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Sex, size, and prey caloric value affect diet specialization and consumption of an invasive prey by a native predator

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Abstract

Escaping the control of natural enemies is thought to heavily influence the establishment success and impact of non-native species. Here, we examined how the profitability of alternative prey in combination with the presence of a competitor and predator aggressive behavior explain individual differences in diet specialization and the consumption of the invasive green porcelain crab *Petrolisthes armatus* by the native mud crab predator *Panopeus herbstii*. Results from bomb calorimetry estimates show that invasive *P. armatus* has high caloric value relative to alternative native prey. Laboratory assays indicated that specialization and consumption of invasive *P. armatus* was mostly exhibited by large, female *P. herbstii*, but the presence of a competitor and predator aggressiveness did not influence diet and the consumption of *P. armatus.* Thus, intrinsic factors (e.g., sex and body size) seem to explain consumption of *P. armatus* and dietary specialization in *P. herbstii*, more generally. Although there are still many predator individuals that do not consume *P. armatus*, the proportion of individuals that have begun to specialize on *P. armatus* suggests that for some, it has become more profitable relative to alternative native prey. Given the high caloric value of *P. armatus*, we suggest that it is likely that differences in the cost of its consumption, including attack, capture, and handling times relative to alternative prey, determine its net profitability to individual predators.

Key words: diet breadth, optimal foraging theory, enemy escape hypothesis, competition, aggressive behavior

Invasive species or species expanding their range can serve as a new, abundant food source for native predators (King et al. 2006; Carlsson et al. 2009; Pintor and Byers 2015a). Although there is increasing evidence that invasive species are consumed by native predators, predators may not be consuming sufficient numbers of non-native prey to limit their spread or regulate population abundance. As a result, invasive prey may still experience escape from natural enemies and reach high abundances. Understanding why some invasive species remain relatively uneaten by native predators thus provides key insights into when an invader may escape top-down control to reach large population sizes and provides the opportunity to examine the mechanisms underlying individual variation in dietary specialization.

Invasive prey species might remain relatively uneaten if predators exhibit a long-term avoidance of the prey or if they are not profitable relative to alternative native prey. For example, although all individuals in a population are typically initially fearful of novel food (e.g., neophobia), some individuals will overcome this neophobic response, whereas others display an active and long-term avoidance of the novel prey (e.g., dietary conservatism; Marples and Kelly 1999). A higher prevalence of dietary conservatism in the predator population may contribute to the non-native's escape from natural enemies. In addition, following optimal foraging theory, if an invasive prey is of lower caloric value per unit handling time than native prey, then the native predator should avoid consuming it (Pyke et al.

1977). The value of the non-native prey could also be low if the predators are naïve to the non-native prey and lack the appropriate foraging skills needed to capture and consume the new prey (Cox and Lima 2006; Li et al. 2010; Sih et al. 2010). Over time, the invasive prey may increase in profitability as predators acquire a search image and learn to consume a new prey species more efficiently, or as the abundance of native prey declines. But in the initial stages of invasion, relative profitability of alternative prey might be one mechanism causing predators to either avoid or limit their consumption of an invasive prey.

Yet the profitability and preferences for alternative prey might vary among individuals within a predator population and may affect the consumption of an invasive prey. For example, individual predators within a population will often consume and specialize on different prey (Bolnick et al. 2002; Newsome et al. 2009) to reduce intraspecific competition for resources. In the context of an invasive prey, if that prey is profitable it may be monopolized by individuals within the predator population that are better competitors. Alternatively, if the invasive prey is not profitable, it might be avoided by individual predators that are better competitors and predominantly consumed by less competitive individuals in order to reduce the cost of competition for more profitable alternative prey. Either scenario implies that only a fraction of the predator population may consume the invasive prey and ultimately this may limit the predation pressure experienced by the invading prey species.

Here in this study, we examined factors that may explain individual differences in dietary niche breadth and the consumption of the invasive green porcelain crab *Petrolisthes armatus* by the native mud crab predator *Panopeus herbstii*. *Petrolisthes armatus* has been expanding its range northward along the southeastern coast of the United States and is frequently observed to reach high abundances when it arrives in a new system (Hollebone and Hay 2007). Native mud crab predators studied near the center of *P. armatus*' introduced range have been shown to consume *P. armatus*, but the continued high abundances of *P. armatus* suggest that it still is escaping control of native predators like mud crabs. Pintor and Byers (2015b) demonstrated that individual native mud crab predators within a single population vary in behavior and consumption of *P. armatus*. Specifically, females and individuals that were less aggressive and less exploratory tended to consume more, and even specialize on invasive *P. armatus*. Whereas, males and individuals that are more aggressive and more exploratory rarely consumed *P. armatus* and instead consumed primarily, or even specialized on, native prey (especially mussels). Based on this work, we hypothesized that consumption of *P. armatus* by native mud crab predators was associated with the profitability of *P. armatus* relative to alternative prey, along with the presence of competition and differences in aggressive behavior among individual mud crab predators.

Here, we aimed to directly test these hypotheses by quantifying the caloric value of alternative prey and experimentally assessing the role of competition and predator individuals' competitive abilities in the consumption of *P. armatus*. First, we examined whether differences in caloric value of *P. armatus* relative to a suite of alternative native prey explain individual variation in the consumption of *P. armatus* by native mud crab predators. We hypothesized that if the limited consumption of *P. armatus* by native predators is due to low caloric value, then *P. armatus* should be of lower energetic value in comparison to a suite of alternative native prey. Second, we examined whether the presence of a competitor and aggressive predator behavior was associated with patterns of *P. armatus* consumption by native mud crab predators. In crustaceans, such as crabs, aggressive

behavior has been shown to vary among individuals and to determine competitive ability and the establishment of dominance hierarchies that determine access to food resources (Hazlett 1981; Brown et al. 2005; Griffen and Byers 2006; Tanner et al. 2011, Pintor and Byers 2015b). Thus, we hypothesized that the presence of a competitor will alter the dietary niche breadth and prey choices of native *P. herbstii* and that individuals that are less aggressive will consume a greater proportion of *P. armatus*, the less preferred prey item.

Materials and Methods

Caloric value of alternative prey

To estimate the caloric densities (i.e., caloric value) of native and non-native prey, we hand collected individuals of 4 prey species, oysters *Crassostrea virginica*, mussels *Geukensia demissa*, and prey crabs *Eurypanopeus depressus* and *Petrolisthes armatus* from a single population within the Bull River off of Skidaway Island in Savannah, Georgia. Although total caloric density can vary seasonally due to changes in carbohydrate, lipid, and protein stores (Gardner et al. 1985), we collected all organisms between July and September 2013 to minimize seasonal variation. We preserved undamaged, individuals by freezing prior to analysis.

