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Selection of an omnivorous diet by the mangrove tree crab *Aratus pisonii* in laboratory experiments

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Abstract

Observational studies on leaf damage, gut content analyses, and crab behaviour have demonstrated that like numerous other mangrove and salt-marsh generalists, the mangrove tree crab *Aratus pisonii* feeds on a variety of food resources. This study is the first that experimentally tests feeding preferences of *A. pisonii*, as well as the first to test experimentally whether chemical composition of food resources is responsible for food selection. Feeding preferences were determined among a variety of plant, algal, and animal resources available in the field both in Florida and Belize, using multiple-choice feeding assays, where male and female crabs simultaneously were offered a variety of food items. To test whether chemistry of food resources was responsible for feeding preferences, chemical extracts of food resources were incorporated in an agar-based artificial food, and used in feeding assays. Results of feeding assays suggest that crabs prefer animal matter from ~ 2.5 to 13× more than other available resources, including leaves of the red mangrove *Rhizophora mangle*, which contribute the most to their natural diet. Artificial feeding assays also demonstrated that chemical cues were responsible for selection of animal matter, up to 25× more than other available resources. Non-polar extracts (derived from extraction in 1:1 ethyl actetate:methanol) stimulated feeding the most, suggesting that fatty acids, triglycerides, or sterols may be important for growth, reproduction, or survival. Results for both sexes were similar across most assays. While chemical composition of food resources appears to play some role in selection, this does not discount the potential role of other factors, such as resource availability, competition, predation, or reproductive requirements in influencing feeding preferences. Bioassay-guided fractionation of extracts should aid in determining chemical constituents that play the greatest role in determining feeding preferences.

Keywords: *Aratus pisonii*; Compensatory feeding; Feeding preference; Mangroves; Omnivores; *Rhizophora mangle*

1. Introduction

While many mangrove and salt-marsh crabs consume significant amounts of plant material, evidence suggests that these organisms are more appropriately considered omnivores rather than herbivores (Malley, 1978; Giddins et al., 1986; Dahdouh-Guebas et al., 1997, 1999; Buck et al., 2003). Many of these crabs appear to be opportunistic feeders on animal matter, supplementing their nutritionally poor plant diet with live or dead animal matter, rich in nitrogen and protein. Some studies show a positive correlation between consumption or feeding preferences and marine plant/algal nutritional value (Camilleri, 1989; Barile et al., 2004); however, others

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demonstrate either no or negative relationships (Duffy and Hay, 1991; Micheli, 1993; Pennings et al., 1998; Cruz-Rivera and Hay, 2001), suggesting that nutrition may not necessarily be the driving factor that results in significant plant consumption. This raises two questions: (1) is significant consumption of plant matter merely compensatory feeding to fulfil caloric requirements until a more valuable food source becomes available; and (2) do these plants contain dietary requirements unrelated to nitrogen and protein content necessitating high feeding on plant matter? If the answer to these questions is yes, this suggests that eating a mixed diet would be more beneficial to the growth, survival, and fitness of mangrove and salt-marsh crabs.

One such crab, the tree crab *Aratus pisonii* is a key organism in the mangrove environment. It is one of the most abundant animals found in Florida and Caribbean mangroves, and it is the primary herbivore of the red mangrove (*Rhizophora mangle*) (Feller and Chamberlain, in review). *A. pisonii* is one of a few crabs in the world that actually feeds on live leaves, and it is able to access leaves all the way up to the top of the canopy (Erickson et al., 2003). Through feeding, mangrove crabs link primary production to higher trophic levels by serving as prey (Beever et al., 1979; Wilson, 1989; Olmos et al., 2001). They also link it to smaller organisms of the same trophic level, and the detrital pathway through leaf shredding and the production of faecal material (Camilleri, 1989; Emmerson and McGwynne, 1992; Steinke et al., 1993; Lee, 1997; Werry and Lee, 2005). Feeding by herbivores can cause premature abscission, elevate soil nutrients, hasten nutrient cycling, and retain nutrients within ecosystems (Risley and Crossley, 1988). Research is currently being conducted to examine how mangrove crabs influence the above parameters regarding nutrient cycling (Nordhaus et al., 2006; S. Chapman, pers. comm., 2005). As a fresh leaf feeder, *A. pisonii* can influence mangrove physiology, chemistry, reproduction, and evolutionary development (Onuf et al., 1977; M. Ball, pers. comm., 2003). Despite this crab feeding mostly on *R. mangle*, observations suggest it is omnivorous, supplementing a low nutritional leaf diet with animal matter (Beever et al., 1979; Erickson et al., 2003; I. Feller, pers. obs., 2003).

