



Multiple prey effects: Agonistic behaviors between prey species enhances consumption by their shared predator

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ABSTRACT

Multiple prey species may affect consumption by their shared predator in a non-additive fashion which cannot be predicted by summing pairwise predator–prey interactions within food webs. By manipulating prey species richness and identity in experimental mesocosms, we sought to explore the effects of interspecific prey interactions between pinfish *Lagodon rhomboides* and pigfish *Orthopristis chrysoptera*, and between blue crabs *Callinectes sapidus* and common mud crabs *Panopeus herbstii* on the consumption rates of a shared predator species, gulf toadfish *Opsanus beta*. We also determined the effects of these interactions on the survivorship of the prey species as mediated by the predator. In both fish and crab prey trials, interspecific interactions between multiple prey increased the overall consumption rate of *O. beta*, and in each case, one prey species was harmed (decreased survivorship) while the other prey species was not significantly affected by these interactions (all relative to single-prey treatments). In trials run with fish prey, behavioral observations revealed that *L. rhomboides* aggressively chased *O. chrysoptera* out of a seagrass refuge and into a surrounding sand matrix where *O. beta* foraged, thus increasing *O. chrysoptera* vulnerability to predation. In trials run with crab prey, *C. sapidus* failed to reduce their activity in the presence of *O. beta*. In addition, *P. herbstii* displaced *C. sapidus* from the seagrass refuge in mixed prey treatments, and so these conspicuous behaviors primarily occurred in the unstructured sand habitat where *C. sapidus* were more vulnerable to predation. We conclude that multiple prey effects, including those involving risk-enhancing, agonistic interactions between prey, can be important determinants of predator–prey outcomes. This may be especially true when refuge space is limited.

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1. Introduction

Many ecological communities harbor diverse trophic guilds in which generalist consumers share multiple prey resources (Schoener, 1989), making complex multispecies predator–prey interactions common in nature (Pimm et al., 1991). Early foundational studies of trophic interactions focused on the consumptive effects of a single predator on a single prey (Sih et al., 1985; Lima and Dill, 1990), and such pairwise interactions have been used to predict the dynamics of more diverse food webs and communities (Levins, 1968; Wilbur and Fauth, 1990). This theoretical framework is only valid under the assumption that pairwise interaction coefficients remain unchanged within a species-diverse community (Vandermeer, 1969), yet more

recently, it has been shown that multispecies interactions can introduce non-linearities via trait-mediated indirect interactions (TMIs) (Kerfoot and Sih, 1987; Wilbur and Fauth, 1990; Werner and Peacor, 2003; Preisser et al., 2005; also termed interaction modifications by Wootton, 1993), and other higher-order interactions (Billick and Case, 1994). TMIs occur when one species elicits trait changes in another (e.g. behavior), which in turn alters the functional relationship of the reacting species with other species in the community (Werner and Peacor, 2003). The discovery and elaboration of these context-dependent, higher-order interactions potentially invalidates the additive community model, and has prompted ecologists to incorporate more diverse predator and prey assemblages into trophic studies that more accurately predict natural population and community dynamics.

The emergent effects of multiple predators have received considerable attention as researchers explore more complex food web interactions [termed multiple predator effects (MPEs), Sih et al., 1998]. Examples of MPEs from both terrestrial (e.g. Losey and Denno, 1998) and aquatic systems (e.g. Soluk, 1993) have been reported, and several interesting results have emerged from these studies. Most importantly, it has been demonstrated that predators can have unpredictable,

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emergent (i.e. non-additive) effects on prey populations while foraging together. Relative to predators foraging alone, predators can interfere with one another to increase prey survival (Crowder et al., 1997; Griffen and Byers, 2006), or facilitate one another to decrease prey survival (Losey and Denno, 1998; Fodrie et al., 2008). Consequently, MPEs can affect energy transfer between trophic levels, and may have important applications for the control of agricultural pests (Losey and Denno, 1998; Cardinale et al., 2003) and invasive species (Harvey et al., 2004).

Despite the apparent influence of MPEs in regulating vertical trophic exchanges (Sih et al., 1998), the effects of non-additive horizontal interactions (i.e. within a trophic level) among prey in determining overall predation rates have received considerably less attention. This disproportionate emphasis is somewhat surprising for several reasons. First, species diversity generally scales inversely with trophic level (Petchey et al., 2004), and therefore situations in which a predator is faced with multiple prey species may be more common than multiple predator scenarios. Additionally, species densities are generally higher at lower trophic levels, increasing the probability of both intraspecific and interspecific prey encounters. Second, within the burgeoning subfield of biodiversity and ecosystem functioning, ecologists have proposed a hypothesis which predicts that prey diversity should enhance resistance to consumption by higher-order consumers (Duffy, 2002); however, empirical support is largely lacking (but see Hillebrand and Cardinale, 2004; Snyder et al., 2008). Lastly, there is a rich empirical and theoretical literature concerning the indirect effects between species that share natural enemies (Abrams, 1987; Holt and Kotler, 1987; Holt and Lawton, 1994). For example, “apparent competition” refers to the situation in which prey species indirectly reduce one another’s equilibrium densities via an increased numerical response of the predator (Holt, 1977; Schmitt, 1987). Still, these shared predator studies have rarely focused on the outcomes of multiple prey interactions from the perspective of the shared predator (e.g. Huang and Sih, 1990; McNeely et al., 1990), or assessed the additivity of these interactions on the predator (as in MPE studies).