Basic sample preparation was the same for all organisms. We thawed, lightly blotted dry, and weighed whole body samples of *E. depressus* and *P. armatus* (including shell) and whole tissue for *G. demissa* and *C. virginica* after shell removal. We used whole body samples of *E. depressus* and *P. armatus* because it was not possible to reliably dissect out all tissue from either because of their small size and this also reflects the way these prey are likely consumed (i.e., in their entirety) by *P. herbstii* predators. However, we accounted for the addition of shell to the sample when estimating the final caloric density of these 2 prey species (see below for details). We dried samples to a consistent weight ($\leq$2% weight change from the previous day) at 60°C for 48–64 h. We randomly selected some samples to estimate caloric density and some to estimate organic content (i.e., ash-free dry weight [AFDM]).

To estimate caloric density, we pooled several individuals of each species in order to achieve the minimum weight needed for the bomb calorimeter to reach a minimal adequate temperature rise for accurate caloric density determination. We ground dried samples into a fine homogenized powered by hand using a mortar and pestle. The homogenized samples were then pressed into pellets of ~0.3 g (*G. demissa* and *C. virginica*) or ~0.7 g (*E. depressus* and *P. armatus*). The difference in pellet weights between species was necessary in order to maximize the amount of combustible material needed to meet the temperature change detection limit whereas minimizing the number of individuals that were needed in each sample. We processed samples in an oxygen bomb calorimeter (Parr model 6400) to determine the calories per gram dry weight (cal g^{-1} DW) in. In total, we ran $N = 43$ replicate runs for *G. demissa*, $N = 35$ replicate runs for *C. virginica*, $N = 9$ replicate runs for *E. depressus*, and $N = 24$ replicate runs for *P. armatus*.

To express the caloric density in terms of calories per gram of AFDM, we dried and weighed additional individuals of each species and placed individuals in a muffle furnace to combust for 1 h at 500°C. The weight loss from combusting is regarded as the organic content. We then calculated the percent organic content per gram of sample (i.e., species) and used this value to express the caloric content of each species in terms of calories per gram AFDM. Because shell will largely not combust, this step was particularly important to remove the influence of shell in the crab samples and have the

caloric value just reflect the energy density per tissue mass. Because the samples for each of the mollusk species were just tissue, nearly 100% of each of those samples combusted; thus, for each of the mollusk species, calories g^{-1} DW and calories per gram AFDM were essentially identical.

In addition to reporting Cal/g AFDM, which depicts the standardized relative energy value of each prey, we also calculated the absolute energy for the given size of prey used in our experiments. This calculation should reflect more of an absolute payoff to a predator in terms of the actual energy gain possible, and thus be more pertinent to foraging decisions in our experimental trial (below). Specifically, we converted the calculated calories per gram of AFDM for each individual per species to calories per individual (cal/ind) of the median sized individual per species used in the experiment. Specifically, we fit polynomial regressions to the relationships between body size and AFDM for each species. We input the median size of our experimental individuals into the equation to yield the AFDM of the median individual. We then multiplied this value by cal/AFDM to yield cal/median individual.

Individual variation in diet and consumption of *P. armatus* in the presence/absence of a competitor

To examine whether the presence of a competitor alters the diet and consumption of *P. armatus* by individual *P. herbstii*, we tested and tracked individual *P. herbstii* (55; 25 females, 30 males) in a simultaneous prey-choice assay both with and also without a conspecific competitor. Specifically, we evaluated the diet breadth of a focal *P. herbstii* when given a choice of the same 4 prey species (3 native, 1 non-native) as used in the bomb calorimetry estimates: the native *E. depressus* (carapace width: 7–12 mm), native *C. virginica* (shell length: 15–25 mm), and the native *G. demissa* (shell length: 20–30 mm) and non-native *P. armatus* (carapace length: 7–10 mm). We hand collected all organisms from a single population from oyster reef clusters within the same site. We recorded the sex and size (carapace width between 25 and 55 mm) of all individual *P. herbstii* used in the experiments, and individually marked them with numbered bee tags. Prior to behavioral assays, we housed organisms by species in flow-through seawater tanks. *P. herbstii* were fed a mixture of live *C. virginica*, *G. demissa*, and *E. depressus* and *P. armatus* ad libitum.

We first quantified the diet of each individual *P. herbstii* predator in the absence of a competitor and then tested the same individual again in the presence of a non-lethal competitor. Specifically, we tested *P. herbstii* individuals singly in 18.9-L aquaria filled with ~6.3 L of aerated seawater. Each aquarium included limited structure (1, 3-piece cluster of bleached oyster halves) to serve as refuge for prey. We withheld food for 24 h to standardize hunger-levels. Five minutes prior to the start of the experiment, we covered the focal predator crab with an isolation chamber (inverted, opaque cup that was weighted to prevent premature escape). Next, we added 3 live individuals from each of the 4 prey species (total of 12 individual prey items) to the tank, opposite that of the predator crab in the isolation chamber. We allowed the prey species to acclimate in the tank for 2 min before releasing the focal predator crab from the isolation chamber. Once the isolation chamber was removed, the experiment began and we checked the tanks every 20 min over a 4-h period (i.e., 12 total observations) to quantify the identity and number of each prey species that was consumed. We replaced any prey items that were consumed during a 20-min interval to keep densities of prey relatively constant. At the end of the 4-h observation period

we removed any remaining prey items from the testing aquaria and the water was completely replaced with fresh aerated seawater.

To examine whether the presence of a conspecific competitor had an effect on the diet breadth of *P. herbstii*, we quantified the diet of same *P. herbstii* individuals tested above in the presence of a single, conspecific competitor. The methodology was exactly the same as described above except for the addition of a size-matched conspecific. Specifically, we introduced a size-matched conspecific (± 5 mm carapace width) to the tank in an isolation chamber and allowed it to acclimate to the tank for 5-min. We rendered the conspecific non-lethal to the prey by gluing the chelae and the mandibles with non-toxic super glue (Griffin et al. 2011). We did this to ensure that any consumption of prey was exclusively by the focal *Panopeus* individual, but still allowed for direct interactions (e.g., interference) between the predator crabs to occur. In addition, to test whether an individual's diet breadth was repeatable (i.e., that an individual's prey choice was consistent over time), we repeated the trial 3 times in one 4-hour trial a day over the course of 3 days. Repeatability of diet in the absence of a competitor had been previously found to be repeatable ($r = 0.39 \pm 0.10$ *SE*; Hostert, Pintor and Byers, unpublished data). Therefore, we only examined diet in the absence of a competitor on one day and did not repeat the trial for an additional 2 days. Finally, logistically it was not possible to test all individuals on the same day, therefore, trials were blocked over time (each week 6–12 individuals were tested over consecutive days, i.e., block = week).