While gut content analyses, studies on crab behaviour, and crab damage patterns on leaves have revealed that this crab consumes a variety of food resources (Beever et al., 1979; Diaz and Conde, 1988; Brogim and Lana, 1997; Erickson et al., 2003), this study is the first that experimentally documents its feeding preferences through the use of multiple-choice feeding assays. In addition, the basis for food choice by this crab remains unknown. Few experimental studies have been conducted on mangrove herbivores and omnivores testing the effect of prey chemistry on feeding preferences (Fratini et al., 2001; Erickson et al., in prep.). While some research has been done that examines how chemistry of mangrove leaves correlates with crab damage (Erickson et al., 2004), experimental studies have not been conducted. Thus, this is the first study to use artificial feeding assays, where extracts of food resources are incorporated into an agar-based diet, to determine whether chemical defences or feeding stimulants are responsible for feeding preferences of this generalist mangrove tree crab. Studies were conducted in Florida and Belize, which are sites of ongoing research examining crab feeding ecology where abundant crab populations are present, to see whether crab populations respond similarly to available food items in each location and whether there is an interaction between chemistry and location in feeding. The questions being asked include: (1) what are the feeding preferences of *A. pisonii*; (2) are feeding preferences linked to food chemistry; and (3) does feeding behaviour of *A. pisonii* include diet mixing or compensatory feeding?

2. Materials and methods

2.1. Collection of organisms

Adult crabs (*Aratus pisonii*) used in feeding assays were collected from Fort Pierce, Florida, in the Indian River Lagoon (Mosquito Impoundment 23) and from Carrie Bow Cay, Belize. This crab is distributed in neotropical mangrove systems ranging from Florida to northern Brazil. Food resources for live assays were collected from the same habitats. *A. pisonii* has been shown in the field to feed on insects, such as crickets (Beever et al., 1979), beetles, and leaf miners (I. Feller, pers. obs., 2003; D. Devlin, pers. comm., 2004). House crickets (*Achetus domesticus*) were purchased from Pet Supermarket in Fort Pierce. They were used because they were readily available as an insect model and could be obtained in a large enough quantity for extraction purposes. The red alga *Gracilaria tikvahiae*, which was used in artificial feeding assays, was obtained from cultures maintained at Harbor Branch Oceanographic Institution by D. Hanisak.

2.2. Multiple-choice feeding assays

Multiple-choice feeding assays were conducted with food resources commonly found in mangrove habitats to gain perspective on how these items rank in palatability and the degree to which crabs maintain a generalized omnivorous diet. Individual adult crabs of each sex ($N=20$) were maintained in their own plastic mesh cages with enough seawater to submerge themselves. Water was replaced after evaporation. For feeding assays, crabs were offered various food items, including: *Rhizophora mangle* fruit, live *R. mangle* leaves, detrital *R. mangle* leaves, detrital *Thalassia testudinum* blades (Belize only), algae (*G. tikvahiae,* which washes up as drift in Florida and mangrove root algae composed primarily of *Bostrychia tenella* and *Caloglossa leprieurii* in Belize), and whole, dead adult *A. pisonii* (average carapace width of 2 cm) and *A. domesticus* (average body length of 1.9 cm). Food items were weighed prior to and after assays, which were run for ~ 3 d. Paired crab-exclusion controls were run simultaneously to account for changes in resources unrelated to feeding (Peterson and Renaud, 1989). Consumption of each item was determined using the following formula:

$$T_i\left(\frac{C_f}{C_i}\right) - T_f$$

where T_i is the initial food mass, T_f is the final food mass, C_i is the initial control food mass, and C_f is the final control food mass. The amount of each item consumed was expressed as the percent of the total food consumed by an individual crab (Lockwood, 1998). Crabs that consumed <10% or >90% of the total food mass cage^{-1} were excluded from statistical analysis given that no choice was demonstrated. Friedman's repeated measures ANOVA and multiple comparison tests (Tukey's and SNK) were used to identify significant differences in the percent of total consumption among food resources (Lockwood, 1998).

2.3. Preparation of extracts

Freshly collected food resources were homogenized in solvent and extracted three times in 1:1 ethyl acetate: methanol producing non-polar extracts and three times in 1:1 ethanol:di H_20 producing polar extracts. Extracts were filtered, dried by rotary evaporator, and stored at 4 °C until used in feeding assays. Non-polar extracts may include compounds such as fatty acids, triglycerides, sterols, and terpenes, while polar extracts may include compounds such as tannins and proteins.

2.4. Artificial feeding assays with extracts

Methods used were adopted from Hay et al. (1998). Artificial diet was made by dissolving 1 g agar in 30 ml di H_20 and heating in a microwave, after which 2 g dried, ground *G. tikvahiae* were added and stirred. Extracts were dissolved in 2 ml ethanol and added at natural concentrations (based on dry weight) into the food. Individual assays were done that tested non-polar extracts of food items against each other, polar extracts of food items against each other, and combinations of *R. mangle* and *A. domesticus* extracts yielding foods at various concentrations (100, 75, 50, 25, and 0%) of plant (*R. mangle*) versus animal (*A. domesticus*) extracts, in which non-polar and polar extracts also were tested separately. Extracts from *A. domesticus* and *R. mangle* leaves were used because crabs frequently encounter insects and feed heavily on *R. mangle* in the field. Ethanol without extracts (2 ml) was added to control food. Artificial food with extracts and control food were spread into a mold with parallel, rectangular wells placed over plastic window screen, allowed to cool, and cut into pieces of ~1 cm^2. Prior to assays, crabs were trained on artificial food to demonstrate that they would feed on it. For artificial assays, male and female crabs ($N=20$) simultaneously were offered one piece of each food type, including controls. They were allowed to feed until half of one piece of artificial food was consumed or 48 h passed. Preference was quantified as the number of window screen squares revealed after food was consumed. Squares that were partially consumed were counted as 0.5 square. Crabs were excluded from statistical analysis if they did not eat or consumed all food. Friedman's repeated measures ANOVA and Tukey's multiple comparison test were used to identify significant differences in the number of squares consumed.

