SIZE DEPENDENT SHIFTS IN THE DIET OF THE MANGROVE TREE CRAB, *ARATUS PISONII*, AS INDICATED BY $\delta^{13}C$ & $\delta^{15}N$ FROM A MANGROVE ECOSYTEM IN INDIAN RIVER LAGOON, FL

By

JONATHAN MICHAEL PAHLAS

(Under the Direction of Samantha Joye)

ABSTRACT

The adult mangrove tree crab, *Aratus pisonii*, is responsible for significant portions of leaf herbivory in mangrove forests. Using the mixing model software, IsoSource, we compare $\delta^{13}C$ & $\delta^{15}N$ of three *A. pisonii* size classes to evaluate their food sources in a mangrove forest along the Indian River Lagoon, FL. We also developed a mass balance method for incorporation of nutrient concentration into the model. Our results suggest that the juvenile *Aratus* diet of MPB and freshly fallen leaves shifts to a diet dominated by senescent leaves and lichens as an adult. This pattern is driven by strong intraspecific restrictions that prevent small *A. pisonii* from entering the mangrove tree canopy. Thus, only the larger crabs have access to nitrogen-fixing, $\delta^{15}N$ depleted lichens in the branches which gives them an unusually low $\delta^{15}N$ value relative to smaller individuals. Similarly, large crab reliance on leaf material results in depleted $\delta^{13}C$ values compared to the small crabs who utilize the $\delta^{13}C$ rich microphytobenthos.

INDEX WORDS: Mangrove; Isotope; *Aratus pisonii*; Indian River Lagoon; Food web; Diet; Lichen; Mangrove tree crab; *Rhizophora mangle*; Nutrient enrichment; Fertilization; Carbon; Nitrogen; IsoSource

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INTRODUCTION

Mangrove forests are distributed within intertidal areas between 30° N and 30° S latitude (Giri *et al.*, 2011). Their prop roots and pneumatophores provide a home for both terrestrial and marine plants, algae, invertebrates, and vertebrates (Nagelkerken *et al.*, 2008). These productive habitats support coastal fisheries by providing a nursing ground for juvenile target species and deliver many valuable ecosystem services, including runoff retention and filtration, protection against shoreline erosion, retention of nutrients benefitting adjacent agriculture, and local subsistence uses as a source of fire-wood, building materials, and nutrition (Hogarth, 1999; Naylor *et al.*, 2000). Mangrove litter and root biomass fuel intense biogeochemical cycling which provides carbon (C) and nutrients for use both within the mangrove forest system and by adjacent habitats (Kristensen *et al.*, 1995). In his pioneering work on mangrove ecosystems, Odum proposed that the consumption of decaying leaf litter drove the forest food web, and the export of this material was a large contributor to adjacent system productivity (Odum, 1971; Odum & Heald, 1972).

Productivity and forest structure have been tied traditionally to abiotic factors acting from the bottom up (Odum, 1971; Cannicci *et al.*, 2008). Nutrient availability in mangroves can range from oligotrophic to eutrophic over relatively short distances as a function of tidal inundation, underlying hydrology, and temperature and salinity regulated microbial decomposition and decay (Feller *et al.*, 2003a). In turn, mangrove trees display highly plastic phenotypic responses to nutrient availability. Stunted stands of the red mangrove tree, *Rhizophora mangle*, grow to an average of only 1.5 m tall under the influence of oligotrophic conditions (Lugo, 1997; Feller *et al.*, 2003a), but *R. mangle* enriched by rivers and bird rookeries can achieve heights as tall as 16 m (Golley, 1975; Pool *et al.*, 1977). Relief of

nutrient limitation has been shown to produce significant physiological effects. Primary productivity, growth rates, and nutritional content of leaf material increase after fertilization treatments (Feller, 1995; Lovelock & Feller, 2003; Feller *et al.*, 2009a), and detritus derived from mangrove trees fertilized with N and P exhibited altered litter quality and decomposition rates (Feller *et al.*, 1999). Because nutrient loading from terrestrial runoff can be a significant source of nitrogen (N) and phosphorus (P) (Yuangen *et al.*, 2012), characterization of enrichment effects on these ecosystems is integral to understanding mangrove forest ecology.

Indian River Lagoon (hereafter IRL) spans 40 percent of Florida's eastern coast and contains more animal and plant species than any other estuary in North America providing an estimated 50% of the east Florida annual fish catch ("Indian River Lagoon: An Introduction to a Natural Treasure," 2007). In 1997, Feller et al. (2003b) began fertilization treatments in an abandoned mosquito impoundment (MI 23), which lies at the southern end of IRL. While most nutrient-limited mangrove forests are limited by P, the IRL is characterized by N-limitation (Feller *et al.*, 2003a; Feller *et al.*, 2003b). Nutrient enrichment at the base of trees produced significant increases in leaf %N, growth, and productivity (Feller *et al.*, 2003b). Mangrove fertilization has also been observed to bring about changes in leaf stoichiometry, photosynthetic productivity, resorption efficiencies, and new growth in other systems (Feller, 1995; Feller *et al.*, 2003a; Feller *et al.*, 2009b). Though fertilization altered plant growth and nutrient dynamics, folivory rates by mangrove associated fauna showed no response to these traits (Feller *et al.*, In Press).

The significant impacts herbivores can have on mangrove forest ecology makes characterization of food web structure of particular interest (Cannicci *et al.*, 2008). Herbivory by the arboreal mangrove crab, *Aratus pisonii*, has been suggested to influence mangrove canopy structure as a function of herbivore population dynamics (Feller *et al.*, In Press). In a study in south Florida, *A. pisonii* herbivory

alone was documented on 30 – 40% of mangrove tree leaves resulting in up to 30% loss of production therein (Erickson *et al.*, 2003). By exerting these top down pressures on primary producers, herbivores can influence net primary productivity and nutrient cycling. Crabs in a SW Atlantic marsh markedly reduced growth rates and increased senescence in fertilized plots (Alberti *et al.*, 2011). Alterations to mangrove forest structure as well as decreases in tree productivity and reproductive success have been linked to herbivory on leaves, wood, and propagules (Farnsworth & Ellison, 1991; Feller & Mathis, 1997; Feller, 2002; Cannicci *et al.*, 2008).

Aratus pisonii, a small Grapsid crab of the subfamily Sesarminae (Warner, 1967), is a widely distributed mangrove invertebrate that has been hypothesized to play a pivotal role in mangrove food webs (Feller *et al.*, In Press). It inhabits shores from Florida to Brazil on the western Atlantic and from Nicaragua to Peru along the Pacific (Rathbun, 1917). *Aratus pisonii* is found commonly in the seaward margins of mangrove swamps climbing among the fringing trees. In a Jamaican forest, *A. pisonii* densities were measured at 15-16 crabs per m^2 (Warner, 1967, 1970), and they appear to be similarly abundant at IRL (Feller *et al.*, In Press). Individuals are generally greenish in color with black and yellow mottling on their carapaces, and their chelae are red with stiff, black bristles. This species can reach a maximum carapace width of about 24 mm, and females become reproductively mature at about 6 mo or 12 mm (Warner, 1967). Frequency of ovulation increases until 15 – 17 mm after which point number of eggs produced continues to increase with body size while frequency of ovulation diminishes.

Aratus pisonii production is responsible for a significant amount of energy, nutrient, and biomass export from the mangrove canopy (Beever *et al.*, 1979; Schwamborn *et al.*, 1999). Warner (1967) estimated an average export of over 6000 eggs per m^2 per month, and shredding of leaf material makes mangrove biomass more accessible for decomposition on the forest floor while also facilitating transfer to pelagic systems if fecal pellets are excreted over water (Robertson & Daniel, 1989; Werry &

Lee, 2005). In addition to export of production and waste, *A. pisonii* directly transfer nutrition to predators such as fish, birds, raccoons, and other crabs (Warner, 1967; McKeon & Feller, 2004). Thus, *A. pisonii* act as an intermediary link between mangrove forest canopies and the rest of the system.

Adult *A. pisonii* utilize a unique niche living among the branches and prop roots of the mangrove trees. Feeding in the canopy provides reduced risk of predation and a reliably abundant food source (Wolcott & O'Connor, 1992), so it is surprising that few crabs worldwide have adapted this strategy (Hartnoll, 1965). Though gut content analyses clearly show a preference for *R. mangle*, *A. pisonii* will eat black or white mangrove leaves as well, *Avicennia germinans* and *Laguncularia racemosa,* respectively (Beever *et al.*, 1979; Erickson *et al.*, 2003). Depending upon availability, *A. pisonii* have been observed to exhibit a diverse array of supplemental feeding strategies. They will graze leaf detritus and algae in the intertidal zone and opportunistically consume animal material, including insect larvae, nematodes, crustaceans, fish scales, forams, and polychaetes (Warner, 1967; Díaz & Conde, 1988; Erickson *et al.*, 2003; McKeon & Feller, 2004; Feller & Chamberlain, 2007).

Due to intense intraspecific competition for space on the branches, only large *A. pisonii* have access to the forest canopy resulting in distinct differences in feeding behaviors between size classes. Any small crabs attempting to move into the upper reaches of the trees are quickly turned away by larger individuals (Warner, 1970). These encounters usually are determined simply by ritualized behaviors that allow smaller individuals to leave unscathed, but adult *A. pisonii* have been observed to kill and consume smaller conspecifics. These vertical migration limitations restrict juvenile *A. pisonii* to the lower prop roots and forest floor where they have access to detritus, algae, and microphytobenthos (MPB) (Feller & Chamberlain, 2007; Giarrizzo *et al.*, 2011). As they mature, they can more freely enter the upper reaches of the trees where they begin to feed on leaves and other prey items (Warner, 1970; Beever *et al.*, 1979; McKeon & Feller, 2004). Though their role as an opportunistic omnivore is well

established, no study has characterized *A. pisonii*'s diet as they shift from juveniles to adults. With such high densities observed where *A. pisonii* reside, these primary mangrove folivores are of particular interest to food web studies (Erickson *et al.*, 2003; Feller *et al.*, In Press).