To quantify and characterize individual differences in diet and consumption of *P. armatus* among *P. herbstii* predators, we used the data collected to calculate a proportional similarity index (PS$_i$) (Equation (3)) for each individual *P. herbstii* tested (see Schoener 1968, Bolnick et al. 2002 for details). More explicit details for the index can be found in Schoener (1968), Feinsinger et al. (1981) and Bolnick et al. (2002). Briefly, let **N** be a matrix of diet data where the element $\mathbf{n}_{ij}$ represents the number of items in individual *i*'s diet that fall into category *j*. This data matrix can then be transformed into a proportional matrix where $\mathbf{p}_{ij}$ (Equation (1)) describes the proportion of resource *j* in individual *i*'s diet and $\mathbf{q}_{ij}$ (Equation (2)) describes the proportion of resource *j* in the population's observed diet and are equal to:

$$p_{ij} = \frac{n_{ij}}{\sum_j n_{ij}} \tag{1}$$

$$q_{ij} = \frac{\sum_i n_{ij}}{\sum_i \sum_j n_{ij}} \tag{2}$$

The PS$_i$ of individual *i* in the population is equal to:

$$PS_i = 1 - 0.5 \sum_j |p_{ij} - q_j| = \sum_j min(p_{ij}, q_j) \tag{3}$$

PS$_i$ values range from 0 to 1, where values near 1 indicate that an individual eats the same proportion of prey types as the population as a whole (i.e., for a generalist species, a generalist individual), whereas values near 0 indicate that an individual does not conform to the diet of the population as a whole (i.e., a specialist). Thus, we used an individual *P. herbstii* predator's PS$_i$ score to characterize the degree of specialization (regardless of prey type) among individuals tested. To examine individual differences in the consumption of *P. armatus*, we also calculated the proportion of *P. armatus* consumed during the simultaneous choice assays with and without a competitor.

Table 1. Scoring system used to determine the average aggression of *P. herbstii* predators in aggression assays

Score	Behavior	Definition
−2	Fleeing	Walking away (rapidly)
−1	Avoidance	Walking away (slowly), turning away
0	Separate	At least one body length apart and not facing each other
1	Initiation (No contact)	Facing, approaching, turning towards opponent
2	Threat display (No contact)	High in legs, claws open, meral spread, claws forward
3	Physical contact (No claw grasping)	Claw touching, claw tapping, claw pushing
4	Physical contact (Claw grasping)	Claws used to grasp opponents claws (claw lock)
5	Unrestrained use of claws	Snapping, ripping, swimming while in claw lock, grasping legs or rostrum

Source: Adapted from Karavanich and Atema (1998) and Pintor et al. (2008).

Individual variation in aggressiveness

To evaluate whether differences in aggressiveness (i.e., a behavior associated with competitive ability) explained variation in the diet and the consumption of *P. armatus* quantified in the diet choice assay described above, we tracked all *P. herbstii* individuals previously tested in the previous assay and ran each through a behavioral assay to quantify aggression. Specifically, we conducted a pair-wise aggression assay between size-matched individuals (±3-mm carapace length). We introduced a focal and non-focal *P. herbstii* into an 18.93-L aquarium in separate, isolation chambers (identical to those used in the diet assays) and allowed them to acclimate for 2 min after introduction to the aquarium. After the 2-min isolation, we lifted the chambers and observed and recorded (Panasonic V100M video camera) aggressive behavior of both individuals for a 20-min period. We assigned a score between −2 and 5 to the behavior of each individual every 5 s following a scoring system similar to that used for assessing aggression in lobsters and crayfish (See Table 1) (Karavanich and Atema 1998; Pintor et al. 2008). We averaged the aggression scores taken every 5 s during a trial to give a mean aggression score for each individual for each day.

In addition, to test whether an individual's aggressiveness was a repeatable behavior (i.e., consistent over time), we performed the above test on each focal individual 3 times in one 20-min trial per day over the course of 3 days. In each trial, we presented the focal individual with a unique size-matched conspecific to avoid habituation and effects of individual identity of the conspecific. Also, the focal individual was removed from the testing tank between trials and the tank was cleaned to remove any cues that might have influenced subsequent trials. In this manner we had 3 daily aggression scores per predator individual used in the diet choice assays.

Statistical analysis

Caloric content of prey

Due to unequal variance between groups, we analyzed differences between prey species in calories per gram AFDM and calories per median sized prey used in the study with a Kruskal–Wallis test using JMP statistical software (JMP Version 12 1989–2007). We then ran multiple *post hoc* comparisons of caloric density of each species using a Wilcoxon multiple comparison test.

Variation in diet and consumption of *P. armatus* predators

We evaluated the distributions of each of the dependent variables (PS_i with and without a competitor and proportion of *P. armatus* consumed with and without a competitor) for normality, skewness, and kurtosis. All were normally distributed.

To evaluate whether the presence of a competitor, aggressive behavior, and size was associated with variation in diet breadth (i.e., PS_i score) of native mud crab predators *P. herbstii*, we ran a series of linear mixed-effects models. Size (carapace width), sex, aggression, and competitor presence were fitted as fixed effects. Block was also fitted as a fixed effect but was not significant and removed from the model. Individual mud crabs were used repeatedly in 2 contexts (with and without a competitor); therefore, mud crab identity was fitted as a random effect in the models. We used Akaike's Information Criterion corrected for small samples sizes (AIC_c) to select the most parsimonious models (Symonds and Moussalli 2011). ΔAIC_c scores were calculated using the difference between the AIC_c value of each model and the model with the lowest AIC_c. ΔAIC_c scores were then used to compute Akaike weights (w_i) in order to rank for top fitting models. We considered models with the $\Delta AIC_c < 2.0$ to be the best fitting models. Each variable was examined for its statistical significance using an F-test. In addition, we calculated the relative variable importance factor (RVI) for all variables of best fitting models by summing Akaike weights (w_i) for each variable across all models containing that variable (Burnham and Anderson 2002; Kittle et al. 2008; Burnham et al. 2011). To evaluate the effects of the same variables (the presence of a competitor, aggressive Behavior, and demographic variation)on the proportion of *P. armatus* consumed by *P. herbstii* (native mud crab predators) we ran the same set of linear mixed-effects models as described above on this different response variable. All linear models were run using the lme4 package for R statistical software (R Core Team 2008).