3. Results

Multiple-choice feeding assays from Florida demonstrated that both male and female crabs significantly preferred animal matter to other food resources (Friedman's $\chi_r^2=95.94$, 41.57, 55.32, for sexes combined, females, and males, respectively, $P\leq0.001$ for all groups; Fig. 1A). Detrital *Rhizophora mangle* leaves and algae (*Gracilaria tikvahiae*) were intermediate in preference, while *R. mangle* fruit and green leaves were low in preference. These patterns differed somewhat from crabs residing in Belize, where preferences were not as well defined (Friedman's $\chi_r^2=37.34$, $P\leq0.001$ for sexes combined; $\chi_r^2=12.83$, $P=0.046$ for females; and $\chi_r^2=35.07$, $P\leq0.001$ for males; Fig. 1B). Root algae were preferred most by male crabs in Belize, closely followed by animal matter. The same trend was found for female crabs despite statistical tests being unable to determine which food types differed. Detritus,

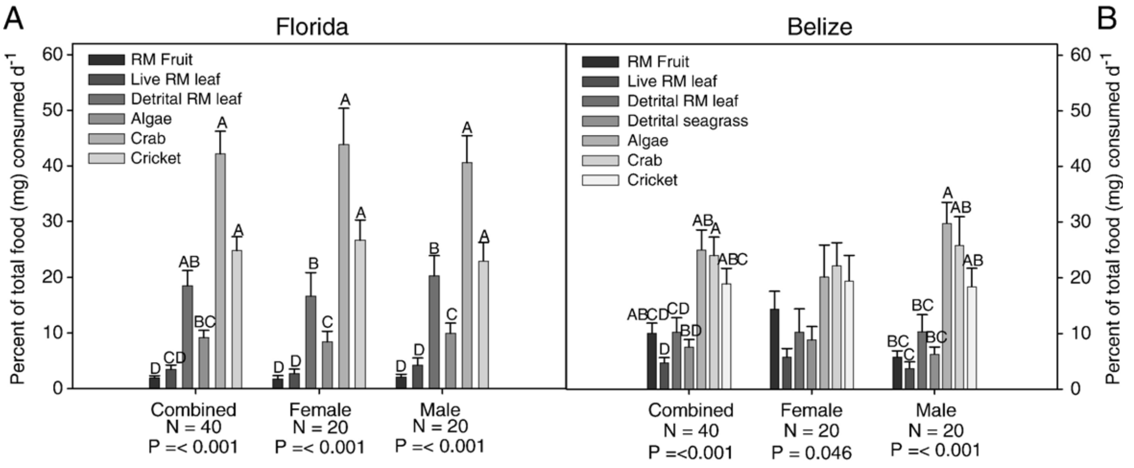

Fig. 1. Feeding preferences from multiple-choice feeding assays where *A. pisonii* were offered fruit, live, and detrital leaves of *R. mangle* (RM), detrital seagrass blades, algae, crabs, and crickets in Florida (A) and Belize (B). Different letters above bars indicate significant differences among treatments.