We used two, single-predator–multi-prey complexes (predator–fishes, predator–crabs) from estuarine ecosystems to explore the non-additive effects of multiple prey on their shared predator, as well as the prey species themselves as mediated by the predator. Pinfish *Lagodon rhomboides* (Linnaeus) and pigfish *Orthopristis chrysoptera* (Linnaeus) are demersal fishes which often co-occur in a wide range of coastal environments throughout the eastern seaboard and Gulf of Mexico, USA (Briggs, 1958; Schimmel, 1977). These fishes are numerically dominant members of the estuarine ichthyofauna, particularly in seagrass meadows (Stoner, 1980) where resource overlap can be high (Livingston, 1982). The highly mobile blue crab *Callinectes sapidus* (Rathbun) and smaller, less-mobile common mud crab *Panopeus herbstii* (Milne-Edwards) also occur sympatrically throughout coastal habitats, although the mud crab is slightly more restricted to structured environments (e.g. oyster reefs or grass beds) (Williams, 1984). In addition to their spatial overlap, these crab species compete for bivalve prey including eastern oysters *Crassostrea virginica* (Bisler and Castagna, 1987) and Atlantic ribbed mussels *Geukensia demissa* (Seed, 1980). All four of these species are intermediate predators within estuarine communities, as well as prey for higher predators such as the gulf toadfish *Opsanus beta* (Goode and Bean), a voracious benthic omnivore (Schwartz and Dutcher, 1963; Wilson et al., 1982). These animals are particularly well-suited for experimental mesocosm settings due to their small size and known intra- and interspecific behaviors; despite being phylogenetically distant, fish and crab prey species pairs are similar in that they both contain a well-known aggressor: *L. rhomboides* and *C. sapidus* (e.g. Jachowski, 1974), and a more subservient species: *O. chrysoptera* and *P. herbstii* (e.g. Brown et al., 2005). Including both fishes and crabs as prey allowed us to evaluate whether qualitative predictions of interaction outcomes could be made based purely on species’ intrinsic traits (e.g. behavior). We expected the aggressive species to win in conflicts for refuge space,

which could increase the competitively-inferior prey’s risk of predation (Jeffries and Lawton, 1984).

Here, we manipulated prey species richness and identity in experimental mesocosms to determine: (1) how prey interactions might influence the overall predation rates of a shared predator (employing the MPE framework, Sih et al., 1998); (2) how interspecific interactions affect the survivorship of each prey species as mediated by their shared predator (acknowledging previous shared predator studies, Holt and Lawton, 1994); and (3) the behavioral mechanisms behind the observed consumptive effects.

2. Methods

We conducted controlled experiments in indoor mesocosms to examine the effects of multiple prey on overall consumption by *O. beta* and the species-specific survival of fish and crab prey. Trials run with fishes as prey were conducted from June through August of 2007, while the crab prey trials were conducted from September through December of 2008 following an identical protocol.

Trials were conducted in 6 cylindrical 665-L (110-cm diameter, 70-cm deep) experimental mesocosm tanks located at the Dauphin Island Sea Lab (DISL), on Dauphin Island, AL, USA. Tanks were filled with seawater to a depth of 50 cm and subjected to an artificial photoperiod (12-h light:12-h dark). Seawater (27‰ salinity, 25 °C; consistent with natural conditions) was continuously filtered, aerated, and circulated throughout the tanks to maintain water quality and mimic estuarine conditions. Each tank contained a circular artificial seagrass patch (34-cm diameter) placed in the center of the tank which covered 10% of the mesocosm bottom. We chose seagrass habitat as a potential refuge because it is utilized in nature by the species as protection from predators (Livingston, 1982; Holmquist et al., 1989; Heck and Orth, 2006) and is an increasingly threatened habitat worldwide, making it a limiting resource in many environments (Waycott et al., 2009). Still, it should be noted that all animals within our study system are habitat generalists and seagrass meadows are just one of many environments in which they occur in sympatry. Artificial seagrass patches were created by tying green ribbons to a circular template of polyethylene plastic sheet-netting at a density of approximately 2400 shoots m^{-2} , which reflects local shoot densities (Byron and Heck, 2006). A sieved sand substrate was added to a depth of 2.5 cm in which the template was buried. The combination of artificial seagrass patches and sand matrix has been effectively employed in previous experiments to emulate natural seagrass habitat (reviewed by Heck and Orth, 2006; Mattila et al., 2008).

