Does relative abundance modify multiple predator effects?

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Abstract

Ecologists have long known that multiple predator species can interact with each other and thereby either strengthen or weaken overall prey regulation. With few exceptions, our understanding of such 'multiple predator effects' (MPEs) is based on experimental combinations of predators at a single relative density (usually 1:1). Because MPEs depend on interspecific interactions between predators, we hypothesized they would vary, potentially non-linearly, with predator species relative abundance. We tested this hypothesis in a southeastern US salt marsh by manipulating two species of predatory crab to generate a continuous relative abundance gradient. After four months, we evaluated the density of two shared prey species (snails and fiddler crabs) across this gradient, before explicitly testing for: (1) the presence of overall MPEs on the densities of these prey; (2) whether (and how) potential MPEs varied as a function of relative abundance; and (3) how indicators of predator–predator interactions (survivorship and limbs lost in contests) were affected by relative abundance. The final density of both prey species varied with relative abundance, but the sign of these effects switched depending on prey identity. The results failed to support an overall MPE on snail density, but final fiddler crab density was higher than expected (i.e., risk reduction, or an overall negative MPE on fiddler crab suppression). Counter to our prediction, this MPE did not vary as a function of relative abundance. Predator survivorship and limb loss indicated asymmetrical negative interactions that strongly impacted the predator species most effective at suppressing fiddler crabs, suggesting an explanation for the negative MPE observed for this prey species. Our findings suggest that MPEs are not always sensitive to species relative abundance, but given that shifts in predator relative abundance are frequently observed in nature, future studies should incorporate this aspect of biodiversity change into their designs wherever possible.

Zusammenfassung

In der Ökologie ist seit langem bekannt, dass mehrere Räuberarten miteinander interagieren und dadurch die Regulation der Beute stärken oder schwächen können. Mit wenigen Ausnahmen basiert unser Verständnis solcher "Mehr-Prädatoren-Effekte" (MPE) auf experimentellen Kombinationen der Räuber bei gleichen Abundanzen (gewöhnlich im Verhältnis 1:1). Da die MPE

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von interspezifischen Interaktionen zwischen den Räubern abhängen, nahmen wir an, dass sie - vermutlich nicht-linear - mit der relativen Abundanz der Räuberarten variieren würden. Wir testeten diese Hypothese in einer Salzwiese im Südosten der USA, indem wir die Abundanz von zwei räuberischen Krabbenarten so variierten, dass ein kontinuierlicher Gradient relativer Abundanz entstand. Nach vier Monaten bestimmten wir die Abundanz von zwei gemeinsamen Beutearten (Schnecken und Winkerkrabben) entlang dieses Gradienten und prüften (1) das Auftreten von MPE auf die Dichte dieser Beutetiere, (2) ob und wie potentielle MPE mit der relativen Abundanz der Räuber variierten und (3) wie die Überlebensraten und Verluste von Extremitäten als Indikatoren von Räuber-Räuber-Interaktionen durch die relative Abundanz beeinflusst wurden. Die Dichte beider Beutearten zu Versuchsende variierte mit der relativen Abundanz, aber das Vorzeichen des Effekts wechselte mit der Beuteart. Die Ergebnisse konnten keinen allgemeinen MPE auf die Schneckendichte untermauern, aber die Enddichte der Winkerkrabben war höher als erwartet (d.h. Risikominderung oder ein genereller negativer MPE auf die Kontrolle der Winkerkrabben). Im Gegensatz zu unserer Vorhersage variierte dieser MPE nicht mit der relativen Abundanz der Räuber. Die Überlebensraten der Räuber und ihre Gliedmaßenverluste zeigten asymmetrische negative Interaktionen an, die stark die Räuberart beeinträchtigten, die besonders effektiv bei der Kontrolle der Winkerkrabben war, was eine Erklärung für den negativen MPE auf diese Beuteart nahelegt. Unsere Befunde legen nahe, dass die MPE nicht immer auf die relativen Abundanzen reagieren, aber da Änderungen der relativen Abundanzen von Räubern in der Natur häufig beobachtet werden, sollten zukünftige Studien den Aspekt der variablen Räuberdiversität in ihre Untersuchungsansätze einbeziehen, wo immer das möglich ist.

Introduction

Predation, and the resultant top–down control on prey populations, is a fundamental process that influences the structure and functioning of ecosystems (e.g., Borer et al., 2005; Estes et al., 2011; Hairston, Smith, & Slobodkin, 1960). Predators are often assumed to act independently in their suppression of prey, but interactions between multiple species of predator can alter the strength of prey suppression. Such interactions are detected by deviations of the predation level of multiple predators from that predicted based on their independent effects (i.e., emergent multiple predator effects [MPEs]; reviewed by e.g., Schmitz, 2007; Sih, Englund, & Wooster, 1998; Vance-Chalcraft, Rosenheim, Vonesh, Osenberg, & Sih, 2007). Positive MPEs (i.e., risk enhancement) are thought to result from mechanisms such as interspecific resource-use complementarity (Griffin, De la Haye, Hawkins, Thompson, & Jenkins, 2008; Northfield, Snyder, Ives, & Snyder, 2010) and conflicted escape behavior of prey (Losey & Denno, 1998). Negative MPEs (i.e., risk reduction), on the other hand, have been ascribed to intraguild predation (IGP; Finke & Denno, 2005) or interspecific interference competition (Griffen & Byers, 2006). Elucidating the nature of MPEs is important for the parameterization of community and ecosystem models (Billick & Case, 1994; McCoy, Stier, & Osenberg, 2012; Sih et al., 1998), and forecasting effects of predator species population depletions and extinctions on ecosystem functioning (Duffy et al., 2007).