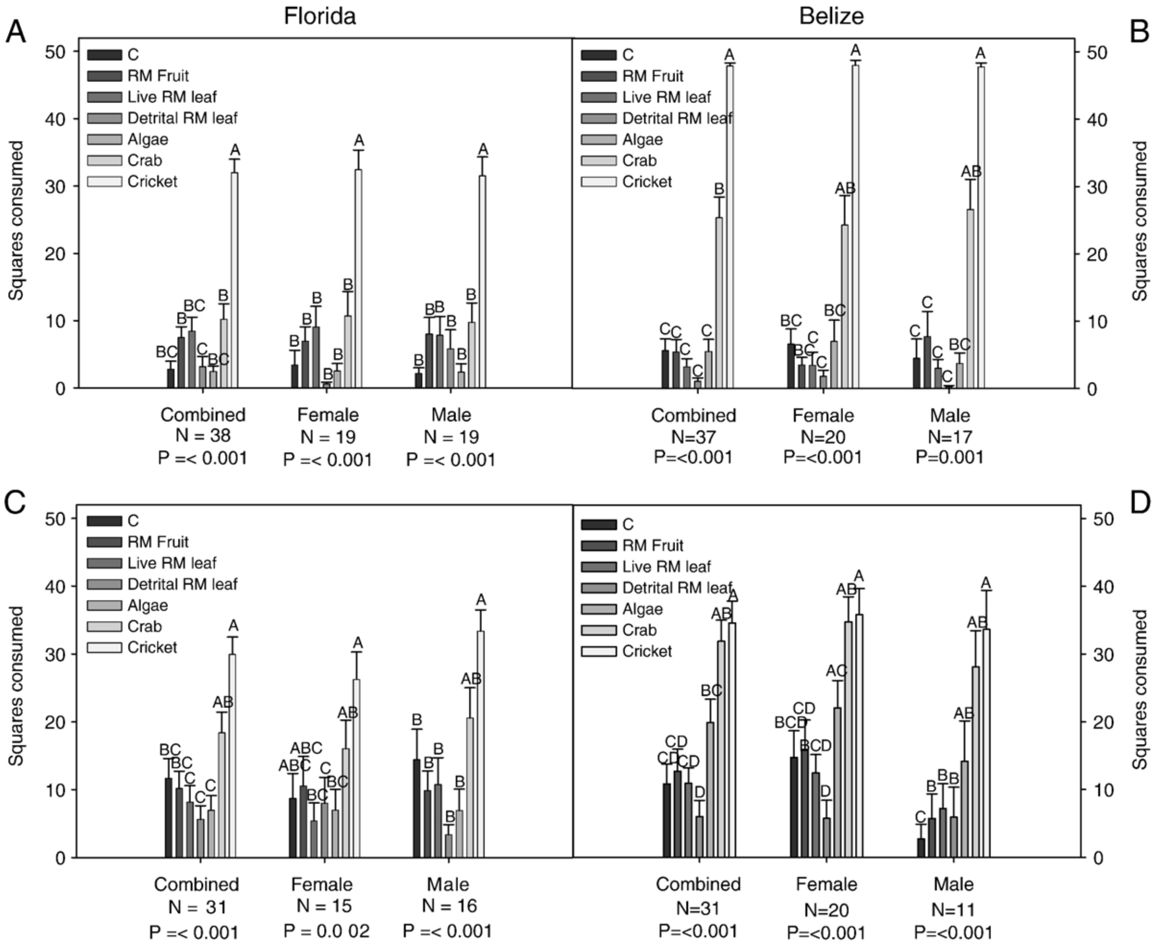

Fig. 2. Artificial feeding assays testing the influence of chemical composition of various food resources on consumption by crabs from Florida and Belize. Non-polar (A, B) and polar (C, D) extracts were incorporated into an agar-based artificial diet. Different letters above bars indicate significant differences among treatments. Abbreviations: see Fig. 1.

live *R. mangle* leaves, and fruits tended to be lower in preference.

Results from artificial feeding assays demonstrated that food with animal extracts (either non-polar or polar) was fed upon significantly more than food with extracts from other resources (Fig. 2) in both Florida and Belize. In Florida, non-polar food with *Achetus domesticus* extracts was consumed the most (Friedman's $\chi_r^2 = 105.70$, 54.18, 53.72, for sexes combined, females, and males, respectively, $P \leq 0.001$ for all groups; Fig. 2A), while in Belize, food with both *A. domesticus* and *Aratus pisonii* non-polar extracts was fed upon the most (Friedman's $\chi_r^2 = 127.69$, 66.02, 63.33 for sexes combined, females, and males, respectively, $P \leq 0.001$ for sexes combined and females, $P = 0.001$ for males; Fig. 2B). Little difference in feeding existed among artificial food with non-polar extracts of other food items, regardless of location. Differences in feeding among food with polar extracts were not as well defined compared to non-polar extracts for both Florida and Belize populations (Friedman's $\chi_r^2 = 59.14$, $P \leq 0.001$ for sexes combined; $\chi_r^2 = 20.67$, $P = 0.002$ for females; and $\chi_r^2 = 41.54$, $P \leq 0.001$ for males; Fig. 2C; Friedman's $\chi_r^2 = 73.14$, 48.88, 35.26, for sexes combined, females, and males, respectively, $P < 0.001$ for all groups, Fig. 2D). Male and female crabs showed similar feeding patterns.

As a follow-up to artificial assays that documented feeding preference of animal matter, artificial feeding assays were conducted that tested preference for food