We used a fully factorial design with prey-base (3 levels) and predator presence/absence (i.e. 2 levels) as fixed factors. For all trials (fish or crab prey), we examined predation rates within three levels of prey: (1) 20 individuals of prey species A; (2) 20 individuals of prey species B; and (3) 10 individuals of prey species A and 10 of prey species B (Table 1). These treatment levels constitute a substitutive experimental design (also known as a replacement series design) where overall species density is kept constant while diversity is manipulated. The substitutive design avoids confounding the effects of increased species densities with increases in diversity, as seen with the additive design, and more appropriately addresses the question of whether interspecific interaction effects outweigh intraspecific effects (Griffen, 2006). However, intraspecific density is decreased in more diverse treatments (Table 1), and so this reduction is confounded with increases in diversity. The prey densities used are well within the natural ranges observed in the northern Gulf of Mexico, USA (May, 1974; Heck et al., 2000; Heck et al., 2001), and particularly from the locations where prey were collected (Toscano, personal observations). We also included predator presence (*O. beta* present, absent) as a factor to help distinguish between mortality generated by prey versus by the predator. Each unique treatment combination was replicated 6 times.

Table 1Substitutive experimental design used in a study of the effects of interactions within fish and crab prey guilds on an *O. beta* predator.

Predator	Prey level	Fish prey	Crab prey	Prey densities
Absent	Single species	<i>L. rhomboides</i>	<i>C. sapidus</i>	20
Absent	Single species	<i>O. chrysoptera</i>	<i>P. herbstii</i>	20
Absent	Mixed	<i>L. rhomboides</i> , <i>O. chrysoptera</i>	<i>C. sapidus</i> , <i>P. herbstii</i>	10, 10
Present	Single species	<i>L. rhomboides</i>	<i>C. sapidus</i>	20
Present	Single species	<i>O. chrysoptera</i>	<i>P. herbstii</i>	20
Present	Mixed	<i>L. rhomboides</i> , <i>O. chrysoptera</i>	<i>C. sapidus</i> , <i>P. herbstii</i>	10, 10

Density values are number of prey species stocked per 665-L experimental tank ($n = 0$, or 1 *O. beta* per tank). Each treatment was replicated 6 times with each prey guild for a total of 72 experimental units.

L. rhomboides (49.49 ± 0.39 mm, mean ± 1 SE; total length, TL) and *O. chrysoptera* (55.32 ± 0.50 mm TL) were collected from Big Lagoon, FL, USA by trawling a net through seagrass meadows during June and July of 2007. To collect *P. herbstii* (18.54 ± 0.27 mm carapace width, CW), we fabricated mesh bags from polyethylene plastic sheet-netting. These bags were then filled with oyster shell and placed beneath the DISL vessel dock. *P. herbstii* naturally settled within the bags and could be easily removed when needed. *C. sapidus* (24.31 ± 0.33 mm CW) were collected by dragging a dip net through seagrass beds nearby DISL. Crab species were collected from September through December of 2008. *O. beta* (181.72 ± 1.85 mm TL) were captured as needed using M-style fish traps baited with fish carcasses and deployed beneath the DISL vessel dock. *L. rhomboides* and *O. chrysoptera* were held in separate cages (approximately 65-L) containing artificial seagrass patches within the mesocosm facility. *C. sapidus* and *P. herbstii* were kept separately in 45-L glass aquaria. Polyethylene plastic sheet-netting cylinders were added to provide refuge, thus reducing possible conspecific antagonistic behaviors and cannibalism. *O. beta* were held in a rectangular acrylic glass tank (approximately 460-L) and randomly fed chopped white mullet *Mugil curema* (Cuvier and Valenciennes) up to 24h before trials.

At the onset of each trial, prey were haphazardly scattered throughout experimental mesocosms and allowed to acclimate for 2h before *O. beta* were introduced as required. The 6 tanks were divided into 2 groups of 3 which used separate circulation and filtration systems. We designated one group as the predator-present tanks and the other as the predator-absent tanks in order to eliminate the possibility of predator chemical cues influencing prey behavior in predator-absent treatments. Predator-present and predator-absent treatments were randomly assigned among the 3 tanks in the appropriate group during each replicate of the experiment. All experimental organisms were measured with Vernier calipers to the nearest 0.01 mm prior to use in trials to minimize differences in body size within and between treatments. Once the experiment began, prey were allowed to interact with one another and *O. beta* foraged for 48h. At the completion of each trial, the sand substrate was sieved to remove buried prey and remaining prey items were enumerated and measured. By matching prey sizes before and after trials, we were able to determine which individuals had been consumed and if there were any size-related effects. After trials, *O. beta* were euthanized with an overdose of MS-222 and their stomachs were excised to verify prey consumption. All experimental fishes and crabs were used only once.

If the prey species influenced predation independently, then their effects on overall consumption rates would be roughly linear. This expectation is appropriate without prior knowledge of the complexity of the trophic system, such as details of the adaptive behaviors of the predators or prey (Sih et al., 1998). Therefore, expected survivorship values for each trial were calculated using an independent effects model that averages the survivorship rates (% recovered) of the two single-prey treatments in trials that were run with a predator (Siddon and Witman, 2004). Specifically, this model predicts that the contributions of prey to the diet of their shared predator in the mixed prey treatment will combine additively. To determine whether multiple prey species had non-independent effects on predation, we compared expected to observed prey survivorship in mixed prey

treatments using paired *t*-tests. An observed survivorship value significantly greater than the expected value would indicate reduced predation (risk reduction among multiple prey), while a significantly lesser value would indicate increased predation (risk enhancement among multiple prey). Because we were interested in predator-mediated mortality, we corrected for any non-predator mortalities by subtracting observed mortalities in the predator-absent treatments from mortalities observed in the predator-present treatments before comparing expected and observed survivorship (i.e. using the predator-absent treatments as controls).