In recent decades, stable isotope analysis has emerged as one of the primary methods of analyzing food web structure (Layman *et al.*, 2011). Although sulphur, oxygen, and deuterium can be useful food web tracers in some circumstances, C and N are the most common isotopic systems employed in this context. For this study, stable isotope ratios are expressed in standard delta notation (Fry, 2006) according to the formula $\delta = 1000[(R_{sample} - R_{standard}) R_{standard}^{-1}]$, where R is the ratio of heavy to light isotope ($^{15}N:^{14}N$ or $^{13}C:^{12}C$), R_{sample} is R measured for animal and plant tissues, and $R_{standard}$ is an international standard (Pee Dee Belemnite for ^{13}C and atmospheric N_2 for ^{15}N). Estimating the trophic position of an organism is effectively achieved by determining its $\delta^{15}N$ value, which shows incremental enrichment through trophic transfer (Minagawa & Wada, 1984; Peterson & Fry, 1987; Post, 2002). Primary producers using different photosynthetic pathways have substantially different $\delta^{13}C$ signatures (Minagawa & Wada, 1984; Peterson & Fry, 1987; Post, 2002). This makes $\delta^{13}C$ a useful tool for identifying primary C sources since $\delta^{13}C$ values exhibit little variation between trophic transfers (DeNiro & Epstein, 1981; Peterson & Fry, 1987; Post, 2002). A $\delta^{13}C$ and $\delta^{15}N$ biplot can reveal ultimate organic matter resources (for C and N) as well as trophic position (Layman *et al.*, 2011). This isotopic signature generally aligns closely with actual trophic position though it must be carefully interpreted to prevent misrepresentations (Layman *et al.*, 2011).

Stable isotope studies have been used to establish the fate of detritus within the mangrove forests and the surrounding ecosystems (Schwamborn *et al.*, 2002; Nguyen *et al.*, 2012; Vaslet *et al.*, 2012), and laboratory rearing experiments have established important aspects of biosynthetic processes that alter the $\delta^{15}N$ and $\delta^{13}C$ for particular organisms and tissues (Martínez del Rio *et al.*, 2009).

Schwamborn *et al.* (2002) determined that Grapsid crab larvae released from mangrove forests did not rely on mangrove derived carbon during development and that these young crabs exhibited assimilatory shifts of up to 1.0 ± 0.2‰ for ^{13}C and up to 1.4 ± 0.6‰ for ^{15}N. Another study using $\delta^{13}C$ and $\delta^{15}N$, determined that young juvenile blue crabs living in the Delaware Bay area fed primarily on zooplankton, while marsh-dwelling individuals, which were enriched in ^{13}C relative to bay juveniles, suggesting they utilized marsh-derived carbon for growth (Dittel *et al.*, 1999). France (1998) used $\delta^{13}C$ and $\delta^{15}N$ values of the fiddler crab, *Uca vocator*, in Puerto Rico to determine that MPB appear to make a proportionally greater contribution to the diets of these crabs than does mangrove carbon. Other isotope studies have shown that ingested material is not always assimilated efficiently by mangrove ecosystem consumers, and other primary producers such as phytoplankton, MPB, and algae may be more important sources of nutrition than previously suspected (Newell *et al.*, 1995; Christensen *et al.*, 2001; Bouillon *et al.*, 2002).

Using C and N stable isotopes, this study attempts to elucidate the primary sources of nutrition for *A. pisonii* across three post-larval life stages: small sized juveniles, intermediate sized individuals, and large adults. We evaluate the hypothesis that *A. pisonii* will ascend to a higher trophic level as they grow and broaden their dietary options. To do so, $\delta^{13}C$ and $\delta^{15}N$ values for the three size classes and potential prey items were measured from IRL and combined using the mixing model, IsoSource, to provide feasible source contributions from each prey item to *A. pisonii's* diet. An alternative mass balance method of using IsoSource to incorporate C and N concentrations of source tissue into the mixing model is utilized as well. In addition, this study assesses whether soil nutrient enrichment has an effect on the greater community of organisms dependent upon the *R. mangle* trees of the IRL mangrove forest. To do so, we evaluate the hypothesis that organisms dependent upon mangrove leaves will exhibit altered nutritional traits corresponding to altered food source quality.

MATERIALS AND METHODS

Site Description

Animal and leaf samples were collected from experimentally nutrient-enriched mangrove tree stands situated along the Intercoastal Waterway in IRL. Located in Avalon State Park on North Hutchinson Island, St. Lucie County, IRL is strongly stratified into three zones, which can be characterized by tree height and species composition, regularity of flooding events, and diversity of fauna. As described by Feller et al. (2003b), the fringe zone, which lies adjacent to the waterway, hosts stands of *R. mangle* rising up to approximately 4 m above the sediment surface. Here, semidiurnal tides regularly flush the sediment, and a variety of invertebrates are found living on the prop roots, in the sediment, and among the branches. The transition and scrub zones exhibit relatively lower faunal abundance and lie inland of the fringe zone by about 5 m and 15 m, respectively (Feller & Chamberlain, 2007).

Experimental Design

Fertilization treatments began January 1997 at IRL, as described in detail by Feller et al. (2003b). The study location is divided into three areas including three treatments per site (Control, Nitrogen (+N), & Phosphorus (+P)) with three replicate trees per treatment within each area for a total of 27 trees within the fringe zone (Feller *et al.*, 2003b). Fertilization treatments were randomly assigned to trees. Small doses of fertilizer (150 g NH_4 or P_2O_5 per cm diameter for +N or +P treatments, respectively) were sealed in dialysis tubing, placed in 30 cm holes cored into the substrate at opposite ends of a tree's canopy, and buried. Control trees received cores, but no fertilizer was added. Fertilization occurred twice per year and was administered into the sediment because surface application would have been washed away during tidal events.

Sample Collection and Preparation

To investigate the effects of nutrient addition on members of the IRL mangrove forest, we measured $\delta^{13}C$, $\delta^{15}N$, C, N, and P concentrations in a variety of leaf and animal tissues collected in June 2010 from the fringe zone. Animals were euthanized in a freezer and all samples were dried in a 70°C convection oven and ground using a Wiley Mill for leaves, Wig-L-Bug for most animal tissues, or mortar and pestle for samples that needed additional refinement.

Green leaf samples were collected from the youngest, fully mature green leaves in penapical positions in sunlit portions of the canopy. Senesced yellow leaves were gathered directly from the trees if they had developed complete abscission layers. Two unidentified lichens (crustose and foliose) from the branches or trunks of *R. mangle* were analyzed using tissue from the whole organism. Spiders (most commonly *Gasteracantha cancriformis*) were collected from within the canopy. *Gasteracantha cancriformis* were generally found on or near webs spanning branches in the canopy while other spiders were found near recesses in dead branch material with webs built around them. Whole spider bodies were used for analyses. Coffee bean snails (*Melampus bidentatus*) were collected off the sediment and debris found underneath the canopy of study trees. Their bodies were removed from their shells and rinsed with 0.6M HCl and distilled water before being prepared for analyses. Filter feeders living attached to treatment trees were also sampled. Barnacles (*Balanus eburneus*) were removed from prop roots that submerge at least during high tides. *Balanus eburneus* were removed from their calcium carbonate housings and rinsed with 0.6M HCl and distilled water. Atlantic ribbed mussels (*Geukensia demissa*) that were attached to prop roots and buried partially in the sediment were collected, and muscle samples were analyzed.

Fiddler crabs (*Uca* spp.) and unidentified mud crabs (Family Xanthidae) were collected directly from or around burrows in the sediment at the base of each tree. *Aratus pisonii* were divided into three

size classes based on carapace width: small (< 9 mm), medium (9 - 12 mm), and large (> 18.00 mm). Small *A. pisonii* were commonly found living among the barnacles and oysters attached to *R. mangle* prop roots or on decaying debris at the base of the trees. Large and medium *A. pisonii* were collected from prop roots and upper branches most often overlooking the water where they would attempt to jump to escape capture. Whole *Uca*, mud crabs, and *A. pisonii* of all 3 size classes as well as muscle tissue extracted from the male *Uca*, mud crabs, and large *A. pisonii* were analyzed.

To remove inorganic carbonates, whole crab samples were washed with 0.6M HCl. For small crabs, 1 mL HCl was applied to samples in 1 mL microvials for 45 min before centrifugation to facilitate separation of the sample from the added acid. This process was repeated three times except the final HCl application remained overnight in the refrigerator. The acid was removed, and the samples were rinsed with distilled water and then centrifuged five times before being returned to the drying oven. Medium crabs were treated with 10 mL 0.6M HCl in 15 mL disposable centrifuge tubes once overnight, and large crabs were similarly treated with 40 mL 0.6M HCl in 50 mL disposable centrifuge tubes. HCl was decanted and replaced with distilled water five times before drying. Samples were then transferred to 2 mL microvials for storage.

To supplement data needed to examine A. pisonii diets, we retrieved $\delta^{13}C$, $\delta^{15}N$, %C, and %N values from Vaslet et al.'s (2012) study at a nearby, non-fertilized site in Indian River Lagoon for MPB, particulate organic matter (POM), *R. mangle* leaf litter, prop root epiphytes, and algae.

Nutrient and Stable Isotope Analyses and Isotopic Mixing Models

Total P (% by mass) for all samples was determined by placing a known mass (~2 mg) of dried, ground tissue in a muffle furnace at 550 °C for 2 h (Miller, 1998) followed by addition of water and subsequent colorimetric analysis using the ammonium molybdate method with an extraction volume of 15 mL by mass (Clesceri *et al.*, 1998) at the Smithsonian Environmental Research Center, Edgewater,

MD. Carbon and N natural abundance isotope ratios as well as %C and %N by mass were determined after combustion of organic matter and chromatographic separation of CO_2 and N_2 using a Finnigan MAT 252 Isotope Ratio Mass Spectrometer at the Center for Applied Isotope Studies, University of Georgia, Athens, GA. Values for C:N are presented as the ratio between mol C and mol N. Adjustments for fractionation and isotopic sorting during digestion, metabolism, and assimilation were applied to the three target mixtures on a sliding scale using C as the exemplary system:

$$\delta^{13}C = \delta^{13}C_{measured} - \delta^{13}C_{correction} \quad (1)$$

where $\delta^{13}C_{measured}$ is the measured isotopic signature of large, medium, and small *A. pisonii* and $\delta^{13}C_{correction}$ adjusts for the change in trophic level and is set to 1.0 (Post, 2002). For N isotopes, the adjustment factor varies with crab size and is set to 3.4 $\delta^{15}N$ for large, 2.4 $\delta^{15}N$ for medium, and 1.4 $\delta^{15}N$ for small crabs, respectively (Post, 2002; Schwamborn *et al.*, 2002).