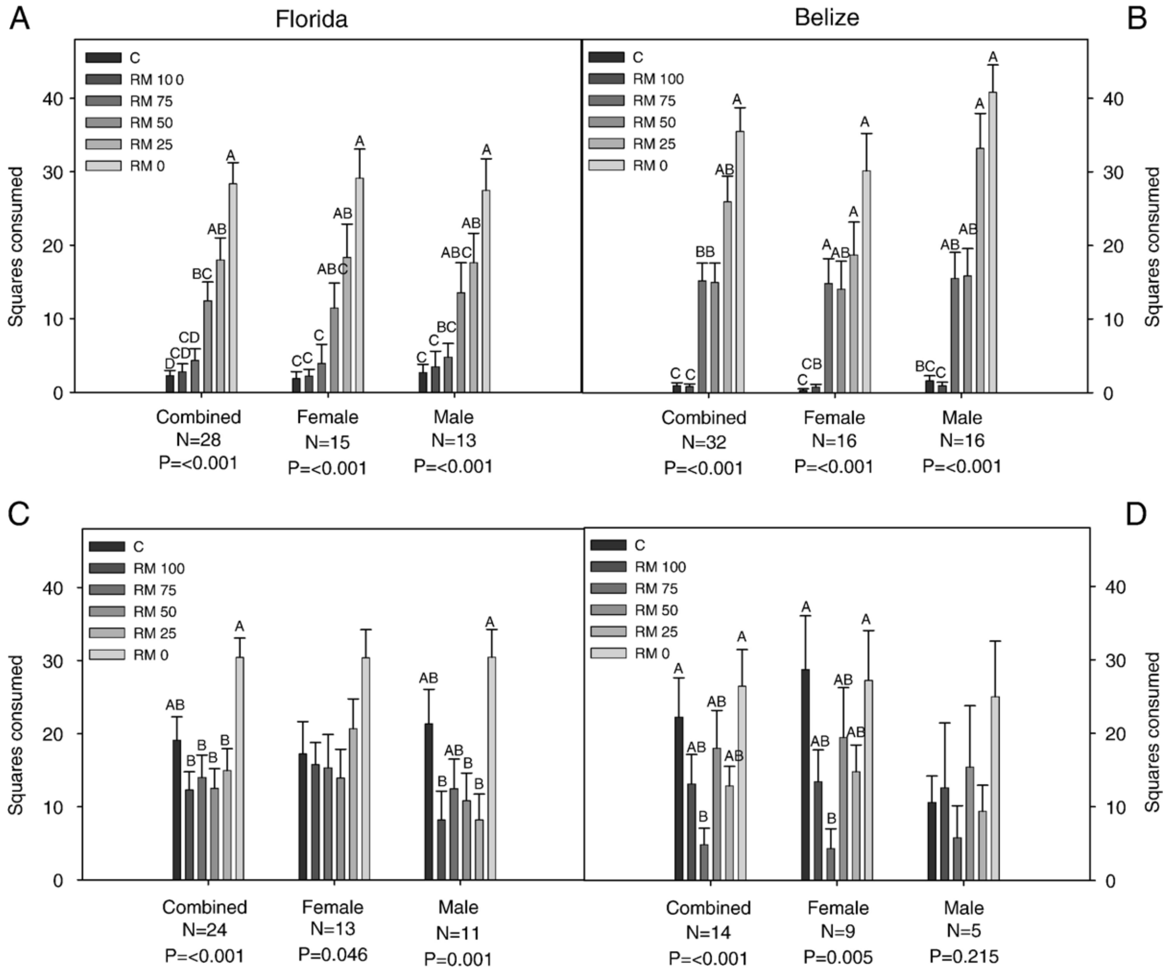

Fig. 3. Artificial feeding assays testing the influence of single and mixed animal and plant diets on consumption by crabs from Florida and Belize. Non-polar (A, B) and polar (C, D) extracts of *R. mangle* (RM) and *A. domesticus* were incorporated into an agar-based artificial diet so that food choices represented 100% RM, 75% RM, 50% RM, 25% RM, and 0% RM, with the remainder being composed of *A. domesticus*. Different letters above bars indicate significant differences among treatments.

with single diets containing animal or plant extracts (using natural concentrations based on dry weight) and mixed diets of both food types combined. Mixed diets ranged from 25 to 75% of either food type. Greater differences in feeding were seen across food with non-polar extracts as opposed to food containing polar extracts (Fig. 3). For crabs from Florida and Belize, feeding on artificial food with non-polar extracts increased as *R. mangle* extract concentration decreased and *A. domesticus* extract concentration increased (Friedman's $\chi_r^2=78.46$, 44.97, 33.99, for sexes combined, females, and males, respectively, $P \le 0.001$ for all groups, Fig. 3A; Friedman's $\chi_r^2=101.29$, 46.75, 56.44, for sexes combined, females, and males, respectively, $P \le 0.001$ for all groups, Fig. 3B). No major differences were found between sexes when non-polar extracts were incorporated into food. For polar extracts, the same strong patterns were not observed. In Florida, the only diet that was fed upon significantly more than others was the single diet of animal extract (Friedman's $\chi_r^2=24.09$, $P \le 0.001$ for sexes combined, $\chi_r^2=11.30$, $P=0.046$ for females; and $\chi_r^2=20.36$, $P=0.001$ for males; Fig. 3C). There was no difference among food types with mixed diets or the single diet with plant extract. While the data from Belize demonstrate differences in feeding among mixed diets with polar extracts, the differences were only between the single diet with animal extract and the mixed diet with 75% plant and 25% animal extract (Friedman's $\chi_r^2=21.57$, $P \le 0.001$ for sexes combined; $\chi_r^2=16.89$, $P=0.005$ for females; and $\chi_r^2=7.07$, $P=0.215$ for males; Fig. 3D). Significant differences were found in Belize among polar food types for females and none were found for males; however, male feeding trends were similar to that of females.