Repeatability of diet and aggressive behavior in *P. herbstii*

To insure that diet specialization and aggressive behavior were consistent within and among individuals used in this study, we examined the repeatability of individual *P. herbstii*'s responses. Namely, we analyzed the degree of specialization in the presence of a competitor, using a one-way ANOVA with individual *P. herbstii* as a fixed-effect to estimate the repeatability of PS_i with a competitor (Lessells and Boag 1987). We estimated standard errors and confidence intervals as in Becker (1984). In addition, we quantified the repeatability of aggressive behavior in *P. herbstii* using a 1-way ANOVA, as just described.

Results

Caloric content of alternative prey

The mean calories per gram of AFDM for the 2 shellfish species, *G. demissa* and *C. virginica*, were 5482.03 ± *SE* 80.47 and 4608.04 ± *SE* 68.41, respectively (Figure 1A). The mean calories per gram of AFDM of the 2 crab species, *E. depressus* and *P. armatus*, were 3624.56 ± 101.20 *SE* and 4597.51 ± 180.18 *SE*, respectively
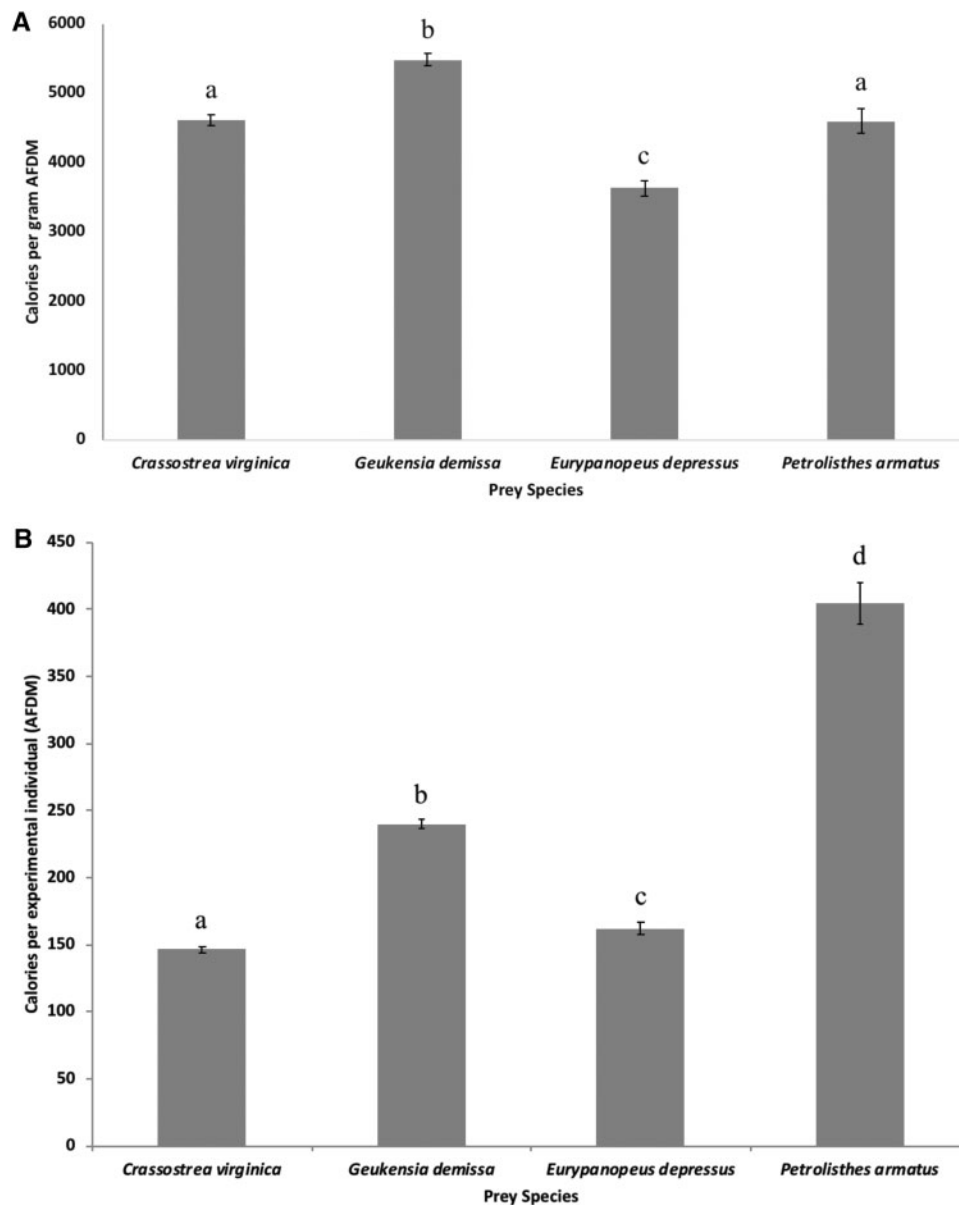
Figure 1. (A) Mean ($\pm$ SE) calories per gram AFDM of alternative prey used in prey choice assay. **(B)** Mean ($\pm$ SE) calories per individual (AFDM) of the median sized prey used in the prey choice assays. For both figures, results of Wilcoxon multiple comparisons are indicated by letters and denote significance at $P < 0.05$.

(Figure 1A). Results of the Kruskal–Wallis test indicated significant differences in the mean calories per gram AFDM between the species ($\chi^2 = 37.97$, $df = 3$, $P < 0.001$). Subsequent Wilcoxon multiple comparison tests indicated that the calories per gram AFDM of *G. demissa* was significantly higher than all other prey ($P < 0.01$ for all pairs). Calories per gram AFDM of *E. depressus* was significantly lower than all other prey ($P < 0.01$ for all pairs). *C. virginica* and *P. armatus* did not differ in calories per gram AFDM ($P = 0.98$).