IsoSource (Phillips & Gregg, 2003) was utilized to assess relative contributions of potential food sources to *A.pisonii* diet. In mathematically underdetermined systems where more sources contribute to a mixture than the number of tracers available (n = 2 in this study), IsoSource determines the range of source combinations consistent with the observed mixture composition. By creating source partitions of 1-2.5% summing to 100%, isotopic signatures of the resulting mixture are calculated and compared to observed signatures with a tolerance of ±0.01-0.1‰. Results are presented as the feasible min to max ranges of source contributions rather than relying on mean or median values which are no more likely statistically than any other point within the range estimates (Phillips & Gregg, 2003; Fry, 2013).

Using $\delta^{13}C$ and $\delta^{15}N$ directly in IsoSource implies that C and N concentrations in each source are the same. Here, we account for concentration variations but assume that the contribution of a source to the mixture is proportional to the fractional contribution of that source times the elemental concentration. Thus, following Phillips and Koch (2002), we transformed our source values and input the

isotopic values ($\delta^{*}C$) of all sources as the difference from the target mixture weighted by its C or N concentration:

$$\delta^{*}C = (\delta^{13}C_{source} - \delta^{13}C_{target})*[C] \quad (2)$$

where $\delta^{13}C_{source}$ is the measured isotopic signature of source material, $\delta^{13}C_{target}$ is the adjusted isotopic signature of the target organism, and [C] is the total C concentration (g C/g sample) in the source material. The reader is referred to Phillips and Koch (2002) for a detailed derivation, but combining N and C isotopes results in the following set of equations implemented in IsoSource:

$$\begin{bmatrix} (\delta^{*}C_1)[C]_1 & (\delta^{*}C_{...})[C]_{...} & (\delta^{*}C_n)[C]_n \\ (\delta^{*}N_1)[N]_1 & (\delta^{*}N_{...})[N]_{...} & (\delta^{*}N_n)[N]_n \\ 1 & ... & 1 \end{bmatrix} \cdot \begin{pmatrix} f_1 \\ f_{...} \\ f_n \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} \quad (3)$$

where $f_{1...n}$ represent the fractions of assimilated biomass (B) from sources 1 to n. This is implemented in IsoSource, using the first two row vectors in the matrix as source descriptors, and 0 as the mixture signature, which then produces a distribution of feasible contributions for each source item.

Statistics

The data were grouped by nutrient treatment (Control, +N, +P) to compare %C, %N, %P, and C:N ratio and by size class (Small, Medium, Large) to compare $\delta^{13}C$ and $\delta^{15}N$. One-way factorial analyses of variance (ANOVA) were applied to each response variable using JMP Pro 9 (JMP). When an ANOVA found significant effects, Tukey's Honestly Significant Difference tests were applied to examine pairwise differences within and among the treatment levels. A multivariate analysis of variance (MANOVA) tested with Pillai, Wilks, and Roy was applied to $\delta^{13}C$ and $\delta^{15}N$ between size classes in R 2.15.3 (www.R-project.org) to confirm differences between groups. Then, $\delta^{13}C$ and $\delta^{15}N$ were used as variables in a quadratic discriminant function analysis (DFA) to examine if individuals could accurately be reclassified

into their predetermined size group. The DFA conducted an a posteriori test to determine the size class in which each crab sample had the highest probability of membership. For all p values, α was set to 0.05.

RESULTS

Nutrient enrichment effects

Nutrient enrichment had little effect on %C, %N, %P, or C:N ratio of any organism sampled with respect to fertilization treatment (Table 1). Large *A. pisonii* displayed an increased C:N ratio as a result of P fertilization somewhat diminishing N concentrations relative to crabs sampled from Control trees (ANOVA, $p = 0.0456$). Xanthid crab claw muscle showed diminished %P with N fertilization relative to individuals sampled from control trees (ANOVA, $p = 0.0398$). No other organisms displayed stoichiometric influences as a function of nutrient enrichment.

Carbon concentrations among organism types ranged from 20 – 50 % and were observed to be as low as 8% for MPB (Table 2). Nitrogen concentrations varied most between primary producers and consumers with %N ranges from 0.5 – 4% and 7 – 12%, respectively. Phosphorus concentrations ranged from 0.05 – 0.70% with leaf material usually less P rich than animal tissues. Values for C:N varied the most between grouped plant, lichen, and invertebrate samples with the latter having some variation between species as well. Green leaf material and lichens displayed C:N ratios around 50 while values for senesced leaves averaged 115. Other primary producers including algae and MPB showed C:N ratios between 6 and 10 while all animal tissues ranged between 4 and 7.

Isotope signatures of the fringe

The 16 types of IRL flora and fauna sampled in this study included three crab species, two filter feeders, one detritivorous snail, one arboreal arachnid, green and senescent *R. mangle* leaves, and two varieties of *R.mangle*-associated lichens (Table 3). Based on known feeding strategies and spatial

distribution within the fringe of the mangrove forest, consumers were expected to show approximately average trophic enrichment of 1.0‰ $\delta^{13}C$ and 3.4‰ $\delta^{15}N$ values relative to leaf material (Figure 1; labels indicated in Table 3). While both green and senescent leaves (labeled GL and SL, respectively) were characterized by depleted $\delta^{13}C$ values relative to all animals sampled, most organisms were much more than 1.0‰ $\delta^{13}C$ enriched. The only exceptions were whole *Uca* spp. and Xanthid crabs whose isotopic signatures signify exceptional fractionation during synthesis (Uw and Xw, respectively). This lack of stepwise trophic enrichment is likely due to multiple dietary sources of C and N other than *R. mangle* leaves.

The only sample organisms with $\delta^{13}C$ values indicative of an *R. mangle* diet were large and medium *A. pisonii* (L and M, respectively). However, $\delta^{15}N$ for large *A. pisonii* were depleted relative to both green and senescent leaves, and medium *A. pisonii* had $\delta^{15}N$ values nearly identical to senescent leaves. These depleted $\delta^{15}N$ values indicate the use of some other N source than mangrove leaves. Crustose and foliose lichens (Cr and Fo, respectively) appear to be the only viable solution to this $\delta^{15}N$ depletion as they are the only members of the food web showing $\delta^{15}N$ values lower than the large and medium *A. pisonii*. The enrichment of large *A. pisonii* muscle tissue relative to whole organism samples reflects differential fractionation during biosynthesis of various tissues.

Showing more intermediate $\delta^{13}C$ and $\delta^{15}N$ values, *M. bidentatus* (Mb) and small *A. pisonii* (S) signify a dependence on *R. mangle* leaves for some of their nutrients and at least some supplemental food sources that are more enriched in $\delta^{13}C$ and/or $\delta^{15}N$ such as MPB, POM, algae, or prop root epiphytes (MPB, POM, Alg, and Epi, respectively). The other members of the food web appear to rely heavily on some external sources of nutrition. Filter feeders (*G. demissa*, Gd, and *B. eburneus*, Be), *Uca*. spp (Um), mud crabs (Xm), and spiders (Gc) exhibit enriched $\delta^{15}N$ values generally compared to other *A. pisonii* which may indicate similar trophic level status, but their intermediary $\delta^{13}C$ values relative to

potential food sources (GL, SL, MPB, POM, Alg, Epi, and in some cases other animals) signifies multiple carbon sources. Overall, these data indicate a community dominated by omnivorous benthic feeders with the exception of large and medium sized *A. pisonii* who spend a significant portion of their time foraging within the branches of the *R. mangle* trees and are probably feeding on leaves, lichens, and some incidental animal prey items.

Ontogenetic shifts in A. pisonii

Isotopic signatures differ among small, medium, and large *A. pisonii* size classes (Figure 2; MANOVA, $p < 0.0005$). For both $\delta^{13}C$ and $\delta^{15}N$, small crabs exhibited higher mean values than medium and large size classes, which did not differ significantly from one another (ANOVA, $p = 0.0004$ and $p < 0.0001$, respectively). To elucidate the differences between size classes, we attempted to reclassify isotope data into their predefined size categories using a discriminant function analysis (Table 4). Large and small crabs were appropriately reassigned to their correct size classes 89% and 85% of the time, respectively. Most reclassification errors were due to medium crabs being incorrectly assigned to the other two groups: 41% to large and 22% to small. Small crabs exhibited a wide range of $\delta^{13}C$ values, but their general isotopic space (indicated by a dashed oval) was confirmed to be independent of the larger individuals (indicated by a solid oval; Figure 4). To further evaluate differences between size class diets, medium *A. pisonii* were omitted due to the statistical similarities they shared with the other two groups.

Isotopic signatures of probable food sources were used with IsoSource to estimate feasible ranges of food source contributions to both small and large *A. pisonii* size classes as described in Table 5 (Phillips & Gregg, 2003). For small crabs, MPB (48 – 58%) and senescent leaves (32 – 42%) were revealed to be significant contributors to their diets (Figure 3). With MPB and senescent leaves accounting for at least 80% of small *A. pisonii* diets, leaf litter (0 – 11%), prop root epiphytes (0 – 7%), POM (0 – 9%), and

algae (0-13%) can only represent a maximum of 20% of small crab diets. The small *A. pisonii* diet displays some distinct differences from that of the larger conspecifics.