4. Discussion

Through the use of multiple-choice and artificial feeding assays, this study is the first to experimentally document feeding preferences of the mangrove tree crab *Aratus pisonii* and demonstrate the role of food chemistry on food selection. The fact that live *Rhizophora mangle* leaves were low preference food items was surprising given that *A. pisonii* consumes significant amounts of *R. mangle* leaf material (Erickson et al., 2003, 2004), and this crab is the primary folivore of *R. mangle* leaves in the field (Feller and Chamberlain, in review). Animal matter was significantly preferred over plant matter. This study provides experimental evidence supporting other observations that *A. pisonii* will feed opportunistically when animal resources become available (Beever et al., 1979; Diaz and Conde, 1988; Brogim and Lana, 1997; Erickson et al., 2003). *A. pisonii* has been known to feed on insects, gastropods, dead fish, and crabs of their own and other species (Beever et al., 1979; Diaz and Conde, 1988; Brogim and Lana, 1997; Erickson et al., 2003). Preliminary work done on carbon stable isotopes in Belize mangrove systems has determined that the dietary signature of *A. pisonii* is of mixed influence (insects and algal/cyanobacterial mats) (M. Fogel, D. Akob, pers. comm., 2001). Supplemental feeding on animal matter has been documented through observational or experimental studies for many other mangrove and salt marsh crabs, such as *Armases cinereum*, *Cardisoma carnifex*, *Chiromanthes onychophorum*, *Neosarmatium meinerti*, *Neosarmatium smithii*, *Selatium elonagtum*, *Parasesarma leptosoma*, and *Perisesarma guttatum* (Malley, 1978; Giddins et al., 1986; Dahdouh-Guebas et al., 1997, 1999; Buck et al., 2003; Thongtham and Kristensen, 2005), as well as other marine herbivores such as sea urchins (McClintock et al., 1982) and amphipods (Cruz-Rivera and Hay, 2000a,b, 2001).

Differences in feeding preferences between sexes could be expected due to sexual dimorphism in chelae size or differential energy and reproductive requirements (Cannicci et al., 1996; Buck et al., 2003). Dimorphism in crab claw size has allowed males to feed on a wider variety of food items (Kyomo, 1992; Brousseau et al., 2001; Buck et al., 2003). Adult *A. pisonii* males tend to have larger club-shaped chelae (Warner, 1979), which may allow greater ability and force with which to grasp food items, and possibly greater breadth in diet as well. Erickson et al. (2003) demonstrated that the male diet of *A. pisonii* is more variable with respect to leaves of different mangrove species but did not address other food types. The female diet may be constrained by energetic requirements or physical location within mangrove habitat that are necessary to maximize fitness. However, in Florida and Belize, there was no difference in preference between males and females. No difference in feeding between sexes also has been found for other marine crabs (Cannicci et al., 1996; Sukumaran and Neelakantan, 1997; Fratini et al., 2000).

Artificial feeding assays, where non-polar and polar food extracts were incorporated into an artificial diet, corroborated feeding preferences from multiple-choice feeding assays and supported the role of chemical composition in food selection. Assays demonstrated that animal extracts stimulated feeding while extract from live *R. mangle* leaves failed to do so. Experimental studies using artificial diets with protein also demonstrated stimulation of feeding by *A. cinereum* (Pennings et al., 1998). The fact that *A. pisonii* preferred animal matter over other resources is not necessarily surprising given that animal matter is significantly higher than plant matter in nitrogen and protein, which are both important for crab growth and reproduction (Wolcott and Wolcott, 1987). Protein in marine animal matter can be as high as 50% dry weight (Duffy and Paul, 1992). For plant matter to be nutritious to marine invertebrates, it needs to have a C:N ratio equal to or less than 17:1 (Russell-Hunter, 1970); however, live *R. mangle* leaves in Tampa Bay, Florida have an average C:N ratio of 38:1, with as little as 1–1.5% nitrogen and 7–8% protein per dry weight (Erickson et al., 2004). Other studies throughout the neotropics have reported similar values for *R. mangle* (Conde et al., 1995; Feller, 1995; Fry et al., 2000). While proteins probably were confined to polar extracts, greater differences were observed among food with non-polar extracts, suggesting these extracts may play more of a crucial role in food selection (Kanazawa, 2001; Sheen and Wu, 2003). Chemical constituents in non-polar extracts include fatty acids, triglycerides, and sterols, which have received significantly less attention than protein in feeding and nutritional studies of marine invertebrates. Experimental feeding assays should be conducted to determine the extent to which these constituents influence feeding preferences, as well as growth, survivorship, and reproduction. Studies on marine crustaceans have demonstrated superior growth when grown on animal versus plant matter (Cruz-Rivera and Hay, 2000a; Buck et al., 2003); however, studies also have yielded species-specific results as far as survivorship and fitness (Cruz-Rivera and Hay, 2000a,b). Alternatively, non-polar extracts of *R. mangle* may have been avoided if they contain feeding deterrents that are lacking in animal tissue, such as terpenes, whose role in mangrove defence have gone virtually unnoticed. *Rhizophora mangle* leaves contain a variety of compounds associated with non-polar extracts, including: n-alkanes, n-alkanoic acids, wax ester acids, hydroxyacids, triterpenones, pentacyclic triterpenoids, and various sterols (Killops and Frewin, 1994; Williams, 1999). Two such compounds, taraxerol and cinnamoyl-lupeol, have demonstrated significant insecticidal activity against agricultural pests (Williams, 1999), therefore having the potential to alter feeding by herbivores in natural systems.