The mean calories per median sized individual used in the experiment for *G. demissa* and *C. virginica* were 239.76 ± 3.52 (*SE*) and $146.70, \pm SE$ 2.18, respectively (Figure 1B). The mean calories per median sized individual used in the experiment for *E. depressus* and *P. armatus* were $162.09, \pm 4.53$ *SE* and $404.36, \pm 15.85$ *SE* cal/ind, respectively (Figure 1B). Results of the Kruskal–Wallis test indicated significant differences in the mean calories per median sized

individual used in the experiment between the species ($\chi^2 = 57.92$, $df = 3$, $P < 0.001$). Subsequent Wilcoxon multiple comparison tests indicated that the calories per median sized individual used in the experiment was significantly different across all prey ($P < 0.01$). Specifically, *P. armatus* had the highest calories per median size prey used, followed by *G. demissa*, *E. depressus* and *C. virginica*.

Individual variation in diet and consumption of P. armatus in the presence/absence of a competitor

In the absence of a competitor, results of the simultaneous choice assay indicated that the sampled population of *P. herbstii* consumed, on average, 35.4% *G. demissa*, 29.2% *P. armatus*, 27.8% *E. depressus*, and 7.6% *C. virginica*. In the presence of a competitor, results of the simultaneous choice assay indicated that the sample

Table 2. Top-ranked linear mixed models testing the effect of morphometric, behavioral, and contextual predictor variables on the PS$_i$ of *P. herbstii* during simultaneous choice assays

Model	# variables	r^2	AIC	ΔAICc	w$_i$
Sex (M)	2	0.0487	−81.79	0	0.37
Sex (M) + Aggression (+)	3	0.049	−80.02	1.76	0.15
Sex (M) + Aggression (+) + Competitor Presence	3	0.0499	−79.85	1.94	0.14

Focal mud crab identity was fitted as a random effect across all models. Top models have Delta_AICc < 2.0. Relative variable important (RVI) scores (over all models) for the variables of the top 3 predictive models for PS$_i$ of *P. herbstii* during simultaneous choice assays are: RVI (sex) = 0.96, RVI (aggression) = 0.28, RVI (competitor presence) = 0.27.

population of focal crabs as a whole consumed 38.6% *G. demissa*, 26.6% *P. armatus*, 22.1% *E. depressus*, and 12.4% *C. virginica*. At the individual level, there was considerable variation in diet breadth (i.e., PS$_i$ scores) among *P. herbstii*. The mean PS$_i$ score without a competitor was 0.58 ± *SD* 0.17, ranging from 0.28 to 0.89. The mean PS$_i$ score with a competitor was 0.57 ± *SD* 0.16, ranging from 0.18 to 0.89.

AICc model comparison indicated 3 top models as the best models to predict diet breadth (PS$_i$) for *P. herbstii* predators (Table 2). The highest weighted model was a single variable model containing only Sex (ΔAICc = 0, wi = 0.301) with male *P. herbstii* exhibiting a more generalist diet (e.g., higher PS$_i$ scores) than females (male mean 0.60, *SD* = 0.17, female mean 0.52, SD = 0.18). There were 2 additional top models with ΔAICc < 2.0 that further indicate that along with sex, aggressive behavior and the presence of a competitor predicted variation in PS$_i$ of *P. herbstii* (Table 3). However, sex was a predictor in all three of the top three models and its relative importance in predicting diet breath (PS$_i$), RVI_Sex = 0.96, is more than 3 times higher than aggression (RVI_Aggression 0.28) and competitor presence (RVI_Competitor Presence = 0.27) (Table 2).

Furthermore, sex was always a significant predictor in the fitted models ($P < 0.05$). In contrast, aggression was not a significant predictor in the fitted model (Estimate = 0.0117, $P = 0.824$), nor was competitor presence (Estimate = − 0.006, $P = 0.729$).

The average proportion of *P. armatus* in the diet of *P. herbstii* when a competitor was absent was 0.28, ± 0.29 SD, whereas the average proportion when a competitor was present was 0.24, ± 0.27 *SD*. AICc model comparisons indicated there were 4 top models with ΔAICc < 2.0 for predicting the proportion of *P. armatus* consumed by *P. herbstii* (Table 3). These top models included a 2-variable model containing sex and size; 2 three-variable models containing sex, size, and aggression and sex, size, and competitor presence; and a 4-variable model containing sex, size, aggression, and competitor presence. Specifically, females consumed a greater proportion of *P. armatus* than males (Female mean 0.41, *SD* = 0.30; Male mean 0.17, *SD* = 0.15) (Figure 2A). In addition, a greater proportion of *P. armatus* was consumed by larger (Figure 2B) and more aggressive individuals, and those individuals in the absence of a competitor. Note that size did not significantly differ between males and females used in the study (female carapace width = 3.63 cm ± 0.107 *SE*; male carapace width = 3.64 cm ± 0.102 *SE*). Both sex and size occurred in all 4 of the top models, however, sex was ranked slightly higher than size in terms of relative variable importance in explaining variation in the proportion of *P. armatus* consumed, RVI_Sex = 0.99 and RVI_Size = 0.88, respectively (Table 3). Sex and Size were both greater than 2 times more important in explaining the proportion of *P. armatus* consumed than aggression or competitor presence (RVI_Aggression = 0.40 and RVI_Competitor Presence = 0.36, respectively). Furthermore, sex and size were significant predictors in

the fitted models (sex $P < 0.001$, size $P < 0.05$). In contrast, aggression and competitor presence were never significant predictors in the fitted models (aggression: Estimate = 0.127, $P = 0.17$; competitor presence: Estimate = −0.018, $P = 0.33$).

Repeatability of diet and aggression

Repeatability in diet breadth of individual *P. herbstii* in the presence of a competitor was significantly repeatable over time ($r = 0.25$, ± *SE* = 0.10). Also, aggression was a repeatable behavior ($r = 0.243 ± SE = 0.098$). Across all trials, *P. herbstii* individual displayed all behaviors possible in the aggression scoring system (e.g., scores ranged from −2 to 5). The average aggression score for females was 1.37 (±0.06 *SE*) and for males 1.44 (±0.07 *SE*).

Discussion

Results from this study have shown that contrary to our predictions based on Pintor and Byers (2015b), non-native *P. armatus* has high caloric value (i.e., per gram AFDM) relative to alternative native prey. Furthermore, although the native mussel, *G. demissa* had a higher caloric value per gram AFDM, for the sizes used in the experiments it had lower dry tissue mass than *P. armatus*. Thus, *P. armatus* was the most calorically profitable prey item used in the experiment. Higher proportions of *P. armatus* were consumed by female *P. herbstii* and individual *P. herbstii* that exhibited more specialized diets on any of the 4 prey species in the experiment were also female (similar to Pintor and Byers 2015b). However, in this study, individuals that were larger consumed higher proportions of *P. armatus*. Furthermore, counter to our hypothesis that *P. armatus* consumption was driven by subordinate individuals, aggression and competition explained little variation in the consumption of *P. armatus* or the diet of *P. herbstii*.