For large *A. pisonii*, senescent leaves account for 68 – 90% of their diets which is more than twice the minimum contribution of senescent leaves to small crab diets while MPB (0 – 3%) are no longer likely contributors to the diets of large crabs (Figure 4). It is evident that senescent leaves are preferred to green leaves whose feasible contribution range only extends from 0 – 18%. Unlikely or minor contributors to large crab diets include small *A. pisonii*, prop root epiphytes, spiders, *Uca* spp., and algae which each range from 0 to no more than 8% feasible contributions. Though leaf material dominates the diet of large *A. pisonii*, neither leaves nor other potential sources described heretofore can account for their $\delta^{15}N$ depletion. Indicated by a dotted line, large *A. pisonii* fall outside of the mixing polygon unless lichens are included. Thus, crustose and/or foliose lichens are required to satisfy the requirements of mass balance and are likely contributors to the diets of large *A. pisonii* (0 – 18% and 0 – 10%, respectively).

Because the C and N concentrations of potential food sources may differ substantially, we adapted our data to still work with IsoSource while accounting for C and N concentrations. While food sources generally retained feasible contribution ranges similar to the original IsoSource model results, this mass balance method produced ranges less precise in most cases but altogether different in some (Table 6).

For small *A. pisonii*, MPB (0 – 73%) still potentially represent a significant contribution to their diets (Figure 5), but this range is much more broad than the well constrained estimate described previously. Similarly, senescent leaves (16 – 61%) exhibit a wider range of feasible contributions. Leaf litter may contribute up to 17%, but algae (0 – 6%) and prop root epiphytes (0 – 4%) still remain

insignificant contributors to small *A. pisonii* diets. However, POM may represent up to a 39% contribution, over four times the previous maximum estimate.

Results for large *A. pisonii* diets did not shift quite as much in most cases (Figure 6). Senescent leaves (33 – 90%) remain significant contributors to large *A. pisonii* diets though their minimum feasible contribution is markedly decreased. In exchange, green leaves (0 – 53%) now may account for a greater portion of large crab diets. Both crustose and foliose lichens maintain low overall potential contributions, but they exhibit slightly higher maximum feasible ranges than before (0 – 20% and 0 – 13%, respectively). Small *A. pisonii*, prop root epiphytes, and algae each maintain feasible contributions of 0 – 8%, and spiders and *Uca* spp. are suggested to contribute no more than 1% each. Perhaps the most notable change from accounting for C and N concentration, MPB may represent up to 28% of the large crab diets indicating potential utilization of a food source that was only attributed to small crabs using the original model.

DISCUSSION

Nutrient enrichment effects

The results of this work suggest that direct enrichment of N and P to the sediment at the base of *R. mangle* trees has little effect on the nutritional makeup of the faunal community that utilizes these mangrove habitats (Table 1). Only 2 sample types exhibited any type of nutritional alterations with respect to fertilization treatment. Large *A. pisonii* living on P fertilized trees tended to have higher C:N ratios than those found on control trees in the fringe zone, and Xanthid crabs collected at the base of N fertilized trees exhibited diminished P content relative to those found at control sites. While studies have shown stoichiometric alterations to invertebrates in other wetlands treated with nutrient enrichment, these results tend to be species specific (Rader & Richardson, 1994; Baggett *et al.*, 2013). Three marsh crab species, *Uca pugnax, Uca pugilator,* and *Gecarcinus lateralis*, grow more rapidly when

offered food with higher N (Wolcott & Wolcott, 1984; Wolcott & O'Connor, 1992), and Spivak et al. (2009) observed that fertilization effects on sea grass nutritional stoichiometry and production can be effectively translated into grazer secondary production. In each of these studies, fertilization effects transferred through primary producers into secondary production.

Because nutrient enrichment did not indicate altered leaf stoichiometry in our study, there appears to be minimal fertilization effects through the food web. Neither green nor senescent *R. mangle* leaves exhibited responses in %C, %N, %P, or C:N ratio with respect to fertilization treatment. This lack of response conflicts with previous studies from the N-limited fringe zone at IRL which reported significant increases in leaf %N, new growth, and productivity (Feller *et al.*, 2003b). Mangrove fertilization has repeatedly been observed to bring about changes in leaf stoichiometry, photosynthetic productivity, resorption efficiencies, and growth (Feller, 1995; Feller *et al.*, 2003a; Feller *et al.*, 2009b). Due to the history of documented nutrient enrichment effects at IRL, it is possible that there were confounding factors influencing nutrient availability to the trees around the time of sample collection. In September 2004, Hurricanes Frances and Jeanne delivered tremendous amounts of nutrients to study site in IRL abating N-limiting conditions for several years (I.C. Feller, personal communication, May 6, 2013). Home ranges wider than the reach of fertilization effects may have also played a role in preventing nutrient enrichment effects to cascade throughout the system. Warner (1970) measured *A. pisonii* home ranges of about 6 m^2 which exceeds the spatial limits of individual tree fertilization effects. Additionally, motile individuals such as crabs and snails are likely to feed on sources derived from outside the fertilization's area of influence (Jordan & Valiela, 1982). Rearing experiments would be ideal for quantifying the bottom-up effects of N and P enrichment on herbivorous and omnivorous members of the mangrove food web allowing for restriction of source contributions.

Isotope signatures of the fringe

Based on their $\delta^{13}C$ and $\delta^{15}N$ values, it is apparent that members of the mangrove fringe community at IRL derive their C and N from a mixture of sources (Figure 1). Large and medium sized *A. pisonii* appear to rely heavily on mangrove C; however, they may obtain N from additional sources, including from lichens for the larger individuals because both types of lichen sampled displayed depleted $\delta^{15}N$ values. Small *A. pisonii* and *M. bidentatus* display isotopic signatures indicative of leaf material and MPB composing a significant portion of their diets. *Balanus eburneus* and *G. demissa* are filter feeders. They derive nutrition from POM and finely processed leaf material, but their enriched $\delta^{15}N$ values indicate reliance upon some externally derived sources. In a more extreme case, *Uca* spp. and Xanthids crab bodies showed extremely depleted $\delta^{13}C$ and enriched $\delta^{15}N$ values when analyzed whole. These values appear to be erroneous possibly due extreme fractionation during chitin formation. Yokoyama et al. (2005) report that high values of fractionation may occur in some bivalves and crustaceans, which makes the consideration of muscle tissue more appropriate for these organisms. The spider *G. concriformis* may be an opportunistically predated food source for Large *A. pisonii* but is unlikely to be feeding on any of the organisms sampled herein due to spatial separation even though they display similar isotopic signatures. More likely, these spiders are another example of an organism deriving it's nutrition from external sources.

Ontogenetic shifts in A. pisonii

Our isotopic data confirmed the presence of niche shifts between *A. pisonii* size classes reflecting changes in diet as a probable result of spatial discrimination and morphological changes between juvenile and adult life history stages (Figure 2). Small *A. pisonii* were more enriched in both $\delta^{15}N$ and $\delta^{13}C$ values relative to large crabs. Medium individuals displayed a wide range of isotopic signatures that overlapped with both large and small crabs about as often as they appeared unique,

consistent with a transitional phase between two distinct diets (Table 4). Isotopic depletion with size is a rather unusual finding as most organisms tend to exhibit increased $\delta^{15}N$ and $\delta^{13}C$ values as they grow, indicating a move toward a higher trophic level (Minagawa & Wada, 1984; Dunton *et al.*, 2012). However, we have documented a shift in the primary consumption habits of *A. pisonii* between small and large conspecifics which can explain this observed isotopic depletion as an artifact of their transition into the canopy as they grow into adults.

As they mature, *A. pisonii* become more arboreal and can freely climb into the canopy where they feed on leaves and other prey items opportunistically (Warner, 1970; Beever *et al.*, 1979; McKeon & Feller, 2004). Our data require that the large crabs are feeding on a $\delta^{15}N$ depleted source apart from those traditionally described in their diets owing to the fact that their own isotopic signature falls below any of those documented food sources (Table 5). The only organisms present with $\delta^{15}N$ values more depleted than the large *A. pisonii* are lichens. Unusually negative $\delta^{15}N$ values in lichens may be the result of foliar uptake of atmospheric NH_3 for subsequent fixation as a response to nutrient limitation (Tozer *et al.*, 2005; Fogel *et al.*, 2008). In an N-limited forest such as this one, feeding on N-fixing lichen symbionts seems to be a logical strategy. Due to their extreme depletion relative to the crabs, lichens need only contribute a small portion of their diets to result in a noticeable decrease in $\delta^{15}N$. Mangrove tree crabs in Africa, *Sesarma leptosoma*, have been documented feeding on lichens growing in the canopy of *Rhizophora mucronata* (Dahdouh-Guebas *et al.*, 1999), and the land crab, *Gecarcinus planatus*, is known to feed on lichens as well (Ortega-Rubio *et al.*, 1997).

Because juvenile *A. pisonii* are found on prop roots and sediment, they do not have access to the $\delta^{15}N$ depleted lichens. Their isotopic values are more consistent with feeding on leaf detritus and MPB (Figure 3), a diet previously suggested in the literature (Feller & Chamberlain, 2007; Giarrizzo *et al.*, 2011). In addition to MPB, senescent leaves, particulate organic matter, and leaf litter appear to have

potential as significant contributors to small crab diets (Figure 5). Though large individuals may still utilize MPB as a C source (Figure 6), isotopic values clearly exhibit a tendency away from MPB with size (Figure 4). Again, the repercussions of strict vertical zonation between size classes are reflected as an unusual isotopic characteristic with increasing size. Reliance on the $\delta^{13}C$ rich MPB, relative to mangrove leaves, results in small *A. pisonii* exhibiting higher $\delta^{13}C$ values than their larger counterparts.

Erickson et al. (2003) determined that mangrove leaves constitute 84% of *A. pisonii* diets, and Feller et al. (In Press) further constrained these observations citing preferential folivory of older leaves in the canopy. Our data agree, indicating that large crabs display a strong tendency toward senescent rather than green leaves (Figures 4 & 6), and small *A. pisonii* tend to prefer freshly fallen leaves over the more aged leaf litter (Figures 3 & 5). Senescent leaves have a markedly higher C:N ratio than leaf litter or green leaves (Table 2); however, they contain far fewer phenolic compounds and condensed tannins than green leaves making older leaves more palatable for large crabs (Lin *et al.*, 2007). While nitrogen content and caloric value increase with decomposition, so do protein-bound and fiber-bound condensed tannins (Lin *et al.*, 2007). These differences in freshly fallen leaves and decomposing leaf litter may explain small crab preferences for freshly senesced leaves.

