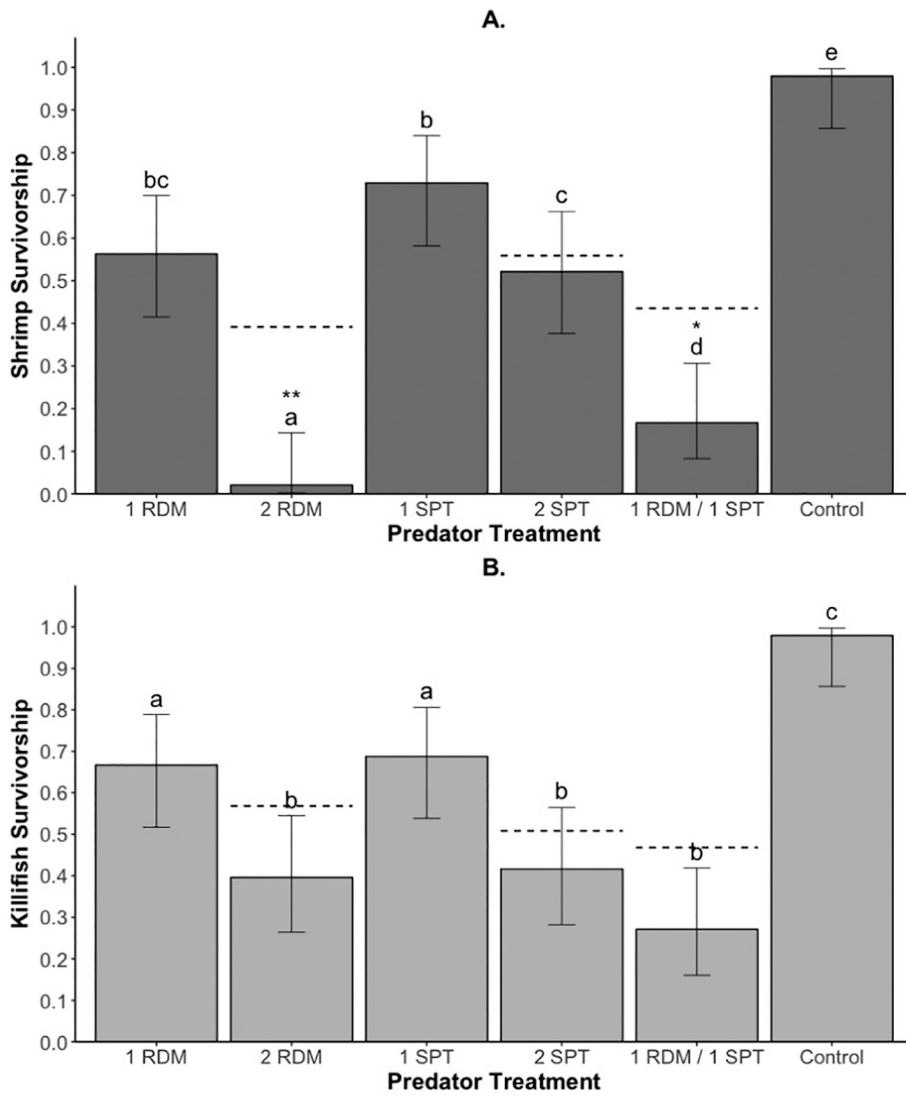


Fig. 3. Predicted values and 95% confidence intervals from binomial models describing the SAV + Reef habitat. Letters in (A) and (B) represent significance among predator treatment levels for shrimp and killifish, respectively, dashed lines denote average expected survivorship from the multiplicative risk model, and asterisks represent significant differences between expected and observed survivorship (single asterisk is marginally significant).