A large body of work has now investigated MPEs with respect to interactions between pairs of predators (e.g., Sih et al., 1998), and increasingly, mixtures of three or more predator species (reviewed by Griffin, Byrnes, & Cardinale, 2013). These studies have been conducted almost exclusively at a single relative abundance of different predator species (usually equal, i.e., 1:1; but see Crowder, Northfield, Strand, & Snyder, 2010; Schmitz, 2009). However, unequal relative abundances of species is the norm in natural communities (Hillebrand, Bennett, & Cadotte, 2008), and human impacts commonly modify species relative abundances – or dominance – as opposed to driving complete and immediate species extinction (Crowder, Northfield, Gomulkiewicz, & Snyder, 2012; Purvis & Hector, 2000). Given a shortage of empirical studies, it is not yet clear whether and/or how the relative abundance of multiple predator species may alter their interactions and resultant MPEs.

Depending on the underlying mechanism, MPEs may vary markedly with species relative abundance and in potentially non-linear ways. For example, if MPEs are driven by interspecific resource-use complementarity, negative interspecific interactions are predicted to be weaker than negative intraspecific interactions (Casula, Wilby, & Thomas, 2006; Finke & Snyder, 2008; Ives, Cardinale, & Snyder, 2005). This would lead to stronger MPEs where predator species abundances are most even, producing a positive hump-shaped relationship in a two-predator system between the relative abundance of one species and the MPE. As another example, if strong asymmetrical interference takes place between predator species, a single individual of the aggressor may be sufficient to intimidate a large number of heterospecifics. This could result in a rapidly saturating relationship between relative abundance and an MPE. Given many possible mechanisms the outcomes of shifts in predator relative abundance are hard to predict, but empirical tests are needed to identify the range of possibilities and ultimately the probability of different outcomes in natural systems.

We investigated the effects of multiple predators, and how this varied with the relative abundance of the predator species, in a coastal salt marsh in the southeastern USA. This highly productive system, dominated by vast expanses of smooth cordgrass (*Spartina alterniflora*), hosts two resident predators—the Xanthid mud crabs *Panopeus obesus* and *Eurytium limosum*. These predators are known to share two species of highly abundant and functionally important prey (Griffin & Silliman, 2011; Kneib & Weeks, 1990; Silliman, Layman, Geyer, & Zieman, 2004), the strongly interacting grazing snail, *Littoraria irrorata* (Silliman & Zieman, 2001) and the ecosystem-engineering burrowing fiddler crab, *Uca pugnax* (Bertness, 1985). Previous studies suggest that interspecific complementarity between crab predators, underpinned by functional differentiation (e.g., in claw strength and attack speed: Seed & Hughes, 1995), could relax interspecific competition relative to intraspecific competition (Griffin et al., 2008; Hillebrand et al., 2008), potentially driving the hump-shaped MPE across a gradient of predator relative abundance as suggested above. On the other hand, strong negative interspecific interactions – including both combat and IGP – between crabs are common (e.g., Griffen & Byers, 2006) and could drive negative MPEs, which may be linear or non-linear depending on the balance of interactions. In crabs, non-lethal combative interactions not only cost time and energy, but also commonly result in loss of limbs (legs and claws), which further impacts individuals' ability to capture prey (reviewed by Juanes & Smith, 1995). Monitoring limb-loss in systems of multiple crab predators can therefore provide an index of the intensity and impacts of such combative interactions (Juanes & Smith, 1995).

To examine impacts of predator relative abundance in our study system, we performed a field experiment in which we manipulated the relative abundance of predatory crabs and measured trophic suppression of two key species of shared prey, as well as lethal (predator survivorship) and non-lethal (predator injury) interactions within the predator guild. This experiment can be viewed as a substitutive multiple predator field experiment with nine multiple predator mixtures varying in the dominance of a focal species. First, we evaluated overall (net) effects of predator relative abundance on response variables, incorporating both the effects of species identity and any emergent multiple predator effects. Second, we isolated any variation that could not be explained by species identity, testing: (1) whether this variation was different than zero (i.e., whether an MPE was detectable) and (2) whether potential MPEs depended on relative abundance in a linear or non-linear fashion.

Materials and methods

Study system

We conducted the following experiment in a salt marsh on Sapelo Island, Georgia, USA during the summer-autumn of 2011. We located our experiment within the monoculture, intermediate-height smooth cordgrass (*Spartina*) zone at Airport marsh (31°25′N, 81°17′W). Xanthid mud crabs (*Eurytium* and *Panopeus*) are abundant (mean $\pm$ SD m^{-2}: 5.87 ± 2.94, $n = 39$) in this spatially extensive zone of the marsh and naturally exhibit spatial variation in relative abundances (Griffin, unpublished data). The most abundant and widespread prey species of the mud crabs are the marsh snail (*Littoraria irrorata*) and the mud fiddler crab (*Uca pugnax*). Both prey species affect important ecosystem processes: grazing snails suppress cordgrass productivity (Kimbro, 2012; Silliman & Newell, 2003; Silliman & Zieman, 2001) and interact with drought to generate and expand cordgrass die-off areas (Silliman, Van de Koppel, Bertness, Stanton, & Mendelssohn, 2005), while active burrowing of fiddler crabs oxygenate the soil, thereby ameliorating anoxic conditions around plant roots and changing biogeochemical pathways (Bertness, 1985; Holdredge, Bertness, Herrmann, & Gedan, 2010; Kristensen & Alongi, 2006). Impacts of mud crabs on the densities of these species may therefore have cascading ecosystem-level consequences in southeastern US salt marshes (Griffin & Silliman, 2011; Silliman et al., 2004).