To determine the effects of the prey-base on each prey species as mediated by the predator, we compared prey survivorship (as both proportion surviving and raw number of prey eaten) between prey levels (single vs. mixed prey) using unpaired *t*-tests. Again, prey survivorship in the predator-present treatments was corrected for any prey-induced mortalities in the analogous predator-absent treatments. The analysis of proportional survivorship data was useful in comparing survivorship between treatments in which intraspecific prey densities were different. We also analyzed these data as the raw number of prey eaten per trial to compare the total number of each prey consumed between treatments.

To elucidate possible behavioral mechanisms accounting for patterns in prey consumption, we periodically observed predator and prey behavior and distribution throughout the experiment. During preliminary trials, we observed aggressive chasing behaviors between *O. chrysoptera* and *L. rhomboides*. We hypothesized that these agonistic behaviors could affect their susceptibility to *O. beta* predation and sought to quantify these interactions. Chases within and between prey species were recorded during 9, 5-minute observational periods distributed evenly over 2 replicates. Chases were defined as any directed movement toward another individual in an aggressive manner, and both the species chasing and the species being chased were recorded. In trials using crabs as prey, we recorded the numbers of active crabs and the distribution of crabs among the seagrass and sand habitats 4 times during 5 of the 6 trials (evaluating each species separately). These counts were performed at approximately 12-h intervals during each 48-h trial. Crabs were designated as "active" if they were observed moving or resting epibenthically ("inactive" crabs were observed buried in the sand matrix). These designations were made largely from the predator's perspective; "active" crabs were not necessarily moving, but were presumably more visible to *O. beta* than buried crabs. All behavioral data (fish prey chases and crab prey distribution and activity proportions) were analyzed separately for each prey species, and compared among treatments using two-way ANOVAs with prey level and predator presence as fixed factors.

Observations of crab prey behaviors were less informative than for fishes, as the crabs were largely sedentary and direct interactions between species were rare. However, based on our observations throughout the experiment, we suspected that the ability of either crab species to occupy the refuge space may determine their susceptibility to predation (i.e. crabs in refuge are less vulnerable). To determine if competition for refuge space played a role in mediating the crab prey interaction, we ran two additional trials of the mixed crab prey with

predator treatment without the seagrass refuge. All other protocol and conditions were identical to those in the original experiment. We compared the difference between the number of each crab species consumed (equal consumption of the prey = 0) by *O. beta* in mixed prey treatments with and without the seagrass refuge using an unpaired *t*-test.

All data were tested for normality and equality of variances (*F*-test, $\alpha = 0.05$) prior to statistical analyses. Raw data (e.g. fish chases, number of prey eaten) were log-transformed and proportional data (e.g. survivorship, crab behaviors) were arcsin-transformed where necessary to generate equal variances between groups. All statistical analyses were performed using R (v. 2.9.2). As previously mentioned, the sizes of all experimental organisms were closely monitored to ensure consistency among treatments. We used one-way ANOVAs to compare the average sizes of individual prey species between treatments. Size differences of *O. beta* were small (<5%), making a test of size differences between treatments unnecessary. In addition, we tested for size-selective predation by *O. beta* by running a paired *t*-test on the average sizes of prey before a trial and those prey remaining after completion of a trial in each predator-present treatment.

3. Results

O. beta consumed on average $3.6 \pm .8$ (mean \pm 1 SE) fish and $4.7 \pm .5$ crabs per 48-h trial (approx. 18 and 23% of prey available). In trials that included a predator, overall prey survivorship was reduced in mixed prey treatments for both fish and crab prey pairs relative to single-prey treatments (Fig. 1). Specifically, the raw number of total prey consumed by *O. beta* in mixed fish prey treatments increased by 79 and 178% (9 and 13% proportional decreases in survivorship) compared to *L. rhomboides* and *O. chrysoptera* single-prey treatments that included a predator, respectively (Fig. 1a). Similar patterns were observed in the crab prey trials; the overall number of prey eaten in mixed prey treatments increased by 111% and 73% (17 and 13% proportional