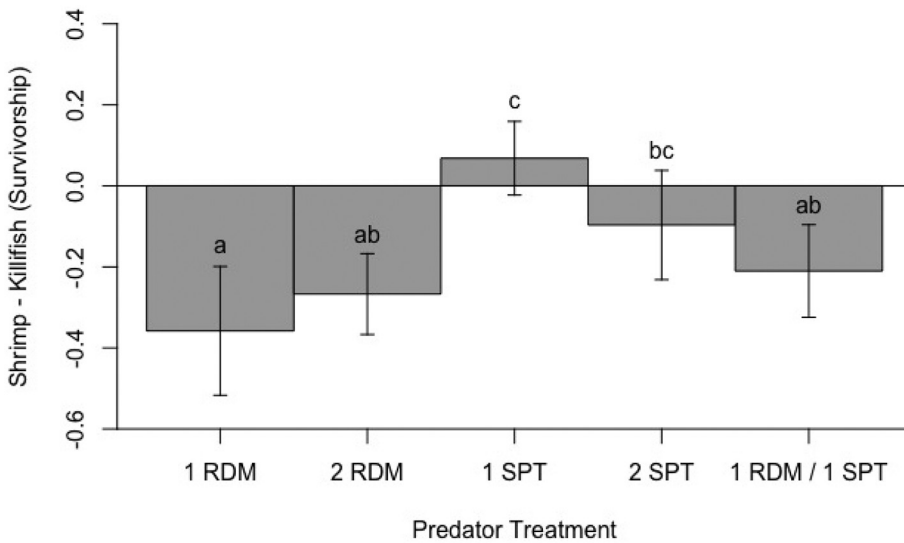


Fig. 4. Mean values and 95% confidence intervals for the difference in survivorship between shrimp and killifish with all habitats pooled. Letters represent significance among predator treatment levels from pairwise post-hoc tests with Bonferroni correction. Negative values indicate higher survivorship of killifish, and therefore higher preference for consuming shrimp.

for killifish, interference behaviors between conspecifics may have been most intense when targeting that species.

The observed risk reduction of killifish in the presence of 2 SPT was most pronounced in trials with moderate habitat complexity in the form of SAV patches. This suggests the SAV may have provided effective refuge for killifish to avoid predation by multiple SPT. However, if killifish were concentrated in the SAV, this may have attracted the SPT to those areas. With both SPT attempting to capture prey in a smaller space (i.e., only at the SAV patches), negative interactions may have occurred more frequently, resulting in heightened survival of killifish. The addition of the artificial reefs, large structures with vertical relief throughout the water column, resulted in a complete mitigation of this risk reduction. One explanation for the mitigation of this emergent MPE is that the structure created by the artificial reefs may have reduced the visual or physical contact between the two predators, thus reducing their negative interactions and allowing them to forage more freely (e.g., Grabowski and Powers, 2004).

Though intraspecific interactions between conspecific RDM and SPT frequently created non-independent predation impacts, exposing prey to both predator species generally resulted in the expected multiplicative predation risk. There was no evidence of either species dominating the observed feeding, and they did not alter or specialize their prey preferences in the presence of the other predator. Therefore, it appears as though they did not interact often, or that their interactions were not consequential. However, shrimp experienced marginally significant risk enhancement in the highest habitat complexity. If the two predators overlap most frequently in areas with complex habitats, then the observed risk enhancement may become important in determining prey survivorship.

The prey preferences of RDM and SPT differed, especially for single individuals, with RDM preferring shrimp and SPT exhibiting a slight preference for killifish. These results align with their recorded dietary habits, since both species are generalist predators but exhibit ontogenetic shifts in prey selection. Although they will consume whatever prey is most abundant and available, RDM and SPT do appear to have separation in their trophic niches based on their prey preferences. Additionally, RDM generally consumed more prey than SPT, especially shrimp. This difference in short-term predation pressure may be due to differences in maturity between the two species at this size. Based on their lengths, the majority of SPT used in this experiment were likely sexually mature and slowing their growth rate, while none of the RDM were close to the species' size at maturity and were likely growing rapidly as subadults (Porch et al., 2002; Bohaboy et al., 2018). This could have influenced their metabolic demands, which would in turn determine their short-term food consumption rates (Buckel et al., 1995).

It has become increasingly apparent that emergent multiple predator effects are not uncommon and may play major roles in shaping ecosystem structure. Creating functional groups of similar or related species has been utilized in ecosystem modelling to simplify food webs, but this may grossly over- or underestimate predation impacts when considering non-substitutable predators. MPEs can thus be important to include when developing models to predict ecosystem-level predation rates and population dynamics. Our understanding of ecosystem functioning, niche separation, and prey regulation will be further strengthened by including the effects of habitat complexity on predator-prey and predator-predator interactions. The results of this experiment provide evidence of non-substitutability and emergent MPEs between and within the populations of two heavily exploited estuarine fish species along a gradient of habitat complexity, which aids in elucidating how they interact with and shape their shared ecosystem as predators.

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