The continued high abundance of *P. armatus* following invasion, despite its caloric value suggests that differences in the time involved in the predation sequence (i.e., attack, capture, handling of prey) may reduce the profitability (i.e., energy per unit handling time) of *P. armatus* relative to native prey. Preliminary data suggest that *P. herbstii* individuals that do not consume high proportions of *P. armatus* take longer to capture and have more failed attacks during predation attempts than individuals that consume higher proportions *P. armatus* (Crosby 2018). Furthermore, naïve *P. herbstii* (from an uninvaded site) take significantly longer to capture *P. armatus* and have more failed attacks than experienced *P. herbstii* from *P. armatus*' invaded range (Crosby 2018). While this may explain why all *P. herbstii* do not consume *P. armatus*, it also suggests that over time, native predators can acquire the skills to reduce search, capture and handling time of a novel prey. Alternatively, predation on *P. armatus* in the experiment may have been lower than what

Table 3. Top-ranked linear mixed models testing the effect of morphometric, behavioral, and contextual predictor variables on the proportion of *Petrolisthes armatus* in the diet of *Panopeus herbstii* during simultaneous choice assays

Model	# variables	r^2	AIC_c	ΔAICc	w_i
Sex (F) + Size	3	0.6844	−4.66	0	0.32
Sex (F) + Size + Aggression (+)	4	0.6846	−4.03	0.63	0.23
Sex (F) + Size + Competitor Presence (−)	4	0.688	−3.62	1.05	0.19
Sex (F) + Size + Competitor Presence (−) + Aggression (+)	5	0.6886	−2.94	1.73	0.14

Focal mud crab identity was fitted as a random effect across all models. Top models have AICc < 2.0. RVI scores (over all models) for the parameters of the top 4 predictive models for proportion of *P. armatus* in the diet of *P. herbstii* during simultaneous choice assays are: RVI (sex) = 0.99, RVI (size) = 0.88, RVI (aggression) = 0.40, RVI (competitor presence) = 0.36.
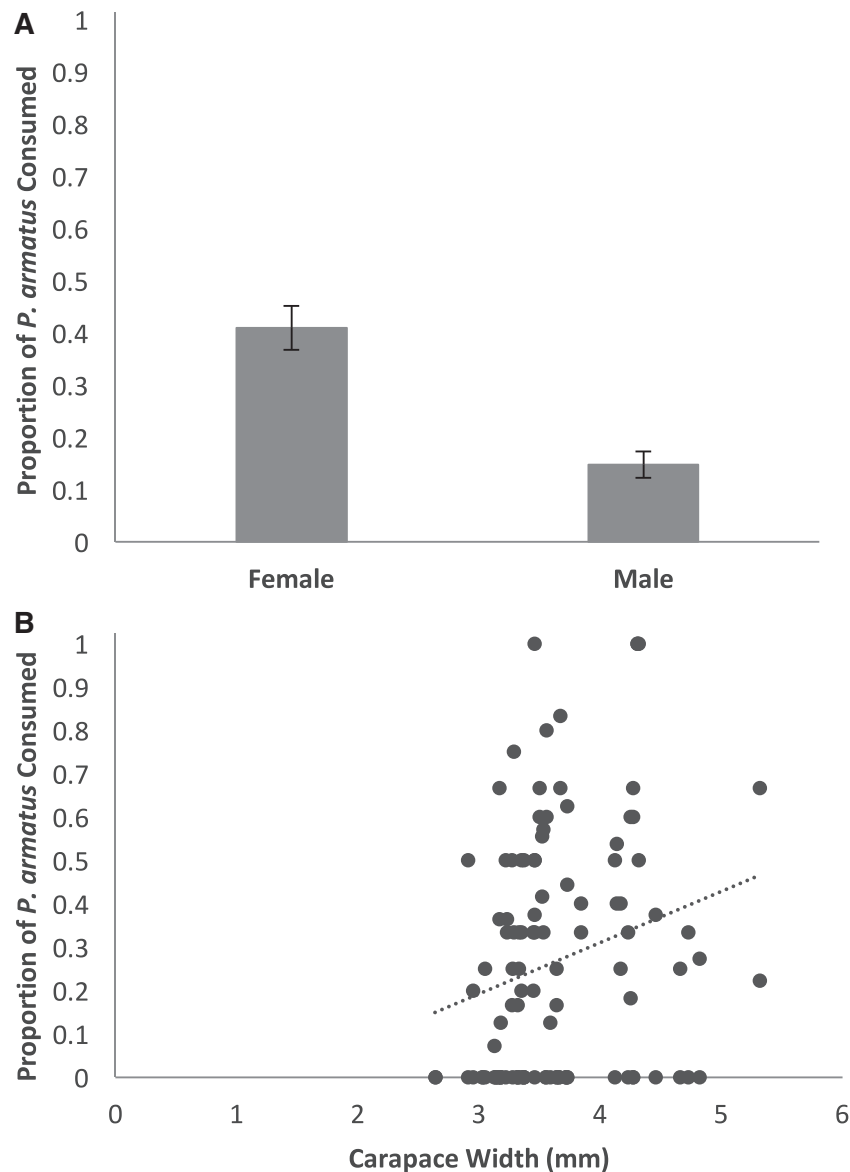
Figure 2. (A) Mean (±*SE*) proportion of *P. armatus* consumed by male and female *P. herbstii* predators. **(B)** Relationship between the proportion of *P. armatus* consumed by an individual *P. herbstii* predator and its carapace width (mm).

may occur in the field because the bivalves in the study were not attached to substrates and easier to capture (i.e., were not attached or cemented to the reef). Thus, energy per unit handling time for bivalves (i.e., with lower caloric value) might have been higher in the experimental arena since they were unattached from substrate

and easier to capture. Finally, *P. herbstii* may not forage optimally (i.e., maximize the energy per unit handling time among alternative prey). For example, *P. herbstii* may not forage optimally if it associates a new crab species of similar shape and size with the native crab, *E. depressus*, which was of lower caloric value relative to the

alternative prey tested in this study. Whether the handling costs associated with *P. armatus* are too great or the predator does not forage optimally, the pattern remains clear that *P. armatus* is not eaten in proportion to what its caloric value alone would suggest. For example, *P. herbstii* predators consumed *P. armatus* in similar proportions to the native crab prey, *E. depressus*, even though *P. armatus* was more than twice as calorically valuable in the experiment. Currently, differences in the attack, capture and handling time among alternative prey, along with evolutionary history may be important factors influencing the overall predation pressure exerted on *P. armatus*.