Opportunistic feeding on smaller conspecifics, other crabs, and insects is well documented in large *A. pisonii* (Warner, 1967; Beever *et al.*, 1979; McKeon & Feller, 2004; Feller *et al.*, In Press). Their robust bodies and freedom to roam between canopy and understory (Warner, 1967, 1969) gives large individuals ample opportunity to forage upon a wide variety of prey items. However, the very small contribution potential of spiders, *Uca* spp, and smaller conspecifics indicates that predatory encounters are uncommon and probably not actively pursued. If such encounters are more frequent than the low feasible range of contribution of these other invertebrates suggests, it is probable that these killing

encounters are motivated by an assertion of dominance rather than for nutritive purposes (Warner, 1970).

Evaluation of IsoSource mixing model results

Adaptation of isotopic data to incorporate C and N concentrations according to equations 2 and 3 produced similar results to those obtained using the intended $\delta^{13}C$ and $\delta^{15}N$ values (Table 6). In most cases, feasible ranges of contribution from source material were less precise using the concentration weighted approach, but some sources that were considered insignificant by the traditional model were brought to light as potentially constituting large portions of the target species' diet. Utilizing both approaches provides a useful proxy for accuracy as well as additional data to help constrain potentially ambiguous diets. Ideally, both approaches would be combined with field observations to validate inclusion of each source material. By including sources which have not been expressly identified as prey species (arboreal spiders for example), IsoSource assigns importance to these species when they may not actually contribute at all (Fry, 2013). Additionally, better understanding prey item diets would allow more confident identification of ultimate organic matter sources, and laboratory rearing experiments to determine isotopic fractionation values would provide a more accurate depiction of isotopic relationships with respect to trophic enrichment.

CONCLUSION

Our data clearly identify a shift in *A. pisonii* feeding habits as they mature, which we suggest indicates a modification in the juvenile diet of MPB and freshly fallen leaves to an adult diet dominated by senescent and to a lesser extent, green, leaves, as well as lichens. Because of strong spatial restrictions preventing small *A. pisonii* from entering the canopy of mangrove trees, larger individuals have access to N-fixing, $\delta^{15}N$ depleted lichens which gives the large crabs an unusually low $\delta^{15}N$ value relative to smaller individuals. Similarly, heavy reliance on leaf material by large crabs results in depleted

$\delta^{13}C$ values compared to the small crabs who utilize the $\delta^{13}C$ rich MPB. Though bottom-up nutrient influences were not identified as playing a significant role in this food web, it is probable that controlled studies would result in direct links to growth rate and nutrient assimilation in some of the primary herbivores sampled herein. In order to better elucidate the part that each food source plays, herbivory of lichens needs to be documented from in situ studies, and thorough observations of foraging strategies for all size classes should be conducted. The elimination of insignificant and addition of primary food sources based on observational data would greatly strengthen the reliability of the IsoSource models which are most useful when considered using both the traditional and mass balance methods.

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Table 1 Fertilization treatment effects among sample types on C, N, and P (% by mass) and C:N ratio (mean ± SD). Values significantly different from one another are indicated by an asterisk (ANOVA; df = 2, $p < 0.05$). *n* = number of samples (%C , %N, & C:N share common *n* values). --- = no samples available.

Sample Type	Treatment	*n*	%C	%N	C:N	*n*	%P
Mangrove tree crabs							
Aratus pisonii Large (> 18 mm)	Control	9	50.3 ± 1.4	8.7 ± 0.7	6.8 ± 0.6*	7	0.2 ± 0.0
	+N	9	50.7 ± 2.0	8.5 ± 0.4	7.0 ± 0.5	8	0.2 ± 0.0
	+P	9	50.7 ± 1.8	8.0 ± 0.8	7.5 ± 0.7*	8	0.2 ± 0.1
Medium (9 - 12 mm)	Control	9	47.3 ± 1.9	8.2 ± 0.6	6.7 ± 0.4	9	0.3 ± 0.1
	+N	9	47.6 ± 3.0	8.5 ± 0.7	6.6 ± 0.6	9	0.2 ± 0.0
	+P	9	43.7 ± 5.5	7.6 ± 1.0	6.7 ± 0.5	8	0.3 ± 0.1
Small (< 9 mm)	Control	9	43.1 ± 5.4	8.4 ± 0.6	6.0 ± 0.7	9	0.3 ± 0.1
	+N	9	44.6 ± 2.3	9.0 ± 0.6	5.8 ± 0.6	9	0.3 ± 0.1
	+P	8	40.4 ± 5.6	8.1 ± 0.8	5.8 ± 0.5	9	0.3 ± 0.1
Claw muscle	Control	6	39.1 ± 1.8	10.1 ± 1.1	4.5 ± 0.4	6	0.7 ± 0.3
	+N	8	35.2 ± 4.6	9.4 ± 1.5	4.4 ± 0.4	8	0.8 ± 0.5
	+P	9	37.7 ± 2.8	10.0 ± 1.1	4.4 ± 0.3	9	0.6 ± 0.2
Red mangrove tree							
Rhizophora mangle Green Leaves	Control	9	49.6 ± 8.9	1.1 ± 0.1	51.6 ± 10.8	9	0.1 ± 0.0
	+N	6	48.5 ± 8.7	1.2 ± 0.2	49.0 ± 14.5	6	0.1 ± 0.0
	+P	9	45.3 ± 8.0	1.1 ± 0.1	51.0 ± 12.3	9	0.1 ± 0.0
Senescent Leaves	Control	9	42.4 ± 2.7	0.4 ± 0.1	115.3 ± 24.8	9	0.05 ± 0.01
	+N	9	43.4 ± 3.5	0.5 ± 0.1	100.9 ± 14.2	9	0.04 ± 0.02
	+P	9	44.5 ± 3.7	0.4 ± 0.1	127.7 ± 15.4	9	0.05 ± 0.02
Lichen (Unidentified)							
Crustose variety	Control	3	47.0 ± 2.3	1.1 ± 0.1	48.2 ± 4.7	3	0.1 ± 0.0
	+N	3	47.3 ± 1.2	1.2 ± 0.1	46.9 ± 4.2	3	0.1 ± 0.0
	+P	0	---	---	---	0	---
Foliose variety	Control	2	43.5 ± 0.5	1.3 ± 0.0	40.6 ± 0.8	2	0.1 ± 0.0
	+N	6	43.8 ± 1.2	1.1 ± 0.1	47.5 ± 4.3	6	0.1 ± 0.0
	+P	6	43.9 ± 1.1	1.1 ± 0.1	47.2 ± 5.9	6	0.1 ± 0.0

Table 1 continued

Sample Type	Treatment	*n*	%C	%N	*n*	%P	C:N
Other crab species							
Uca spp. Fiddler crab bodies	Control	9	47.6 ± 2.3	8.4 ± 0.7	9	0.1 ± 0.0	6.6 ± 0.7
	+N	9	47.9 ± 2.0	8.3 ± 0.5	9	0.1 ± 0.0	6.7 ± 0.4
	+P	9	49.7 ± 1.6	8.8 ± 0.6	9	0.1 ± 0.0	6.6 ± 0.6
Male claw muscle	Control	9	39.3 ± 2.9	11.8 ± 1.2	9	0.6 ± 0.1	3.9 ± 0.2
	+N	9	37.3 ± 4.6	10.7 ± 1.9	9	0.8 ± 0.2	4.1 ± 0.3
	+P	7	39.0 ± 3.6	11.7 ± 1.5	7	0.5 ± 0.2	3.9 ± 0.2
Xanthidae Mud crab bodies	Control	2	46.7 ± 12.2	7.6 ± 0.1	2	0.5 ± 0.1	7.2 ± 2.0
	+N	5	49.0 ± 5.5	8.3 ± 1.0	5	0.7 ± 0.4	6.9 ± 0.6
	+P	4	50.5 ± 3.3	8.9 ± 1.1	4	0.5 ± 0.4	6.7 ± 1.2
Mud crab claw muscle	Control	2	41.8 ± 2.4	11.5 ± 2.2	2	0.13 ± 0.0*	4.3 ± 0.6
	+N	4	34.7 ± 6.8	9.5 ± 2.9	4	0.08 ± 0.0*	4.4 ± 0.6
	+P	3	40.9 ± 2.9	12.2 ± 0.6	3	0.09 ± 0.0	3.9 ± 0.1
Other invertebrates							
Spiders *Gasteracantha cancriformis*	Control	9	49.2 ± 1.2	12.0 ± 0.5	9	0.6 ± 0.1	4.8 ± 0.3
	+N	8	49.0 ± 1.4	11.8 ± 1.0	9	0.8 ± 0.1	4.9 ± 0.6
	+P	6	48.7 ± 1.6	12.0 ± 0.5	6	0.7 ± 0.1	4.8 ± 0.3
Coffee bean snail *Melampus bidentatus*	Control	9	37.9 ± 3.1	6.9 ± 0.8	9	0.6 ± 0.1	6.5 ± 0.6
	+N	8	38.4 ± 4.0	7.0 ± 1.0	9	0.6 ± 0.1	6.5 ± 0.5
	+P	7	38.3 ± 2.3	7.1 ± 0.8	8	0.6 ± 0.1	6.3 ± 0.4
Ivory Barnacle *Balanus eburneus*	Control	5	39.7 ± 1.2	12.2 ± 0.5	6	0.3 ± 0.1	3.8 ± 0.1
	+N	7	41.4 ± 1.7	12.8 ± 1.0	7	0.3 ± 0.1	3.8 ± 0.2
	+P	7	41.5 ± 0.6	12.8 ± 0.6	7	0.3 ± 0.0	3.8 ± 0.1
Atlantic ribbed mussel *Geukensia demissa* muscle	Control	3	35.4 ± 5.9	8.8 ± 2.3	1	0.3	4.8 ± 0.6
	+N	2	35.4 ± 0.4	9.2 ± 0.8	0	---	4.5 ± 0.3
	+P	3	36.8 ± 1.9	9.3 ± 0.9	1	0.5	4.6 ± 0.3

Table 2 Total C, N and P (% by mass) and C:N ratios (mean ± SD) of flora and fauna collected at IRL. Values reported by Vaslet et al. (2012) from IRL samples are labeled as such. *n* = number of samples analyzed. --- = not available.