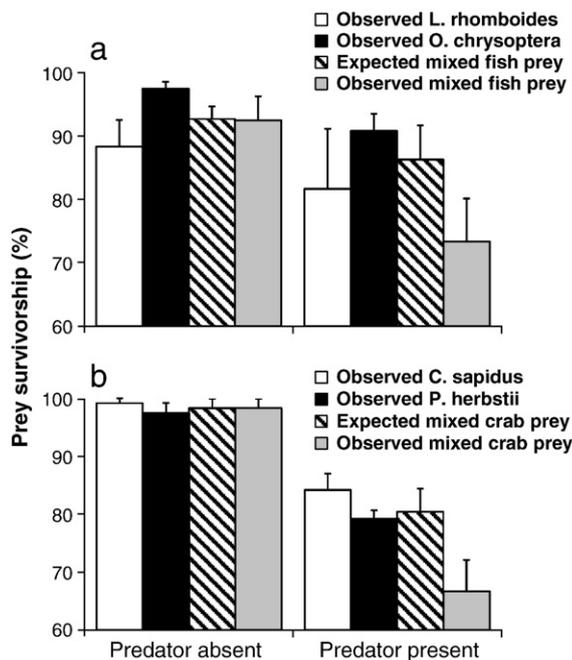


Fig. 1. Overall prey survivorship (as proportions) in experimental tanks with *O. beta* ($n = 0$ or 1 *O. beta* per tank) as a predator: a) fish prey guild trials: *L. rhomboides* only ($n = 20$ fish per tank, white bars), *O. chrysoptera* only ($n = 20$ fish per tank, black bars), and *L. rhomboides* and *O. chrysoptera* ($n = 10$ of each fish species per tank, gray bars); b) crab prey guild trials: *C. sapidus* only ($n = 20$ crabs per tank, white bars), *P. herbstii* only ($n = 20$ crabs per tank, black bars), and *C. sapidus* and *P. herbstii* ($n = 10$ of each crab species per tank, gray bars). Data were recorded following a foraging interval of 48 h, and all bars represent the mean of 6 replicates (± 1 SE). Expected survivorship values in mixed prey treatments are provided (striped bars) as calculated with the independent effects model.

Table 2

Differences between expected and observed prey survivorship in mixed prey treatments (paired *t*-tests) and influence of interspecific interactions on species-specific prey survivorship in both fish and crab prey guilds (unpaired *t*-tests).

Factor	df	MD	<i>t</i>	<i>p</i>
Overall fish prey survivorship				
Expected vs. observed survivorship	5	0.112	1.939	0.1101
Overall crab prey survivorship				
Expected vs. observed survivorship	5	0.15	4.174	0.0087
<i>L. rhomboides</i> survivorship: alone vs. mixed prey				
Proportional survivorship	10	0.033	0.428	0.6775
Raw number consumed	10	1.5	1.138	0.2818
<i>O. chrysoptera</i> survivorship: alone vs. mixed prey				
Proportional survivorship	10	0.258	3.316	0.0078
Raw number consumed	10	1.833	1.938	0.0813
<i>C. sapidus</i> survivorship: alone vs. mixed prey				
Proportional survivorship	10	0.217	4.914	0.0006
Raw number consumed	10	1	1.369	0.2009
<i>P. herbstii</i> survivorship: alone vs. mixed prey				
Proportional survivorship	10	0.05	0.741	0.4759
Raw number consumed	10	1.333	1.606	0.1393

Note: MD is the mean difference between groups.

decreases in survivorship) compared to *C. sapidus* and *P. herbstii* single-prey treatments (Fig. 1b). If the prey species affected overall predation independently, we would expect 90 ± 4 (mean \pm 1 SE) of fish prey and $83 \pm 3\%$ of crab prey to survive to the end of trials in mixed prey with predator treatments (as predicted by the independent effects null model, Fig. 1). Instead, observed prey survivorship in mixed fish and crab prey treatments was only 79 ± 6 and $68 \pm 5\%$, respectively, indicating risk enhancement within both multiple prey systems (Fig. 1). Differences between expected and observed values were marginal for fish prey ($p = 0.110$, Table 2) and significant for crab prey ($p = 0.009$, Table 2).

Unpaired *t*-tests on individual prey species survivorship revealed that prey level (single or mixed prey) had a strong negative effect on the survivorship of one prey in both single-predator–multi-prey systems (Fig. 2). Specifically, *O. chrysoptera* and *C. sapidus* were consumed by *O. beta* at a disproportionately higher rate in mixed versus single-prey treatments (122 and 133% increases in raw number consumed, respectively) and prey composition had significant effects on the proportional survivorship of both *O. chrysoptera*

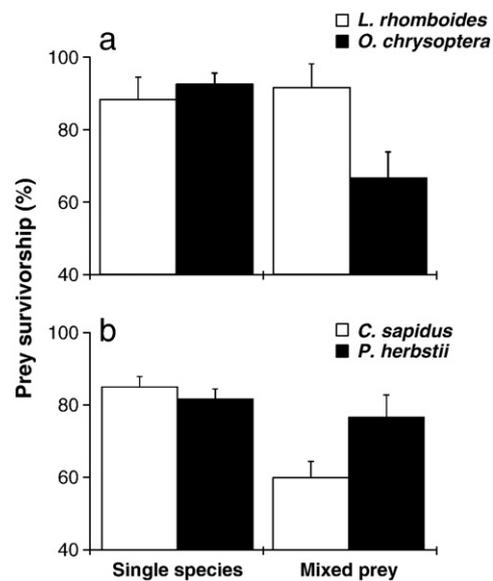


Fig. 2. Individual prey species survivorship (as proportions) corrected for mortalities in predator-absent treatments: a) fish prey guild trials: *L. rhomboides* (white bars) and *O. chrysoptera* (black bars); b) crab prey trials: *C. sapidus* (white bars) and *P. herbstii* (black bars). All bars represent the mean of 6 replicates (± 1 SE).