Field experiment set-up

To test the effects of predator species relative abundance, we included the complete and continuous gradient of relative proportions (i.e., ratio) of the two predator species at a constant density of 10 individuals in a substitutive design (De Wit, 1960; Schmitz, 2009), giving a total of 11 treatments. We also had a predator-free control. Controls to examine the effects of cages were not included for two reasons. First, all treatments (including the consumer-free treatment) were fully caged. Second, testing the interaction between cages and experimental factors (predator relative abundance) was impossible because this factor could not be manipulated in the absence of full cages. This design reflects previous experiments in laboratory mesocosms (e.g., Cardinale, 2011) and field mesocosms (e.g., Schmitz, 2008). All treatments and the control were replicated four times (total of 48 field enclosures). Note that mesh screening came loose from one enclosure during the course of the experiment and that replicate was therefore excluded from analyses (treatment *Panopeus* 0: *Eurytium* 10 was thus reduced to $n = 3$, final $n = 47$). We conducted this experiment between June and October (four months) 2011.

The assemblages of predatory crabs were enclosed within experimental cages constructed of galvanized wire mesh stapled to wooden frames that were 2 m × 1 m × 1 m (length, width, height; elongate to allow side-access to the center of plot) and extended 50 cm belowground to prevent escape of predatory crabs. We located each enclosure to incorporate a pre-existing mound (40–70 cm in diameter) of ribbed mussels (*Geukensia demissa*) because relatively high densities of mud crabs associate with these habitat-forming species

(Silliman et al., 2004). Enclosures were separated by >2 m and experimental treatments and the controls were randomly assigned to enclosures.

We collected adult mud crabs (32–38 mm carapace width) during tidal emersion from burrows in local marshes and randomly assigned individuals to appropriate treatments. Mean initial body size (carapace width) did not vary with species (mean ± 1 SD, *Panopeus* = 35.03 ± 1.79 mm; *Eurytium* = 34.99 ± 1.81 mm; *t*-test, $P > 0.5$) or according to predator relative abundance (proportion *Panopeus*) (linear regression: $P > 0.5$). Note that these similarities in initial predator carapace sizes can be extended to body mass, as these species show indistinguishable carapace width–body mass relationships (Griffin, J.N., unpublished data).

We set initial densities of adult (14–17 mm length) snails to 1500 per enclosure (750 m^{-2}), creating naturally occurring high densities of grazers (Silliman et al., 2005). We were unable to equalize initial densities of fiddler crabs within experimental plots due to their small body sizes, cryptic nature, and deep burrows; fortunately, this species was at ambient naturally occurring densities and relatively evenly distributed across field enclosures in the experiment. Initial densities were estimated by counting the total number of distinctive *Uca* burrows (Bertness, 1985; Holdredge et al., 2010) within two 30 × 30 cm^2 quadrats (1 located on the mussel mound, 1 on flat benthos). These burrows were classified in the field according to size (small: <5 mm diameter, large: 5+ mm diameter). The initial densities of fiddler crab burrows in each size class (mean ± 1 SD, per quadrat) on mussel mounds were: 3.02 ± 3.01 (small) and 17 ± 6.21 (large); and on flat benthos were: 3.21 ± 2.94 (small) and 0.88 ± 0.89 (large). These initial densities did not vary with assigned predator relative abundance levels (linear regression: $P > 0.5$ in all cases).

Response variables

Prey level: To assess predator treatment effects on the strength of top–down control of snails and fiddler crabs, we quantified the final density of snails and fiddler crab burrows (a widely-used proxy of fiddler crab density, e.g., Bertness, 1985) within each enclosure upon termination of the experiment. All snails in each enclosure were manually removed and counted. To quantify densities of small and large fiddler crab burrows, we employed the same methods as to assess initial densities (see *Field* experiment set-up), using the mean density across both microhabitat types within each replicate enclosure as our response variable. Note that relatively small fiddler crab individuals (<8 mm) were able to move into and out of field enclosures through the mesh (finer mesh or sheet material was not used because of effects on drainage, light and humidity). Therefore, the final densities of this species (and thus the effects of predators) included net effects of predation, immigration and emigration of individuals <8 mm. While the strength of top–down control of this species may

not be entirely attributable to feeding interactions, it nonetheless provides a measure of the overall affect of predators on local prey density (see methods of O'Gorman & Emmerson, 2009).