Based on these crab feeding preferences, optimal foraging theory would predict that animal matter would be consumed most in the field (Schoener, 1971); however, this is not the case. In the feeding assays conducted in this study, adult crabs were offered dead prey as food. While *A. pisonii* are highly mobile, it is possible that they only feed on animal matter when it is easy to obtain or catch. The fact that more animal matter was not found in crabs guts (Erickson et al., 2003) could relate to the difficulty in catching mobile insects as well as associated energy costs. It also could relate to the fact that animal matter tends to digest more quickly than plant matter (Williams, 1981).

Other food items also were high or intermediate in preference. For instance, root algae were highly preferred in Belize but not in Florida. Algal root communities in Florida are not developed to the same extent as they are in Belize. Mangrove tree crabs spend a significant amount of time crawling among roots especially at receding, low, and incoming tides (Erickson, pers. obs.). Algae likely are of greater nutritional value than mangrove leaves. Other mangrove crabs that frequently consume root algae along with mangrove leaves are *Metopograpsus* spp. (Dahdouh-Guebas et al., 1999; Fratini et al., 2000). In Florida, artificial assays demonstrated that algae, *R. mangle* fruit, and live leaves were similar in palatability. At the same time, in Belize, *R. mangle* fruit, live leaves, and detritus were of similar palatability. In Florida, detritus was of intermediate palatability, but detritus was of low palatability in Belize. It is not surprising that detrital leaves were fed upon more than live leaves. Aging of leaves may increase nitrogen content through bacterial or fungal growth or it may increase digestibility through the leaching of tannins (Neilson et al., 1986). Litter-feeding crabs from the paleotropics have been suggested to cache leaves in burrows to age them, thus reducing tannins and increasing nitrogen content (Giddins et al., 1986); however, this theory was disproved by Skov and Hartnoll (2002), who demonstrated no difference in carbon, nitrogen, and C/N ratio between cached and freshly fallen senescent leaves.

One discrepancy from this study between whole food and artificial assays was found for detrital *R. mangle* leaves. While they were fed upon readily in whole food assays, food with non-polar extract was fed upon the least in artificial assays. The fact that chemical composition failed to support feeding on detrital leaves suggests that some other quality may be responsible for feeding on detrital leaves, such as toughness or water content. For instance, plant toughness was shown to correlate negatively with leaf damage by *A. pisonii* (Erickson et al., 2004) and to be a major determinant of feeding preferences by the salt marsh crab *A. cinereum* and the opisthobranch gastropod *Dolabella auricularia* (Pennings and Paul, 1992; Pennings et al., 1998). In addition, feeding preferences documented in this study may have resulted based on the decision to use specific organisms as food resources and results may have been different if other animal or algal species had been used.

While crabs feed significantly on *R. mangle*, the extent to which they feed on animal matter in the field is unknown. Given significant feeding on these leaves and the opportunistic feeding behaviour of *A. pisonii*, we expected that a mixed diet would be fed upon more than single diets where extracts of either plant or animal matter were incorporated into artificial food. However, a single diet where non-polar extract of animal matter was incorporated into artificial food was fed upon most across locations and sexes, which suggests that something other than protein is important. Between 25–50% of non-polar animal extract increased feeding. Polar extracts did not necessarily support the same pattern or show the same magnitude of differences among the mixed diets. Based on these results, it cannot be determined whether there are basic dietary requirements present in *R. mangle* leaves or whether mixed diets of both animal and plant matter would yield greater growth, survival, and fitness. Mixed diets with complementary resources have been consumed in the field or preferred in experimental trials by other marine organisms, such as crustaceans, gastropods, and fish, beyond single diets of either plant or animal matter (Lobel and Ogden, 1981; Pennings et al., 1993; Cruz-Rivera and Hay, 2000a,b, 2001; Buck et al., 2003; Hall et al., 2006). While mixed diets tend to increase growth, evidence suggests they may be of limited benefit to survivorship and reproduction of some amphipod species (Pennings et al., 1993; Cruz-Rivera and Hay, 2000a,b, 2001; Buck et al., 2003).

So why does *A. pisonii* feed so heavily on *R. mangle* leaves in the field? As *R. mangle* is a nutritionally poor food source (based on nitrogen and protein) that is relatively unpalatable in feeding assays, it may be fed upon because it is a readily available and highly abundant food source. Feeding behaviour in the field suggests that crabs may be using compensatory feeding, consuming greater amounts of *R. mangle* leaf material than expected given its nutritional value. Compensatory feeding has been observed for other herbivorous and omnivorous crustaceans (copepods, amphipods, crabs), sea urchins, and fish (Stachowicz and Hay, 1996, 1999; Poore and Steinberg, 1999; Cruz-Rivera and Hay, 2000a, 2001, 2003; Valentine and Heck, 2001; Thongtham and Kristensen, 2005; Prince et al., 2006). However, effects of compensatory feeding on growth, survivorship, and fitness have been species-specific in the above studies, demonstrating that it may not always be an effective feeding strategy. Further, to determine whether this is actually occurring, no-choice experiments need to be performed in conjunction with multiple-choice ones to demonstrate increased consumption when *R. mangle* is the only food choice available.