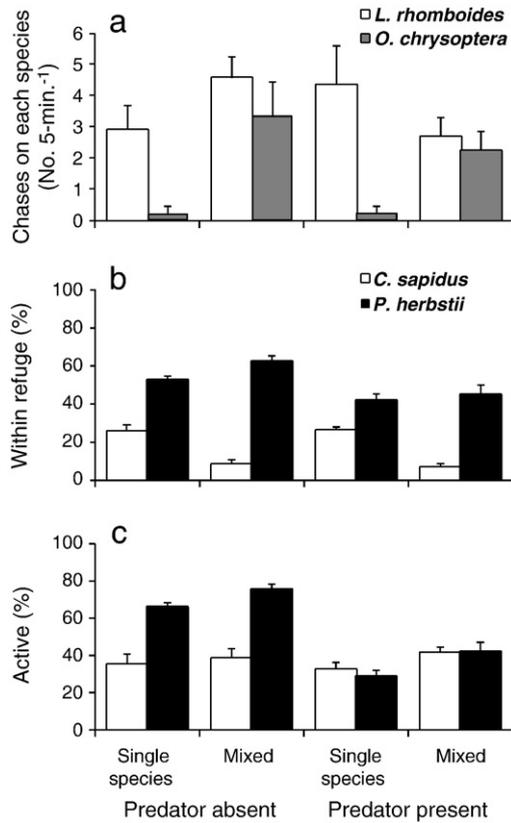


Fig. 3. Investigating behavioral mechanisms behind observed patterns in predation: a) chases on fish prey species (by prey): mean pursuits of *L. rhomboides* (white bars) and *O. chrysoptera* (gray bars) per 5 min observation. Nine observations were made during 2 of 6 total replicates. All bars represent the mean number of chases observed during 9 observations (± 1 SE); b) proportion of *C. sapidus* (white bars) and *P. herbstii* (black bars) observed in refuge. All bars represent the mean proportion observed over 20 observations (± 1 SE); c) proportion of *C. sapidus* (white bars) and *P. herbstii* (black bars) observed as active. All bars represent the mean proportion observed over 20 observations (± 1 SE).

($p = 0.008$, Table 2) and *C. sapidus* ($p = 0.001$, Table 2). Prey level had no significant effect on *L. rhomboides* and *P. herbstii* survivorship.

L. rhomboides exhibited considerable intraspecific aggression. On average, 3.6 ± 0.7 (mean ± 1 SE) chases were recorded per 5-minute observational period in *L. rhomboides* single-prey treatments compared to 0.2 ± 0.2 chases in *O. chrysoptera* single-prey treatments (Fig. 3a). Predator presence had no significant effect on chases for either species (*L. rhomboides*: $F_{1,34} = 0.066$, $p = 0.799$; *O. chrysoptera*: $F_{1,34} = 0.466$, $p = 0.500$). The total number of chases on *O. chrysoptera* increased significantly in mixed prey treatments ($F_{1,34} = 24.875$, $p < 0.001$) (Fig. 3a), and in these treatments, 100% of pursuits on *O. chrysoptera* were by *L. rhomboides*. Within predator-present treatments (i.e. when these behaviors could potentially affect predation), chases on *L. rhomboides* decreased while chases on *O. chrysoptera* increased in the mixed prey treatment (versus single prey with a predator) (Fig. 3a), possibly increasing the susceptibility of *O. chrysoptera* to *O. beta* predation.

P. herbstii showed a greater affinity for refuge habitat than *C. sapidus*. In single-prey treatments without a predator, only $26 \pm 3\%$ (mean ± 1 SE) of *C. sapidus* occupied the seagrass patch compared to $52 \pm 2\%$ of *P. herbstii* (Fig. 3b). In mixed prey treatments, *P. herbstii* increased their refuge use (although statistically marginal; $F_{1,78} = 3.235$, $p = 0.076$) while *C. sapidus* decreased their refuge use ($F_{1,78} = 67.125$, $p < 0.001$), suggesting a possible displacement. *P. herbstii* significantly reduced their activity in predator-present treatments ($F_{1,78} = 60.177$, $p < 0.001$) while *C. sapidus* failed to do so ($F_{1,78} = 0.001$, $p = 0.9779$) (Fig. 3c), perhaps increasing their conspicuousness to *O. beta*.

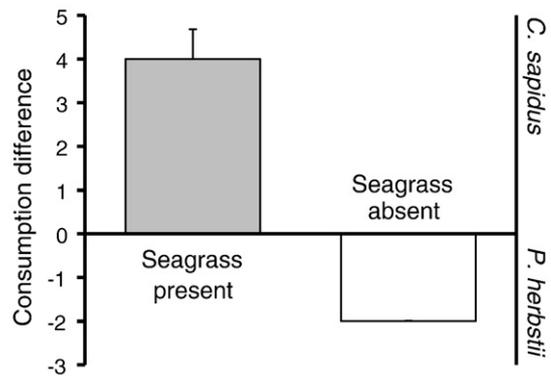


Fig. 4. Difference in consumption of crab prey species by *O. beta* with seagrass (gray bar) and without seagrass refuge (white bar). Consumption difference is the difference in prey species consumed per 48 h trial where preference towards *C. sapidus* is positive and preference towards *P. herbstii* is negative. The gray bar (seagrass present trials) represents the mean of 6 replicates (± 1 SE) while the white bar (seagrass absent trials) represents the mean of 2 replicates. The consumption difference was the same (-2) in both seagrass absent trials and as a result, no SE was generated.