Predator level: We assessed whether predator relative abundance influenced predator survivorship and injury level (limbs lost) as indicators of the negative interactions between predators. To assess treatment effects on predator survivorship throughout the experiment, we regularly (every 17 days) checked the surviving number of individuals of both crab species within each enclosure. Surveys were not more regular to avoid excessive disruption of crab behavior. Missing individuals were replaced following each survey to compensate for loss and prevent accumulated density changes. Our experimental cages therefore approximated a situation where predators move into an area following a decrease in conspecific density (i.e., density-dependent immigration). In systems where predator relative abundance gradients can occur on small spatial scales and predators exhibit mobility (e.g., in our system predators show evidence of migration to new burrows depending on availability), such immigration and density homogenization is likely to occur on a regular basis. For each experimental enclosure, we calculated the mean survivorship (proportional) for each component predator species separately (accounting for the starting/target species-specific densities) and for both species combined. Since predatory crabs in general are known to engage in both cannibalism and IGP, and we have directly observed these interactions in the field in our system, we interpret predator mortality (1—survival, if both are expressed as proportions) to be largely the result of directly fatal and consumptive crab–crab interactions (i.e., cannibalism or IGP). Importantly, crab escape could be ruled out because the cages extended deep belowground and there was no evidence of crabs burrowing out (i.e., exit holes on the exterior of cages), indicating that they remained sealed.

To provide a measure of physical damage (injury) associated with crab-crab combat, we recorded the number of missing legs and claws for each individual crab (these were summed because claw loss was rare) at the end of the experiment, and calculated average limb loss per crab within each replicate. Only crabs with full limb sets were added at the start of the experiment and when replacements were made. Individuals that were added as replacements within the final month were marked and excluded from analyses. However, regrowth of limbs, which occurs during moulting, may have occurred during the experiment. Therefore, estimates of limb loss integrate possible regrowth and are thus conservative estimates.

Treatment of independent variables: To allow us to test the role of predator relative abundance on response variables and emergent effects, we operationally defined our independent variable of interest (relative abundance) simply as the proportional abundance of *Panopeus* (see Schmitz, 2009). Although the sign of model coefficients must be interpreted accordingly, we used a substitutive design and thus the abundances

of *Eurytium* were perfectly negatively correlated with those of *Panopeus* and therefore the abundance of *Panopeus* also indicates the effects of changing the abundance of *Eurytium*.

Analysis

Prey level: We assessed the overall effects of predators on the final densities of snails and both small and large fiddler crab burrows using *t*-tests comparing control replicates to all pooled replicates containing predators. Where predators had a significant effect on a prey type, we proceeded to test whether this was determined by the overall effect of relative abundance. We fitted linear regression models with relative (i.e., proportional) abundance of *Panopeus* as the independent variable (11 levels, from 0–1 at increments of 0.1) and the density of each prey type as a response variable in respective models. We then evaluated multiple predator effects (MPEs) for each prey type by calculating the expected final prey density based on the average of observed final prey densities within predator species monocultures weighted by the proportion of each predator species in each treatment (Schmitz, 2009). Next, we calculated the deviation from expected final prey density (i.e., observed–expected) for each replicate at each level of predator relative abundance in mixed-species treatments (9 levels, from 0.1–0.9 *Panopeus*). We then tested: (1) whether these deviations differed overall (on average) from zero using a one-sample *t*-test (Schmitz, 2009); and (2) whether and how MPEs varied with relative abundance by fitting a variety of linear models (linear, polynomial, logistic, exponential) if deemed appropriate after visual examination. If multiple fits were possible, we chose the most parsimonious model according to Akaike Information Criterion (AIC). Both prey response variables satisfied assumptions of normality and variance homogeneity.

Predator level: Similar to the analysis of prey level responses, we first tested for an effect of relative abundance on predator level responses, including overall and single species predator survivorships, and overall and single species limb loss. We further calculated expected overall predator responses based on the average of observed predator responses within predator species monocultures weighted by their proportion in each treatment (Schmitz, 2009). For single species predator responses, we used the mean single species predator response to generate the expected response for multi-species treatments, as these measures were initially calculated taking into account differences in abundance of each species. We tested whether deviations from expected were significantly different from zero using one-sample *t*-tests. For overall survivorship and leg loss, we also tested whether this deviation depended on dominance in a linear or non-linear fashion. Survivorship response variables (proportions) were logit transformed and limb loss variables square root transformed to meet assumptions of linear regression. All statistical analyses were performed using the statistical program R (R Core Team, 2014).

Results

Net effects of predators on prey

In predator-free controls, the density of snails declined by 35% over the four-month experiment through causes of mortality other than predation (likely competition for fungal food; Silliman & Newell 2003). Predators further reduced snail density by an average of 53% relative to controls (*t*-test: $t = -6.55$, $P < 0.001$) over the course of the experiment. Fiddler crab burrow densities increased in all plots, but more so in predator-free treatments. In predator-free controls, the density of small fiddler crab burrows increased by ∼six-fold and the density of large fiddler crab burrows increased by two-fold (relative to mean initial conditions) due to recruitment/immigration and growth over the four-month experiment. By comparison, the density of small and large fiddler crab burrows increased on average only ∼3- and 1.6-fold, respectively, in predator treatments. Therefore, final small fiddler crab burrow densities were 50% lower than final densities in controls (*t*-test: $t = -3.005$, $P = 0.001$), while final large fiddler crab burrow density was only 30% lower and not significantly different to controls at an alpha level of 0.05 (*t*-test: $t = -1.912$, $P = 0.062$). The following results only apply to snails and small fiddler crab burrows (hereafter simply 'fiddler crab burrows').