Another possibility, rather than compensatory feeding, is that these crabs are capable of breaking down metabolites of significant concentration that may be nutritionally unavailable or toxic. Many invertebrate herbivores harbour gut symbionts, including bacteria or fungi, which contribute enzymes, further breaking down food and making it more available for absorption (Gulmann and Mullineaux, 2001; Zimmer et al., 2001; Kimura et al., 2002). Red mangrove leaves have high concentrations of condensed tannins (which often range between 20–40% dry weight) (Hernes et al., 2001; Erickson et al., 2004). While these tannins do not deter feeding by this crab, tannins have the ability to reduce digestion of protein by binding to it (Hagerman et al., 1992). *Aratus pisonii* has been shown to have significant gut populations of trichomycete fungi in Tampa Bay, Florida, populations (Mattson, 1988); however, it is unknown how prevalent these fungi are in *A. pisonii* and whether they influence digestion, absorption, or availability of resources.

A third possibility is that while high levels of feeding on *R. mangle* are not correlated to nitrogen or protein (Erickson et al., 2004), feeding on leaves may be related to other constituents such as amino or fatty acids and lipids (Harrsion, 1990; Jonasdottir, 1994). For instance, lipid concentration has been shown to be important to copepod egg production (Giese, 1966). Hall et al. (2006) demonstrated significant uptake and tissue incorporation of fatty acids by the mangrove crab *Parasesarma erythodactyla* through a series of feeding trials that alternated feeding and starvation. The authors also suggest that the crabs are capable of synthesizing and storing highly unsaturated fatty acids de novo from chemical precursors in mangrove leaves. Given this example, it is possible that *A. pisonii* also produces similar fatty acids and derives a significant amount of nutrition from the leaves. Meziane et al. (2006) demonstrated fatty acid signatures representing either mangrove or microbial origin in the mangrove crab *P. erythodactyla*; they also suggested that crabs may selectively feed on the microbial community of mangrove leaves, rendering consumption of leaf material merely as incidental. Silliman and Newell (2003) also document that salt marsh snails (*Littoraria irrorata*) found on live rather than detrital *Spartina alterniflora* feed within and around self-created wounds made by their radulae. Interestingly, this same behaviour has been observed by *A. pisonii* (Erickson et al., in preparation); in feeding trials, *A. pisonii* selectively fed 70% of the time in and adjacent to wounds that they previously created on live *R. mangle* leaves. Thus, it appears that these crabs may be able to promote the generation of more abundant and nutritious microbial resources for their consumption.

It also is possible that factors unrelated to nutrition constrain feeding preferences. For instance, high levels of feeding on mangrove leaves may relate to the crabs' proximity to the shoreline. *A. pisonii* releases fertilized eggs at new and full moon into the water column, where larval stages develop as plankton before emerging as juveniles into the mangroves (Warner, 1967). In addition, *A. pisonii* may feed on a less than optimal food (*R. mangle* leaves) as this may provide a refuge from predation. Predators, such as birds and raccoons may have difficulty moving through the prop roots of *R. mangle* allowing crabs time to escape up to the canopy. Use of associational defences has been demonstrated by other small herbivores (Duffy and Hay, 1994; Poore and Steinberg, 1999; Stachowicz and Hay, 1999; Cruz-Rivera and Hay, 2000a). However, *A. pisonii* is fairly mobile and does not restrict itself completely to *R. mangle*. The crabs have been seen in high number resting on the black mangrove *Avicennia germinans*, of which they consume very little leaf material (Erickson et al., 2004). It also has been suggested that *A. pisonii* feeds predominantly at night (Warner, 1977), thus avoiding diurnal predators that rely on visual cues. This behaviour has been observed by other small marine herbivores that may be susceptible to predation if moving about to acquire a mixed diet (Rogers et al., 1998).

In conclusion, more studies need to be conducted that examine how single and mixed diets, as well as individual nutritional characteristics, influence crab growth, survival, and fitness. Bioassay-guided fractionation will aid in identifying chemical constituents responsible for stimulating feeding by *A. pisonii*. Finally, efforts should be made to identify the extent to which crabs actually consume animal matter and to which dietary mixing or compensatory feeding occurs in the field. Techniques including stable isotope analyses (Bouillon et al., 2004) as well as fatty acid tracers (Jonasdottir, 1994; Hall et al., 2006) should facilitate a better understanding of feeding preferences as well as trophic dynamics. These studies should help to identify nutritional requirements of an abundant mangrove crab and aid in understanding the true diet of *A. pisonii* and its trophic significance in mangrove food webs. Through studying trophic relationships, a greater understanding of mangrove ecosystem function can be gained.

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