Although many individual *P. herbstii* predators still do not readily consume non-native *P. armatus*, the proportion of individual predators that do consume a high percentage of *P. armatus* relative to native prey suggests that for some individual predators (e.g., female *P. herbstii*), *P. armatus* either was or has become more profitable relative to alternative native prey. The high proportion of *P. armatus* consumed by female *P. herbstii* confirms previous work demonstrating that female *P. herbstii* eat significantly more *P. armatus* than males (Pintor and Byers 2015b) and could be explained by multiple reasons. Given the high caloric value of *P. armatus* relative to native prey tested in this study, the increased proportion of *P. armatus* consumed by female *P. herbstii* could be associated with the metabolic demands of females (Smith et al. 2015). Alternatively, predator differences in diet choices among native prey prior to invasion could promote the high consumption of non-native *P. armatus* by female *P. herbstii* predators. For example, future work could examine whether females that had diets consisting of greater proportions of the native crab, *E. depressus*, or other small crab species on the reef prior to invasion, are able to transfer the foraging skills to *P. armatus* more quickly than males. For example, shore crabs (*Carcinus maenas*) with previous experience handling dogwhelks handle mussels, a similar but novel prey, more efficiently than those with previous experience only handling fish (Hughes and O'Brien 2001). In addition, morphological differences in chelae shape, size, or strength between males and females are common in crabs (Sneddon et al. 2000; Juanes et al. 2008) and may cause males and females to choose prey species suited to the constraints of their respective chelae morphology and strength. Although chelea size does not typically differ between males and females, it is unknown whether there is a difference in claw strength between the sexes. Finally, not only did females consume higher proportions of *P. armatus* more often than males, but females were also more likely to specialize on any of the prey used in the study in comparison to males. Differences between males and females in morphological, physiological and behavioral capacity to handle alternative prey will influence the relative value of prey and can certainly drive individual variation in diet (Bolnick et al. 2003). Here, in our study, female *P. herbstii* predators not only appear to be the individuals more likely to specialize on *P. armatus*, but to specialize on any of the alternative prey used in the study.

Contrary to our expectations, the presence of a competitor and aggressive behavior did not explain a biologically meaningful amount of variation in prey specialization and the high consumption of *P. armatus*. For example, although these variables were in some of the top statistical models, the presence of a competitor resulted in a negligible shift in the average diet breadth (e.g., $PS_i = 0.58$ without a competitor, 0.57 with a competitor) and the average proportion of *P. armatus* consumed by predators (e.g., proportion of *P. armatus* consumed was 0.28 without a competitor and 0.24 with a competitor). Although competition is partially thought to drive individual

diet specialization as a means to reduce the cost of competing over prey (Svanback and Bolnick 2007, Catrya et al. 2014) and was a reasonable hypothesis here in this study, recent empirical evidence from other studies does not consistently support this hypothesis. For example, dietary niche breadth in lizards is thought to be a function of the variety of resources available in the environment and not associated changes in the density of lizards (i.e., not associated with changes in intraspecific competition for resources) (Novosolov et al. 2017). Alternatively, diet specialization may arise as a consequence of competitors diversifying in their use of physical habitat and the subsequent access to different prey present across different habitats (Jackson et al. 2017). The relative abundance of preferred prey has also been shown to play a role in a predator's consumption of an invasive prey (Liu et al. 2018; Kinney et al. 2018). But more often, there is increasing evidence that diet diversification and intraspecific variation in traits is associated with morphological variation within a population (Kristjansson and Leblanc 2018; Marklund et al. 2018). Across this study and Pintor and Byers (2015b), variation in intrinsic traits (e.g., size and sex) consistently explained the majority of variation in the consumption of *P. armatus*, and thus may be the more important factors driving access to prey, and variation in diet and the consumption of *P. armatus*. Few studies have examined the link between personality traits, such as aggressiveness, and diet, including specialization (Toscano et al 2016). Although the behaviors measured in Pintor and Byers (2015b) suggested that competition and aggressiveness might have been associated with the consumption of *P. armatus* they were less important variables in the top statistical models.

Here, we have seen that there is considerable variation in the diet of *P. herbstii* predators yet only a selective proportion of the population are consuming the non-native prey species *P. armatus* despite its high caloric value. The tested population of *P. herbstii* in 2013 as a whole consumed 29.2% *P. armatus* in the absence of a competitor, which is greater than previous observations in 2011 by Pintor and Byers (2015b) where *P. herbstii* predators were consuming only 14% *P. armatus*. Although this increase may have multiple explanations, a contributing factor could be that with a longer time since invasion, *P. herbstii* predators might continue to switch and consume more non-native *P. armatus*. Ongoing research is comparing multiple populations of *P. herbstii* along the invaded range of *P. armatus* and evaluating how learning may influence the relative value of *P. armatus* to native prey by decreasing the capture and handling time and thereby, increasing the overall profitability of *P. armatus*.

References

Becker W, 1984. *Manual of Quantitative Genetics*. Pullman: Academic Enterprises.

Bolnick DI, Svanback R, Fordyce JA, Yang LH, Davis JM et al., 2003. The ecology of individuals: incidence and implications of individual specialization. *Am Nat* **161**:1–28.

Bolnick DI, Yang LH, Fordyce JA, Davis JM, Svanback R, 2002. Measuring individual-level resource specialization. *Ecology* **83**:2036–2941.

Brown KM, Keenan SF, Banks PD, 2005. Dominance hierarchies in xanthid crabs: roles in resource-holding potential and field distributions. *Mar Ecol Prog Ser* **291**:189–196.

Burnham KP, Anderson DR, 2002. *Model Selection and Multimodel Inference: A Pratical Information-Theoretic Approach*. 2nd edn. New York: Springer Verlag.

Burnham KP, Anderson DR, Huyvaert KP, 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behav Ecol Sociobiol* **65**:23–35.

Carlsson NOL, Sarnelle O, Strayer DL, 2009. Native predators and exotic prey: an acquired taste? *Front Ecol Environ* **7**:525–532.

Catrya T, Alves JA, Gill JA, Gunnarsson TG, Granadeiro JP, 2014. Individual specialization in a shorebird population with narrow foraging niche. *Acta Oecol* **56**:56–65.