Final snail density declined linearly with relative abundance of *Panopeus* (linear regression: $R^2 = 0.172$; $F_{1,41} = 8.54$; $P = 0.005$; Fig. 1A; Appendix A: Table 1). This indicates that a higher relative abundance of *Panopeus* resulted in greater suppression of snail density. In contrast, final fiddler crab burrow density increased linearly with *Panopeus* relative abundance (linear regression: $R^2 = 0.164$; $F_{1,41} = 8.04$; $P = 0.007$; Fig. 1B; Appendix A: Table 1), showing that lower proportional abundance of *Panopeus* (and thus higher proportional abundance of *Eurytium*) suppressed fiddler crab densities most strongly.

Multiple predator effects

We next evaluated MPEs (observed–expected prey density) across the continuous gradient of relative abundance in multiple predator treatments. For snails, we did not detect an MPE (one-sample *t*-test: $t_{35} = 1.168$, $P = 0.251$; Fig. 1A) and there was also no detectable effect of relative abundance on MPE values (linear regression on O–E: $R^2 = 0.03$; $F_{1,34} = 1.035$; $P = 0.316$). For fiddler crab burrows, however, there was an overall negative MPE (one-sample *t*-test: $t_{35} = 4.580$, $P < 0.001$; Fig. 1B), showing that prey density was higher (i.e., predators were less effective at regulating fiddler density) in enclosures containing both predator species than expected based on the species' independent effects. There was no detectable relationship between predator relative abundance and the MPE values (linear regression on O–E: $R^2 = 0.004$; $F_{1,34} = 1.137$; $P = 0.713$; Appendix A:
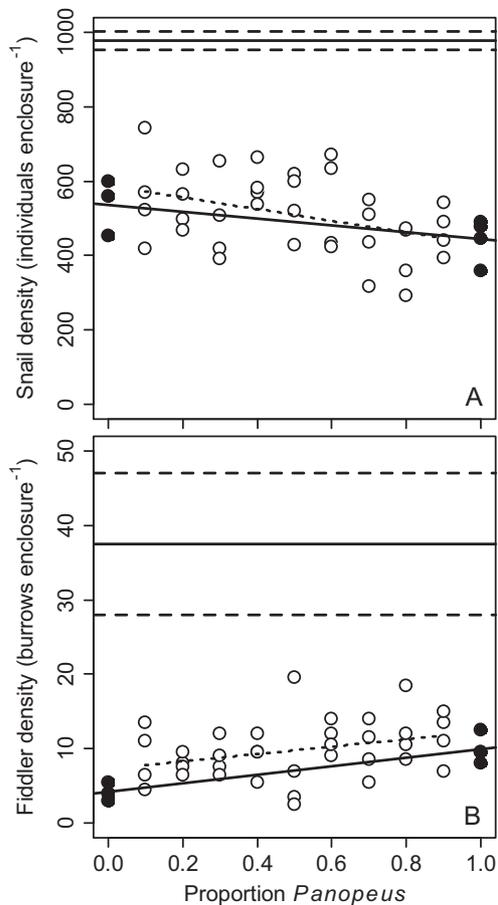
Fig. 1. Effects of predator dominance (proportion of *Panopeus obesus*) on the final density of marsh snails (*Littoraria irrorata*) (panel A) and small mud fiddler crabs (*Uca pugnax*) (panel B) measured at the end of a four month field experiment. Black horizontal lines show density (solid lines) ± S.E (dashed lines) of respective prey types in predator-free controls at the end of the experiment. Black dots indicate prey density in single species predator treatments while white dots indicate density in multi-species treatments. Black lines fitted to data depict expected prey density based on final prey density in single species predator treatments (see *Methods* for calculation). Dotted lines depict linear model fits across the entire range of dominance treatments.

Table 1) indicating that this MPE was not highly sensitive to the relative abundance of predator species.

Indicators of predator-predator interactions

Mean aggregate predator survivorship (mean ± SD) was 86.1 ± 5.5% between each monitoring occasion (17 days). Predator survivorship was relatively invariable across single species treatments (Fig. 2A). Across all treatments, predator survivorship was best described by a negative polynomial (U-shaped) relationship (polynomial linear regression: $R^2 = 0.444$; $F_{2,40} = 15.95$; $P < 0.001$; Fig. 2A; Appendix A: Table 2) with the lowest levels of overall survival at intermediate levels of *Panopeus* relative abundance (i.e., equitable
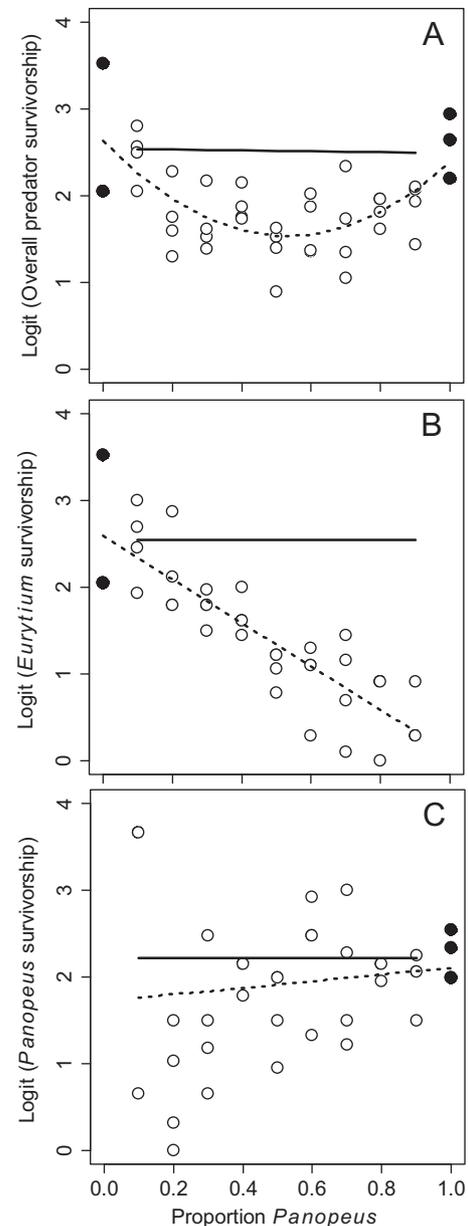