Sample Type	*n*	%C	%N	%P	C:N
Mangrove tree crabs					
Aratus pisonii					
Large (> 18 mm))	27	50.6 ± 1.7	8.4 ± 0.7	0.19 ± 0.1	7.1 ± 0.6
Medium (9 - 12 mm)	27	46.2 ± 4.0	8.1 ± 0.8	0.24 ± 0.1	6.7 ± 0.5
Small (< 9 mm)	26	42.8 ± 4.8	8.5 ± 0.8	0.27 ± 0.1	5.9 ± 0.6
Claw muscle	23	37.2 ± 3.6	9.8 ± 1.3	0.70 ± 0.4	4.4 ± 0.3
Red mangrove tree					
Rhizophora mangle					
Green Leaves	24	47.7 ± 8.4	1.1 ± 0.1	0.10 ± 0.0	50.7 ± 11.8
Senescent Leaves	27	43.4 ± 3.3	0.5 ± 0.1	0.05 ± 0.0	114.6 ± 21.2
Lichen (Unidentified)					
Crustose variety	6	47.1 ± 1.6	1.2 ± 0.1	0.11 ± 0.0	47.6 ± 4.1
Foliose variety	14	43.8 ± 1.0	1.1 ± 0.1	0.06 ± 0.0	46.4 ± 5.2
Other crab species					
Uca spp.					
Fiddler crab bodies	27	48.4 ± 2.1	8.5 ± 0.6	0.09 ± 0.0	6.7 ± 0.5
Male claw muscle	*25*	38.5 ± 3.7	11.4 ± 1.6	0.63 ± 0.2	4.0 ± 0.2
Xanthidae					
Mud crab bodies	11	49.1 ± 5.7	8.4 ± 1.0	0.57 ± 0.3	6.9 ± 1.0
Mud crab claw muscle	9	38.4 ± 5.7	10.8 ± 2.3	0.10 ± 0.0	4.2 ± 0.5
Other invertebrates					
Spiders					
Gasteracantha cancriformis	24	49.0 ± 1.3	11.9 ± 0.7	0.70 ± 0.1	4.8 ± 0.4
Coffee bean snail					
Melampus bidentatus	*26*	38.2 ± 3.1	7.0 ± 0.8	0.63 ± 0.1	6.4 ± 0.5
Ivory Barnacle					
Balanus eburneus	8	35.9 ± 3.4	9.1 ± 1.4	0.41 ± 0.1	4.7 ± 0.4
Atlantic ribbed mussel					
Geukensia demissa muscle	20	41.0 ± 1.4	12.6 ± 0.8	0.30 ± 0.1	3.8 ± 0.1
Vaslet et al. (2012)					
Microphytobenthos	3	7.7 ± 0.6	0.9 ± 0.1	---	10.4 ± 0.1
Prop Root Epiphytes	3	20.9 ± 0.3	2.6 ± 0.0	---	9.3 ± 0.1
Litter (R. mangle)	4	48.8 ± 0.6	0.7 ± 0.0	---	80.9 ± 2.8
Algae	3	20.8 ± 2.0	3.8 ± 0.7	---	6.4 ± 0.6
POM	2	22.4 ± 2.9	3.8 ± 0.6	---	6.9 ± 0.2

Table 3 $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD) of flora and fauna collected at IRL. Labels correspond to isotopic signatures in Figure 1. Values reported by Vaslet et al. (2012) from IRL samples are labeled as such. *n* = number of samples analyzed. --- = not available.

Sample Type	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Fig. 1 Label
Mangrove tree crabs				
Aratus pisonii				
Large (> 18 mm))	27	-26.8 ± 0.5	-0.6 ± 0.8	L
Medium (9 - 12 mm)	27	-26.3 ± 0.8	0.0 ± 1.5	M
Small (< 9 mm)	26	-25.1 ± 0.9	2.0 ± 0.8	S
Claw muscle	23	-23.8 ± 0.6	3.3 ± 1.2	Ap
Red mangrove tree				
Rhizophora mangle				
Green Leaves	24	-28.1 ± 0.8	3.1 ± 0.8	GL
Senescent Leaves	27	-27.6 ± 0.6	0.1 ± 0.6	SL
Lichen (Unidentified)				
Crustose variety	6	-22.6 ± 0.7	-5.5 ± 1.1	Cr
Foliose variety	14	-19.0 ± 1.1	-10.3 ± 1.8	Fo
Other crab species				
Uca spp.				
Fiddler crab bodies	27	-52.3 ± 8.7	27.7 ± 9.0	Uw
Male claw muscle	*25*	-23.0 ± 0.8	5.0 ± 0.6	Um
Xanthidae				
Mud crab bodies	11	-56.4 ± 1.2	34.5 ± 1.2	Xw
Mud crab claw muscle	9	-22.9 ± 1.6	7.2 ± 1.1	Xm
Other invertebrates				
Spiders				
Gasteracantha cancriformis	24	-24.0 ± 0.5	6.1 ± 0.5	Gc
Coffee bean snail				
Melampus bidentatus	*26*	-25.0 ± 0.6	2.4 ± 0.5	Mb
Ivory Barnacle				
Balanus eburneus	8	-23.3 ± 0.7	7.8 ± 0.3	Be
Atlantic ribbed mussel				
Geukensia demissa muscle	20	-23.5 ± 0.6	7.0 ± 0.5	Gd
Vaslet et al. (2012)				
Microphytobenthos	3	-19.7 ± 0.1	3.0 ± 0.4	MPB
Prop Root Epiphytes	3	-25.3 ± 0.1	5.8 ± 0.2	Epi
Litter (R. mangle)	4	-28.1 ± 0.2	3.1 ± 0.5	LL
Algae	3	-22.2 ± 0.7	4.8 ± 0.6	Alg
POM	2	-21.6 ± 1.3	5.9 ± 0.5	POM

Table 4 Reclassification summary of trophic groups from discriminant function analysis of *A. pisonii* size classes: large (> 18 mm), medium (9 – 12 mm) and small (< 9 mm).

	Predicted size group reclassification success (%)			Error
	Large	Medium	Small	
Large	88.8	11.1	0.0	11.1
Medium	40.7	37.0	22.2	62.9
Small	3.8	11.5	84.5	15.4

Table 5 $\delta^{13}C$, $\delta^{15}N$, δ*C and δ*N values used to determine dietary contribution ranges in IsoSource. δ*C and δ*N values were calculated using equations 2 and 3. *n* = number of samples.

Sample Type	*n*	δ13C (‰)	δ15N (‰)	δ*C	δ*N
Target Organism					
Small *A. pisonii*	26	-25.1 ± 0.9	2.0 ± 0.8	0	0
Sources					
Microphytobenthos	3	-19.7 ± 0.1	3.0 ± 0.4	0.4132	0.0089
Leaf litter	4	-28.1 ± 0.2	3.1 ± 0.5	-0.6363	0.0297
Particulate Organic Matter	2	-21.6 ± 1.3	5.9 ± 0.5	1.6885	0.0277
Prop root epiphytes	3	-25.3 ± 0.1	5.8 ± 0.2	-0.0503	0.1459
Algae	3	-22.2 ± 0.7	4.8 ± 0.6	0.6393	0.1070
Senescent leaves	27	-27.6 ± 0.6	0.1 ± 0.6	-1.0840	-0.0087
Target Organism					
Large *A. pisonii*	27	-26.8 ± 0.5	-0.6 ± 0.8	0	0
Sources					
Prop root epiphytes	3	-25.3 ± 0.1	5.8 ± 0.2	0.3175	0.1688
Green leaves	24	-28.1 ± 0.8	3.1 ± 0.8	-0.5977	0.0414
Senescent leaves	27	-27.6 ± 0.6	0.1 ± 0.6	-0.3195	0.0031
Lichen (Crustose)	6	-22.6 ± 0.7	-5.5 ± 1.1	1.9681	-0.0571
Lichen (Foliose)	14	-19.0 ± 1.1	-10.3 ± 1.8	3.4197	-0.1076
Algae	3	-22.2 ± 0.7	4.8 ± 0.6	0.9592	0.2065
Microphytobenthos	3	-19.7 ± 0.1	3.0 ± 0.4	0.5490	0.0314
G. cancriformis	24	-24.0 ± 0.5	6.1 ± 0.5	1.3911	0.7964
Uca spp. muscle	25	-23.0 ± 0.8	5.0 ± 0.6	1.4703	0.6444
Small *A. pisonii*	26	-25.1 ± 0.9	2.0 ± 0.8	0.7532	0.2201

Table 6 Feasible source contributions to diets of small and large *A. pisonii* using the traditional method and mass balance method to determine inputs for IsoSource.