Cox JG, Lima SL, 2006. Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators. *Trends in Ecol Evol* **21**:674–680.

Crosby C, 2018. The role of individual learning and dietary preference in the consumption of the invasive green porcelain crab Petrolisthes armatus by Native Crab Predators [Master's thesis]: The Ohio State University

Feinsinger P, Spears EE, Poole RW, 1981. A simple measure of niche breadth. *Ecology* **62**:27–32.

Gardner WS, Nalepa TF, Frez WA, Cichocki EA, Landrum PF, 1985. Seasonal patterns in lipid-content of Lake Michigan macroinvertebrates. *Can J Fish Aquat Sci* **42**:1827–1832.

Griffen BD, Byers JE, 2006. Intraguild predation reduces redundancy of predator species in multiple predator assemblage. *J Anim Ecol* **75**:959–966.

Griffin JN, Butler J, Soomdat NN, Brun KE, Chejanovski ZA et al., 2011. Top predators suppress rather than facilitate plants in a trait-mediated tri-trophic cascade. *Biol Lett* **7**:710–713.

Hazlett BA, 1981. The behavioral ecology of hermit crabs. *Annu Rev Ecol Syst* **12**:1–22.

Hollebone AL, Hay ME, 2007. Population dynamics of the non-native crab *Petrolisthes armatus* invading the South Atlantic Bight at densities of thousands m^{-2}. *Mar Ecol Prog Ser* **336**:211–233.

Hughes RN, O'Brien N, 2001. Shore crabs are able to transfer learned handling skills to novel prey. *Anim Behav* **61**:711–714.

Jackson MC, Evangelista C, Zhao T, Lecerf A, Britton JR et al., 2017. Between-lake variation in the trophic ecology of an invasive crayfish. *Freshw Biol* **62**:1501–1510.

JMP®, Version 12. Cary, NC: SAS Institute Inc., 1989–2007.

Juanes F, Lee KT, McKnight A, Kellogg K, 2008. Claw allometry in green crabs *Carcinus maenas*: heterochely, handedness, and sex. *Mar Biol* **153**:523–528.

Karavanich C, Atema J, 1998. Individual recognition and memory in lobster dominance. *Anim Behav* **56**:1553–1560.

King RB, Queral-Regil A, Stanford KM, 2006. Population size and recovery criteria of the threatened Lake Erie watersnake: integrating multiple methods of population estimation. *Herpetol Monogr* **20**:83–104.

Kinney KA, Pintor LM, Byers JE, 2018. Does predator-driven, biotic resistance limit the northward spread of the non-native green porcelain crab, *Petrolisthes armatus*? *Biological Invasions*. Doi:10.1007/s10530-018-1821-1.

Kittle AM, Fryxell JM, Desy GE, Hamr J, 2008. The scale-dependent impact of wolf predation risk on resource selection by three sympatric ungulates. *Oecologia* **157**:163–175.

Kristjansson BK, Leblanc CA, 2018. Variation in the magnitude of morphological and dietary differences between individuals among populations of small benthic Arctic charr in relation to ecological factors. *Ecol Evol* **8**:1573–1581.

Lessells CM, Boag PT, 1987. Unrepeatable repeatabilities: a common mistake. *Auk* **104**:116–121.

Li YM, Ke ZW, Wang SP, Smith GR, Liu X, 2011. An exotic species is the favorite prey of a native enemy. *PLoS ONE* **6**: e24299.

Liu X, Wang SP, Ke ZW, Cheng CY, Wang YH et al., 2018. More invaders do not result in heavier impacts: the effects of non-native bullfrogs on native anurans are mitigated by high densities of non-native crayfish. *J Anim Ecol* **87**:850–862.

Marklund MHK, Svanback R, Zha YH, Scharnweber K, Eklov P, 2018. The influence of habitat accessibility on the dietary and morphological specialisation of an aquatic predator. *Oikos* **127**:160–169.

Marples NM, Kelly DJ, 1999. Neophobia and dietary conservatism: two distinct processes? *Evol Ecol* **13**:641–653.

Newsome SD, Tinker MT, Monson DH, Oftedal OT, Ralls K et al., 2009. Using stable isotopes to investigate individual diet specialization in California sea otters Enhydra lutris nereis. *Ecology* **90**:961–974.

Novosolov M, Rodda GH, Gainsbury AM, Meiri S, 2017. Dietary niche variation and its relationship to lizard population density. *J Anim Ecol* **87**: 285–292.

Pintor LM, Byers JE, 2015a. Do native predators benefit from non-native prey? *Ecol Lett* **18**:1174–1180.

Pintor LM, Byers JE, 2015b. Individual variation in predator behavior and demographics affects consumption of non-native prey. *Behav Ecol* **26**:797–804.

Pintor LM, Sih A, Bauer ML, 2008. Differences in aggression, activity and boldness between native and introduced populations of an invasive crayfish. *Oikos* **117**:1629–1636.

Pyke GH, Pulliam HR, Charnov EL, 1977. Optimal foraging: a selective review of theory and tests. *Q Rev Biol* **52**:137–154.

R Core Team, 2008. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. URL http://www.R-project.org

Schoener TW, 1968. The *Anolis* lizards of Bimini: resource partitioning in a complex fauna. *Ecology* **49**:704–726.

Sih A, Bolnick DI, Luttbeg B, Orrock JL, Peacor SD et al., 2010. Predator-prey naiveté, antipredator behavior, and the ecology of predator invasions. *Oikos* **119**:610–621.

Smith EAE, Newsome SD, Estes JA, Tinker MT, 2015. The cost of reproduction: differential resource specialization in female and male California sea otters. *Oecologia* **178**:17–29.

Sneddon LU, Huntingford FA, Taylor AC, Orr JF, 2000. Weapon strength and competitive success in the fights of shore crabs *Carcinus maenas*. *J Zool* **250**:397–403.

Svanback R, Bolnick DI, 2007. Intraspecific competition drives increased resource use diversity within a natural population. *Proc R Soc B-Biol Sci* **274**:839–844.

Symonds MRE, Moussalli A, 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behav Ecol Sociobiol* **65**:13–21.

Tanner CJ, Salalt GD, Jackson AL, 2011. Feeding and non-feeding aggression can be induced in invasive shore crabs by altering food distribution. *Behav Ecol Sociobiol* **65**:249–256.

Toscano BJ, Gownaris NJ, Heerhartz SM, Monaco CJ, 2016. Personality, foraging behavior and specialization: integrating behavioral and food web ecology at the individual level. *Oecologia* **182**:55–69.