Fig. 2. Logit-transformed overall predator survivorship (panel A), *Eurytium limosum* survivorship (panel B), and *Panopeus obesus* survivorsip (panel C) as a function of predator dominance (proportion of *Panopeus obesus*). Survivorship of predators was measured over the course of a four month field experiment. Black dots indicate survivorship in single species predator treatments while white dots indicate survivorship in multi-species treatments. Black lines depict expected survivorship based on predator survivorship in single species predator treatments (see *Methods* for calculation). Dotted lines depict linear model fits across the entire range of dominance treatments.

species abundances). Observed overall survival in predator species mixtures deviated significantly and negatively from expected (one-sample *t*-test: $t_{35} = 12.448$, $P < 0.001$; Fig. 2A), and these deviations conformed to a negative polynomial relationship (polynomial linear regression:

$R^2 = 0.322$; $F_{2,33} = 7.822$; $P = 0.002$; Fig. 2A; Appendix A: Table 2), driving the overall pattern.

The predator species showed different responses to *Panopeus* relative abundance. *Eurytium* survivorship in species mixtures was lower than expected (*t*-test: $t_{35} = -9.408$, $P < 0.001$; Fig. 2B) and showed a linear decrease in survivorship with increasing *Panopeus* relative abundance (linear regression: $R^2 = 0.749$; $F_{1,37} = 15.95$; $P < 0.001$; Fig. 2B; Appendix A: Table 2), suggestive of IGP of *Panopeus* on *Eurytium*. *Panopeus* survival was also lower than expected in the species mixtures (*t*-test: $t_{35} = -2.193$, $P = 0.035$; Fig. 2C); however, note that there was no positive trend in survivorship with *Panopeus* dominance (linear regression: $R^2 = 0.018$; $F_{1,38} = 0.692$; $P = 0.411$; Fig. 2C; Appendix A: Table 2), likely due to an outlying point at the 0.1 *Panopeus* level.

Predator limb loss across both species (overall) tended to increase with *Panopeus* relative abundance (linear regression: $R^2 = 0.145$; $F_{1,41} = 8.121$; $P = 0.006$; Fig. 3A; Appendix A: Table 3). Observed overall limb loss in predator species mixtures was on average greater than expected (one-sample *t*-test: $t_{35} = 2.561$, $P = 0.015$), but this deviation did not vary with the proportion of *Panopeus* in the mixture (linear regression: $R^2 < 0.001$; $F_{1,35} = 0.024$; $P = 0.876$; Fig. 3A; Appendix A: Table 3). We then examined patterns of limb loss within the individual species. *Eurytium* lost an average of 2.46 ± 1.62 limbs and this was higher than expected in the mixed species treatments (one-sample *t*-test: $t_{35} = 4.569$, $P < 0.001$; Fig. 3B), and tended to increase with *Panopeus* relative abundance, though not significantly at an alpha of 0.05 (linear regression: $R^2 = 0.09$; $F_{1,35} = 3.501$; $P = 0.070$; Fig. 3B; Appendix A: Table 3). These patterns suggest that *Panopeus* may have inflicted limb damage on *Eurytium*. *Panopeus* lost an average of 1.15 ± 0.90 limbs; the number of limbs lost in this species was lower than expected when it occurred in mixtures with *Eurytium* (one-sample *t*-test: $t_{35} = -6.447$, $P < 0.001$; Fig. 3C), and the negative deviation from expected was greatest at low *Panopeus* relative abundance (linear regression: $R^2 = 0.335$; $F_{1,37} = 20.17$; $P < 0.001$; Fig. 3C; Appendix A: Table 3); these results indicate that *Eurytium* individuals were less harmful to *Panopeus* individuals than were *Panopeus* individuals to other *Panopeus*.