In the refuge removal experiment performed with the crab prey species, there was a significant shift in prey preference by *O. beta* with the removal of the seagrass patch (unpaired t -test: $t = 3.166$, $df = 6$, $p = 0.019$). With the removal of the seagrass patch, the mean consumption difference changed from $+4 \pm 0.7$ (mean ± 1 SE) *C. sapidus* to $+2$ *P. herbstii* consumed per 48 h (Fig. 4).

4. Discussion

In both systems, interspecific prey interactions decreased overall prey survivorship (Fig. 1), thereby benefiting the shared predator. These increases in net predation rate were primarily driven by disproportionately higher predation rates on one prey species (*O. chrysoptera* and *C. sapidus*), while the co-occurring prey (*L. rhomboides* and *P. herbstii*) was not significantly affected in mixed prey treatments (all relative to single-prey treatments) (Fig. 2). Behavioral interactions within the three-species trophic systems caused reduced, non-additive prey survivorship in mixed prey treatments which could not be predicted based on survivorship in single-predator–single-prey treatments. Interactions were indirect and behaviorally-mediated, as in both systems, one prey species altered the behavior of a second which in turn increased the second species' vulnerability to predation.

Furthermore, in pairwise treatments, *O. beta* consumed more *L. rhomboides* and *P. herbstii* individuals than either of the co-occurring prey species (*O. chrysoptera* and *C. sapidus*, respectively) (Fig. 2), and so it could be expected that this "preference" would be maintained within mixed prey treatments. Interestingly, we observed the opposite pattern in multiple prey situations; *L. rhomboides* and *P. herbstii* were not significantly affected by increases in prey richness while *O. chrysoptera* and *C. sapidus* were more heavily preyed upon by *O. beta*. This reversal exemplifies the importance of prey interactions in modifying the effects of predation within these experimental three-species systems. In interpreting these predation rates on individual prey species it should be noted that intraspecific density was reduced in the mixed prey treatments (from 20 to 10 individuals), and so our comparison of individual prey survivorship is somewhat confounded by these reductions in density which should result in decreased encounter rates with predators.

The asymmetrical predation of *O. beta* in mixed prey treatments can largely be explained by behavioral interactions within the three-species trophic systems. *O. beta* are ambush predators (Schwartz and Dutcher, 1963; Wilson et al., 1982) and not surprisingly, we failed to record an actual predation event during our observations. In our

experiment, *O. beta* were primarily observed resting motionless and partially buried on the edge or within the seagrass patch during the 12-h day periods. *O. beta* were seen, however, in the sand matrix in an active state (exposed with pectoral fins undulating) on several occasions shortly after the start of the dark period of the artificial photocycle (observed with LED light). As these were the only observed habitat shifts or movements of *O. beta* throughout the experiment and no predation events were observed during the day, we speculate that *O. beta* foraged during these low-light conditions and in the sand matrix where prey were more visible and less protected. It is well known that prey often restrict their activity to microhabitats where predators are less effective (Kerfoot and Sih, 1987), and the capture success of *O. beta* could have been considerably enhanced by foraging in the sand matrix rather than the structurally complex seagrass patch (e.g. Heck and Orth, 2006).

Predators can increase (Kerfoot and Sih, 1987; Mittelbach, 1988) or alter the outcome (Persson, 1991) of competitive interactions between prey species by forcing them into common refuge habitats. We did not measure the effects of competition for refuge space between prey species *per se* (e.g. reduced growth rates), but we did monitor interference behaviors between prey and how these behaviors affected relative predation rates by *O. beta* (i.e. apparent competition). Based solely on chase data in single-prey with predator treatments, *L. rhomboides* were clearly more aggressive towards conspecifics while *O. chrysoptera* on *O. chrysoptera* chases were rare (Fig. 3a). Although intraspecific *L. rhomboides* chases remained high in both single and mixed prey treatments, chases on *O. chrysoptera* increased dramatically in mixed treatments (Fig. 3a) with all chases by *L. rhomboides*. These chases primarily occurred within or around the periphery of the seagrass patch, leading us to believe that *L. rhomboides* directly excluded *O. chrysoptera* from the seagrass refuge (a form of interference competition). As noted by Sih et al. (1988), high rates of emergence from a refuge alone can increase attack rates on a given species, even if the species is rarely observed outside of the refuge. Because *O. beta* presumably foraged in the surrounding sand matrix, these prey conflicts could increase *O. chrysoptera* vulnerability to predation via increased encounter rates with *O. beta* in the sand matrix. We suspect that these agonistic interactions could also have induced a change in *O. chrysoptera* behavior (e.g. reduced vigilance), rendering them more susceptible to predation regardless of their location within the tank.