Sample Type	n	Traditional Method	Mass Balance Method
Target Organism			
Small *A. pisonii*	26	---	---
Sources			
Microphytobenthos	3	48 - 58%	0 - 73%
Leaf litter	4	0 - 11%	0 - 17%
Particulate Organic Matter	2	0 - 9%	0 - 39%
Prop root epiphytes	3	0 - 7%	0 - 4%
Algae	3	0 - 13%	0 - 6%
Senescent leaves	27	32-42%	16 - 61%
Target Organism			
Large *A. pisonii*	27	---	---
Sources			
Prop root epiphytes	3	0 - 5%	0 - 8%
Green leaves	24	0 - 18%	0 - 53%
Senescent leaves	27	68 - 90%	33 - 90%
Lichen (Crustose)	6	0 - 18%	0 - 20%
Lichen (Foliose)	14	0 - 10%	0 - 13%
Algae	3	0 - 3%	0 - 5%
Microphytobenthos	3	0 - 3%	0 - 28%
G. cancriformis	24	0 - 3%	0 - 1%
Uca spp. muscle	25	0 - 3%	0 - 1%
Small *A. pisonii*	26	0 - 8%	0 - 5%

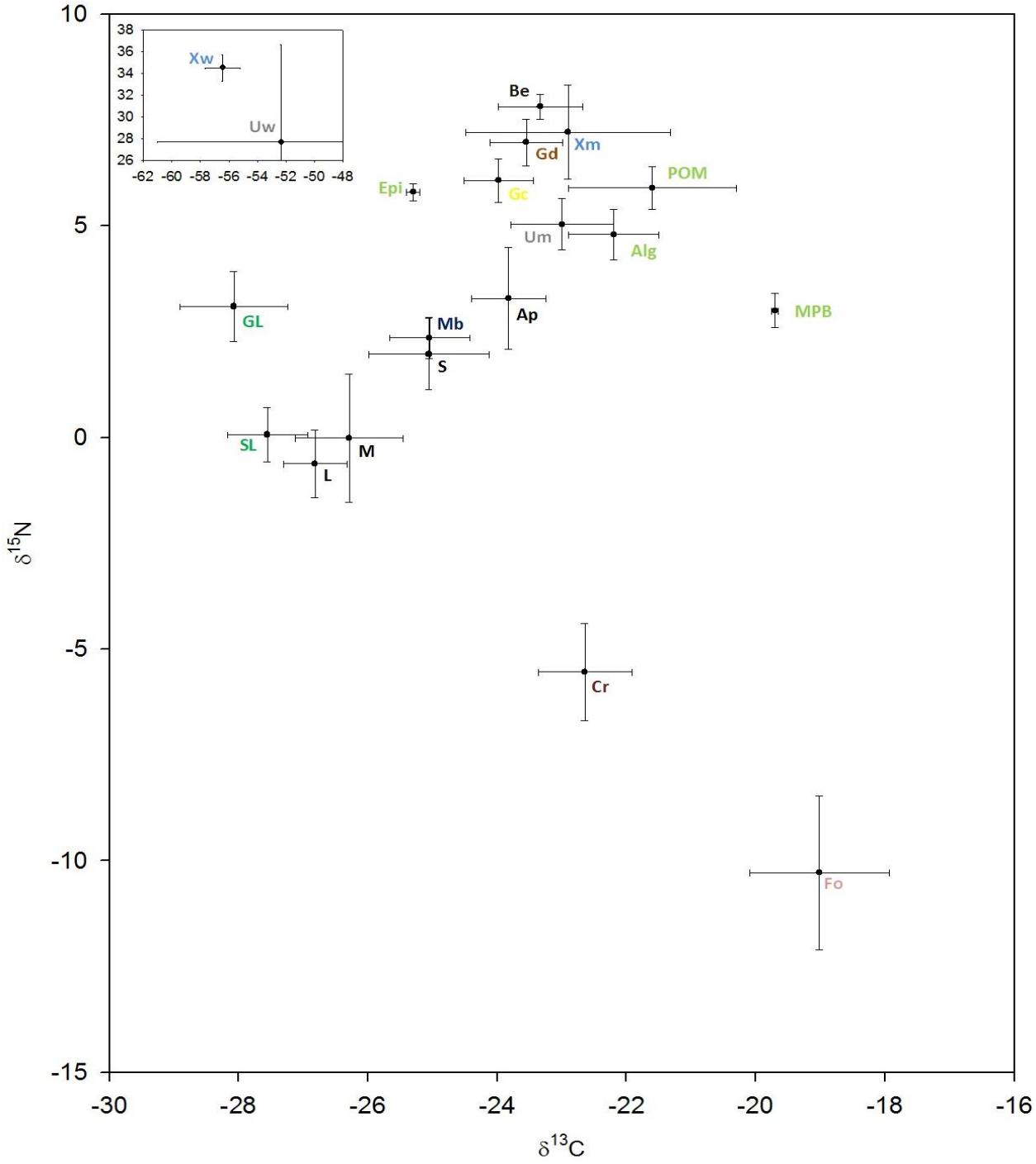

Figure 1 Biplot of $\delta^{13}C$ v. $\delta^{15}N$ values for all samples collected from IRL (denoted by letters, see Table 3). The smaller biplot represents samples whose isotopic space was much further from the rest and are subject to different scales using the same axis labels. (Xw – Xanthid crab bodies; Uw – *Uca* spp. bodies; Epi – Prop root epiphytes; Be – *B. eburneus*; Gd – *G. demissa*; Xm – Xanthid crab muscles; Gc – *G. cancriformis*; POM – Particulate organic matter; Um – *Uca* spp. muscles; Ap – *A. pisonii* muscles; Alg – Algae; GL – Green *R. mangle* leaves; Mb – *M. bidentatus*; S – Small *A. pisonii*; MPB – Microphytobenthos; SL – Senescent *R. mangle* leaves; L – Large *A. pisonii*; M – Medium *A. pisonii*; Cr – Crustose lichen; Fo – Foliose lichen)

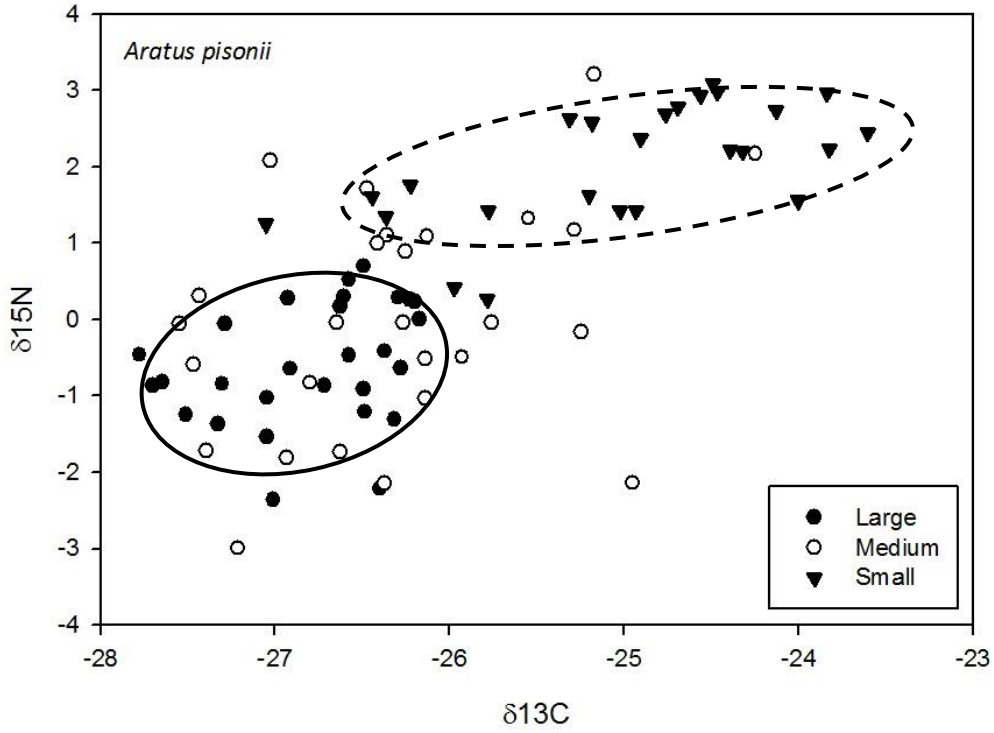

Figure 2 The isotopic differences between large (> 18 mm), medium (9 - 12 mm) and small (< 9 mm) *A. pisonii* size classes. The average isotopic space occupied by small immature individuals (solid triangles, dashed outline, *n* = 26) is distinct from that of large individuals (solid circles, solid outline, *n* = 27). Medium individuals (open circles, *n* = 27) exhibit isotopic characteristics of both small and large individuals.