Discussion

A rich history of experiments shows that interactions between multiple predator species can have important consequences for top–down control of prey populations (reviewed by Griffin et al., 2013; Sih et al., 1998; Vance-Chalcraft et al., 2007). However, these studies have largely overlooked the potential for multiple predator effects to depend on the relative abundance of predator species–a common gradient in natural and human-perturbed systems–even when both additive and substitutive designs have been used (e.g., Byrnes &
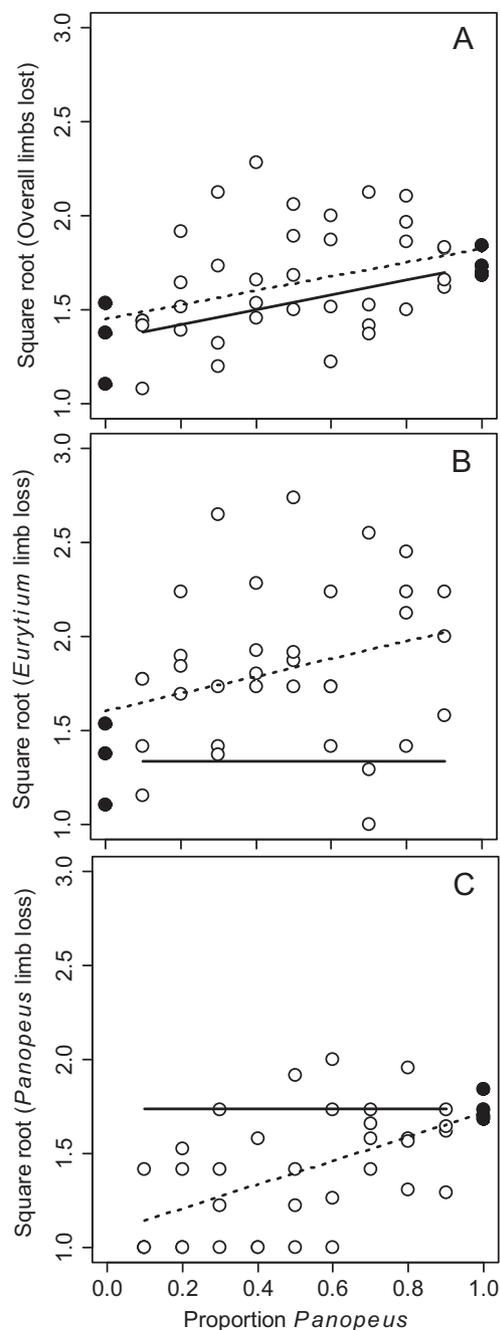
Fig. 3. Square root-transformed overall predator limb loss (panel A), *Eurytium limosum* limb loss (panel B), and *Panopeus obesus* limb loss (panel C) as a function of predator dominance (proportion of *Panopeus obesus*). Black dots indicate limb loss in single species predator treatments while white dots indicate limb loss in multi-species treatments (see *Methods* for calculation). Black lines depict expected limb loss based on limb loss in single species predator treatments. Dotted lines depict linear model fits across the entire range of dominance treatments.

Stachowicz, 2009). Our results show that, in a southeastern US salt marsh, multiple predator effects between two dominant infaunal predators were negative or were not detected, depending on the identity of prey considered. Counter to our

hypothesis, MPEs did not vary in any consistent way across nine levels of predator relative abundance. Below, we discuss these results in more detail and in light of the indicators of interactions between predators.

We did not detect an MPE on the top–down control of snail density i.e., observed snail density in mixed predator treatments did not differ from that expected based on a weighted average of the predators' independent effects (a 'linear' effect of multiple predators, sensu Schmitz, 2007). This suggests, in light of the substitutive design we used, that interspecific interactions between predators did not differ from intraspecific interactions with respect to suppression of snail density. This conclusion diverges from a previous study in the same system, which showed less negative interspecific – relative to intraspecific – interactions between predatory crabs and a resulting positive MPE on snail suppression (Griffin & Silliman, 2011). A possible explanation for the different conclusions is that this earlier study incorporated a wider range in environmental conditions across seasons, allowing expression of interspecific temporal complementarity of predators and an insurance effect of biodiversity (Griffin & Silliman, 2011; Yachi & Loreau, 1999). Furthermore, we cannot rule out that if higher levels of replication had been used in the present study, subtle effects of multiple predator interactions might have been detected.

In contrast to snails, we found a higher density of fiddler crab burrows (a proxy of fiddler crab density) compared to that predicted from the independent effects of predators. This negative MPE indicates that interactions between heterospecifics, with respect to fiddler crab suppression, were more negative than between conspecifics, reducing their combined effects. Our finding that MPEs varied across prey types may be in part explained by differences in the impact of negative predator–predator interactions across the two species. With an increasing abundance of *Panopeus* we found that *Eurytium* lost more limbs and also suffered reduced survivorship, suggesting that *Panopeus* individuals were disproportionately inflicting damage to *Eurytium* individuals through combat events and also killing – and most likely consuming – them. Since *Eurytium* was the most effective predator on fiddler crabs, the negative effects of *Panopeus* on *Eurytium* may help to explain the reduced suppression of fiddler crabs in mixed predator treatments. Differences in habitat domains of prey types may also have contributed to differences in MPEs across them. *Eurytium* and *Panopeus* share the benthic domain with fiddler crabs and might therefore adopt a more active foraging mode to increase encounter rates with them, which would strengthen predator-predator interference and may cause a negative MPE for this prey type. In contrast, the benthic predator crabs do not share the entire domain of the climbing snails, and thus likely consume them opportunistically while using a sit-and-wait mode, which has reduced potential for predator-predator interference. More generally, our study highlights the possibility (or even the probability) that MPEs differ among prey species in natural systems. We now need studies identifying how characteristics

of prey species (e.g., habitat domain, foraging mode) interact with the foraging modes and associated interactions between predator species, to help explain and predict how MPEs vary across prey species in natural food webs (see also Schmitz, 2007).

The negative MPE on fiddler crabs did not, however, vary in any consistent way across the continuous gradient of predator relative abundance in our study, suggesting that negative interspecific interactions were insensitive to relative abundance; this could occur if, for example, a single *Panopeus* individual was able to suppress the predation efficiency of *Eurytium* to a similar degree as a larger number of *Panopeus*. Our findings of both neutral and negative MPEs, which are both insensitive to predator relative abundance, add to the short list of case studies testing the role of predator relative abundance. Schmitz (2009) reported linear effects of multiple predators across a gradient of relative abundance in an old-field system (Schmitz, 2009), while Crowder et al. (2010) showed that high evenness of natural enemies increased pest control and yield in an agricultural system. Including our study therefore, general conclusions regarding effects of predator relative abundance are yet to emerge and will likely require many more studies.