Crabs were rarely seen moving and direct behavioral interference between crab species was never observed. Still, *P. herbstii* showed a greater affinity for the seagrass refuge than *C. sapidus* (Fig. 3b), and their refuge-holding potential was made apparent in mixed prey treatments; *P. herbstii* occupancy of the refuge increased while *C. sapidus* occupancy declined, suggesting a habitat displacement. Additionally, *C. sapidus* failed to reduce their activity level in predator-present treatments (unlike *P. herbstii*) (Fig. 3c). As activity levels were designated based on crab exposure and visibility, it is likely that this lack of an anti-predator response by *C. sapidus* resulted in their higher susceptibility to predation. Interestingly, the activity levels of *C. sapidus* remained constant regardless of the presence of the other species (Fig. 3c), which is indicative of an individual response rather than a prey interaction.

It appears that the displacement of *C. sapidus* by *P. herbstii* outside of the refuge habitat acted to increase predation on *C. sapidus*. For this explanation to be accurate, structurally complex refuge space must have played a critical role in mediating the interaction between prey. To test this, we performed two additional trials without the seagrass patch. As expected based on the behavioral data, the prey consumption difference of the predator shifted from *C. sapidus* with seagrass (+4) to *P. herbstii* without seagrass (+2) (Fig. 4). This result, as well as the higher rates of mud crab consumption compared to blue crab consumption in pairwise treatments corroborates the hypothesis that refuge space, and specifically the refuge-holding potential of *P. herbstii* increased the susceptibility of *C. sapidus* to predation in mixed prey treatments.

In the present study, we intentionally chose prey pairs with possible competitive asymmetries in order to make qualitative predictions on how prey interactions might affect relative predation rates on the prey species. We expected to see the more aggressive species win in a conflict for refuge space, thus forcing the more docile prey into the precarious unstructured habitat. This interaction was observed in the fish prey pair, yet in the crab prey pair it was the more aggressive *C. sapidus* that suffered increased predation due to their displacement by *P. herbstii* in mixed prey treatments and failure to become less active in the presence of *O. beta*. Brown et al. (2005) found *P. herbstii* (referred to as *P. simpsoni*) to be the most subservient species in a guild of xanthid crabs, yet xanthids are well known for their use and defense of complex, structured habitats (McDonald, 1982; Williams, 1984). In this case, refuge-holding potential proved more important than aggressive behaviors in the competition for enemy-free space between crab prey species.

Within the subfield of biodiversity and ecosystem functioning, researchers have demonstrated that horizontal diversity (i.e. within a trophic level) can exert a strong influence on community dynamics, and synergistic interactions between species have been proposed as a possible mechanism driving such diversity effects (Duffy, 2002; Ives et al., 2005; Stachowicz et al., 2007). Specifically, authors have hypothesized that prey diversity should increase the resistance of the prey trophic level to consumption by higher predators. For example, in a meta-analysis, Hillebrand and Cardinale (2004) showed that the effects of herbivore consumption decreased with increased algal diversity over a range of diversity levels and community types. The authors suggest positive, facilitative interactions between algal species as a possible mechanism (amongst others) causing consumer effects to decline with prey diversity. In another recent study by Snyder et al. (2008), prey diversity was found to have no effect on predation by a multiple predator guild.

While the present study only manipulated prey diversity from 1 to 2 species and did not allow for species turnover (and likely does not provide a true test of prey diversity-consumer effects theory), our results should be of general interest to ecologists studying such diversity effects. Using either prey guild, we failed to show a reduction in consumption rates with the addition of prey species. At the interaction level, our study demonstrates that risk enhancement between prey species is also possible (in addition to facilitation), which could result in increased predation with diversity. In contrast to Hillebrand and Cardinale (2004), we used animal species as prey; highly mobile animal species with evolved behaviors can introduce further complexities, which studies of plant diversity may fail to capture (Duffy, 2002).

In the present study, a structurally complex seagrass patch provided refuge from a predator on a local scale, and interspecific prey interactions associated with this refuge had meaningful consequences for prey survivorship. In addition, we showed that interactions between prey can have unpredictable reciprocal effects on their shared predator. Pairwise predator-prey relationships were fundamentally altered by the presence of additional prey species, and knowledge of such relationships is vital to predicting how communities might respond to the loss or gain of species. An obvious next step is to expand both the spatial and temporal scales of such prey interaction studies. For example, studies which measure the effects of these interactions on both predator and prey growth or fecundity as well as multigenerational studies in laboratory systems would yield new insight on how such interactions can structure communities and influence population dynamics. Field studies over larger spatial scales are necessary to determine whether these interactions persist in more complex, heterogeneous environments and among a more speciose background environment.

Many recent empirical studies of horizontal diversity in predator-prey systems have explored the non-additive effects of consumers on prey in unusual inverted trophic pyramids (multi-predators-single-prey) (Sih et al., 1998). Emergent multiple prey scenarios have been

overlooked in comparison, yet are equally common in nature and thus worthy of thorough investigation. We advocate 1) further empirical exploration of the possible interactions within single-predator–multiple-prey systems, in order to keep pace with the rich body of theoretical work (Holt, 1977; Holt and Lawton, 1994), and; 2) that future studies in such systems also determine the effects of multiple prey on predators (rather than prey only) using the non-additive MPE framework (Sih et al., 1998).

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