Interestingly, aggregate survivorship across both species showed a negative hump-shaped relationship with species relative abundance, reaching its lowest when species were most even in abundance. This suggests that fatal interspecific interactions (IGP) exceeded fatal intraspecific interactions (cannibalism) on average, across both species. This pattern also demonstrates that the aggregate survival of predators can be determined not just by the presence of multiple predators (e.g., Finke and Denno, 2005), but also by their relative abundances. IGP is common in natural systems (Gagnon, Heimpel, & Brodeur, 2011; Polis, Myers, & Holt, 1989) suggesting that the patterns reported here, ostensibly mediated by IGP, could be pervasive. However, in some systems IGP may be outweighed by cannibalism, which would reverse the sign of the U-shaped relationship reported here. Indeed, both positive and negative effects of multiple predators on predator survival have been reported (Takizawa & Snyder, 2011; Vance-Chalcraft et al., 2007), suggesting that the strength and sign of relative abundance effects are also likely to depend on the specific system and nature of interactions between the particular predators.

The opposing effects of *Panopeus* dominance on suppression of snails and fiddler crabs can be interpreted as species identity effects and suggest an interspecific trade-off in the foraging capabilities or preferences of the two predators. Such species identity effects have been widely documented in studies of multiple predators (e.g., O'Connor, Grabowski, Ladwig, & Bruno, 2008). We speculate that *Panopeus* has stronger claws that allow it to break open snail shells more easily, whereas *Eurytium* is faster and therefore more able to capture fast-moving fiddler crabs. In light of such dietary complementarity, high species evenness (intermediate levels of *Panopeus* abundance) would be expected to reduce

interspecific competition relative to intraspecific competition and to generate positive MPEs on prey suppression, but we found no evidence of such an effect. A possible explanation for this is the high level of prey abundance in the system in general, and in our experimental units specifically. Such high levels of prey abundance effectively eliminate strong intraspecific competition that is important in generating positive effects of resource complementarity (see Griffin et al., 2008; Griffiths, Wilby, Crawley, & Thomas, 2008; Northfield et al., 2010). An additional consideration is that predator survivorship was lowest at intermediate levels of *Panopeus* abundance and the behavioral interference associated with it could be expected to reduce foraging efficiency and therefore counteract any potential increases in prey suppression resulting from dietary complementarity. Finally, apparently limited functional differences between predators in terms of other traits such as foraging domain and body size might have led to a high frequency of interspecific interference irrespective of differences in diets. This similarity may reflect the close taxonomic relatedness of the predator species in our study (they are co-familial); indeed, this explanation is consistent with a recent meta-analysis that MPEs tend to grow more positive with increasing taxonomic distinctness of predators (Griffin et al., 2013). Our results add to a growing body of evidence indicating that effects of resource complementarity are context-dependent, relying on factors such as the degree of functional differentiation among species (Griffin, Mendez, Johnson, Jenkins, & Foggo, 2009; Heemsbergen et al., 2004), community context (e.g., density, Griffin et al., 2008; Northfield et al., 2010) and the occurrence and strength of other, counteracting mechanisms, such as strong negative interactions within trophic groups (this study; DeLong & Vasseur, 2012; Jousset, Schmid, Scheu, & Eisenhauer, 2011).

Our results reveal interspecific differences in the ability of predator species to suppress key intermediate interactors in southeastern US salt marshes and therefore potentially influence plant growth and associated ecosystem functions through multiple channels. By more effectively regulating the density of a plant antagonist (grazing snails), *Panopeus* should promote plant growth more strongly than *Eurytium* through a positive trophic cascade (Silliman & Bertness, 2002). Meanwhile, by more strongly suppressing densities of a known plant facilitator (burrowing fiddler crabs; Bertness, 1985), *Eurytium* should more strongly suppress plant growth by indirectly reducing oxygen availability (see Wu, Duffy, Reich, & Sun, 2011 for an analogous cascade in alpine meadows). Combined these results suggest that dominance by *Panopeus* should promote net positive cascading effects of predators, although this prediction could not be adequately assessed in our relatively short-term study. Longer-term experiments are needed to illuminate the relative importance of regulation of negative and positive interactors in this system and therefore net effects of different predator species on plant biomass.

The relative abundances of species within guilds vary continuously in space and time in response to local environmental variation, stochastic factors, and location relative to the center of species' respective biogeographic ranges. Researchers are just beginning to explore the effects of predator species' relative abundances on the functioning of predator guilds. Separate previous studies have shown that predator evenness (Crowder et al., 2010) and predator composition (Schmitz, 2009) can both affect prey suppression in agricultural settings and old fields, respectively. Effects may be highly context-dependent—and we show that it can even depend on the identity of prey within the same system. Nevertheless, predicting specific outcomes could become possible with further development and testing of theory based on the functional traits of interacting species (Chalcraft & Resetarits, 2004; Schmitz, 2007; Schmitz & Price, 2011). Until then, ecologists and environmental managers must be mindful that shifting relative abundances of predator species – even when richness remains unchanged – may have unexpected effects on the functioning of predator guilds and ultimately ecosystems.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.baae.2015.05.003.

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