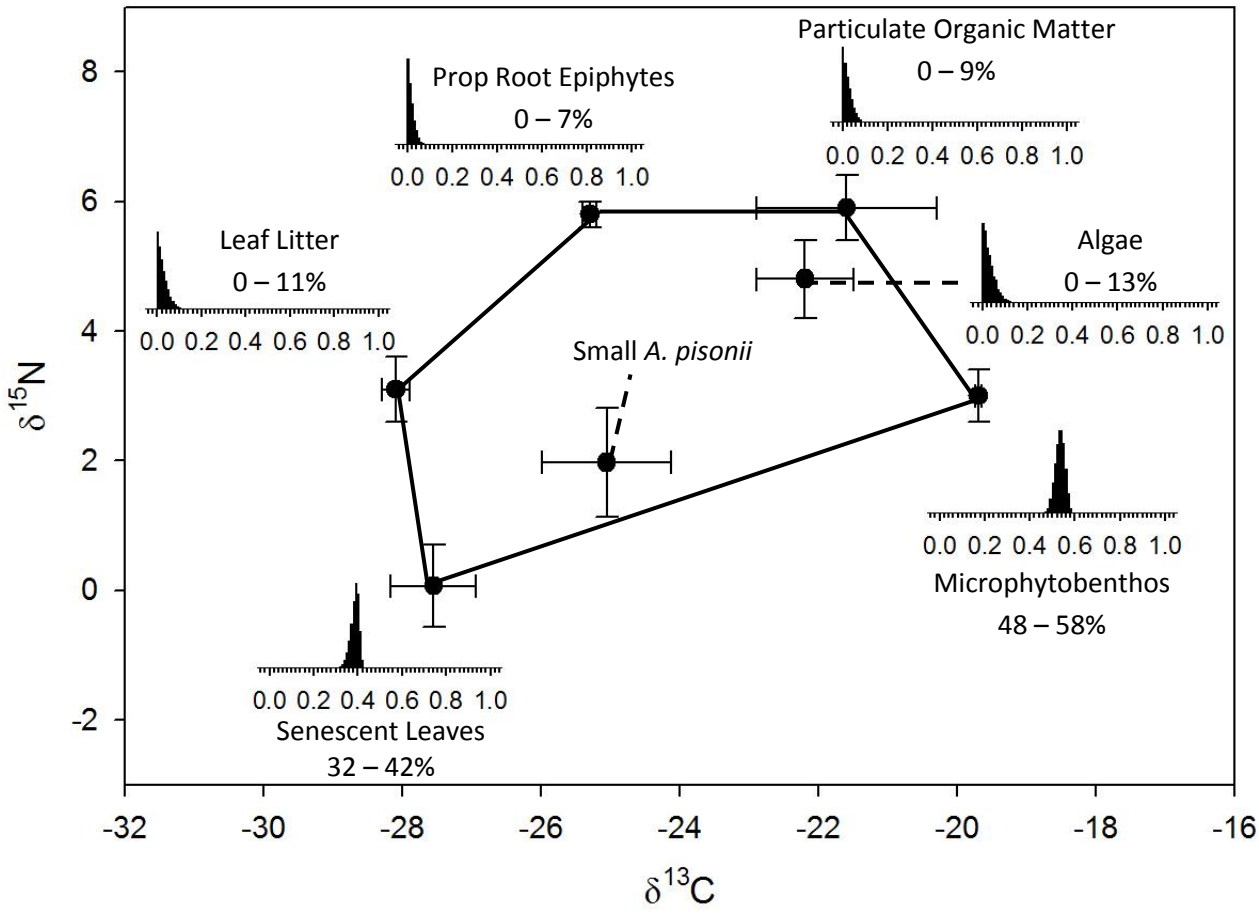

Figure 3 Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ signatures of 6 food sources for small *A. pisonii*. Histograms show the distribution of feasible contributions from each source for the small *A. pisonii* diet generated in IsoSource using $\delta^{13}C$ and $\delta^{15}N$ values displayed in Table 5. Dashed lines indicate which histograms correspond to their appropriate data points when not positioned with obvious proximity. Values shown in boxes are 1-99 percentile ranges for these components.

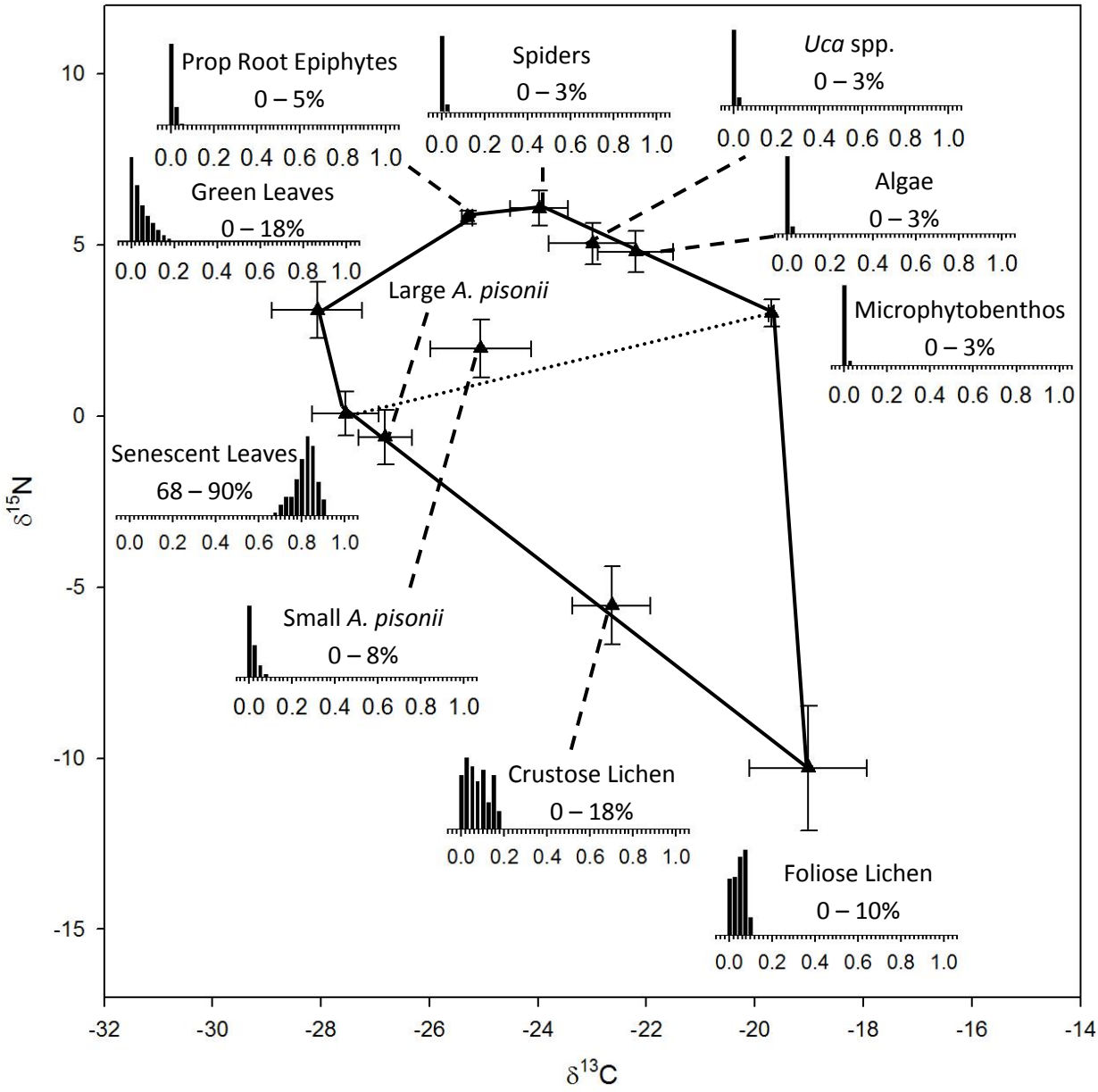

Figure 4 Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ signatures of 10 potential food sources for large *A. pisonii*. Histograms show the distribution of feasible contributions from each source for the large *A. pisonii* diet generated in IsoSource using $\delta^{13}C$ and $\delta^{15}N$ values displayed in Table 5. Dashed lines indicate which histograms correspond to their appropriate data points when not positioned with obvious proximity. The dotted line shows that large *A. pisonii* fall outside the mixing polygon if lichens are not included as potential food sources. Values shown in boxes are 1-99 percentile ranges for these components.

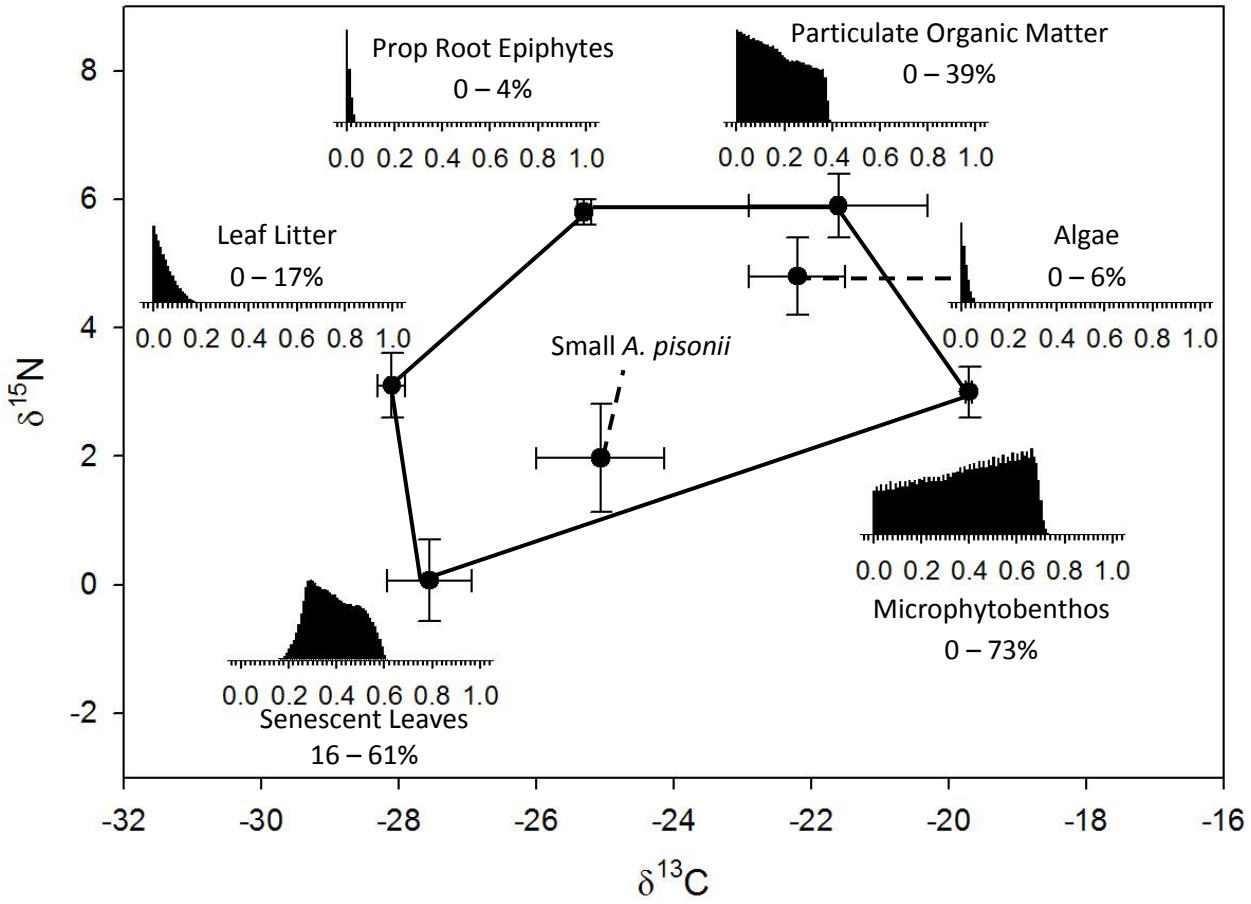

Figure 5 Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ signatures of 6 potential food sources for small *A. pisonii* using the mass balance method. Histograms show the distribution of feasible contributions from each source for the small *A. pisonii* diet generated in IsoSource using $\delta^{13}C_{\Delta}$ and $\delta^{15}N_{\Delta}$ values displayed in Table 5. Dashed lines indicate which histograms correspond to their appropriate data points when not positioned with obvious proximity. Values shown in boxes are 1-99 percentile ranges for these components.

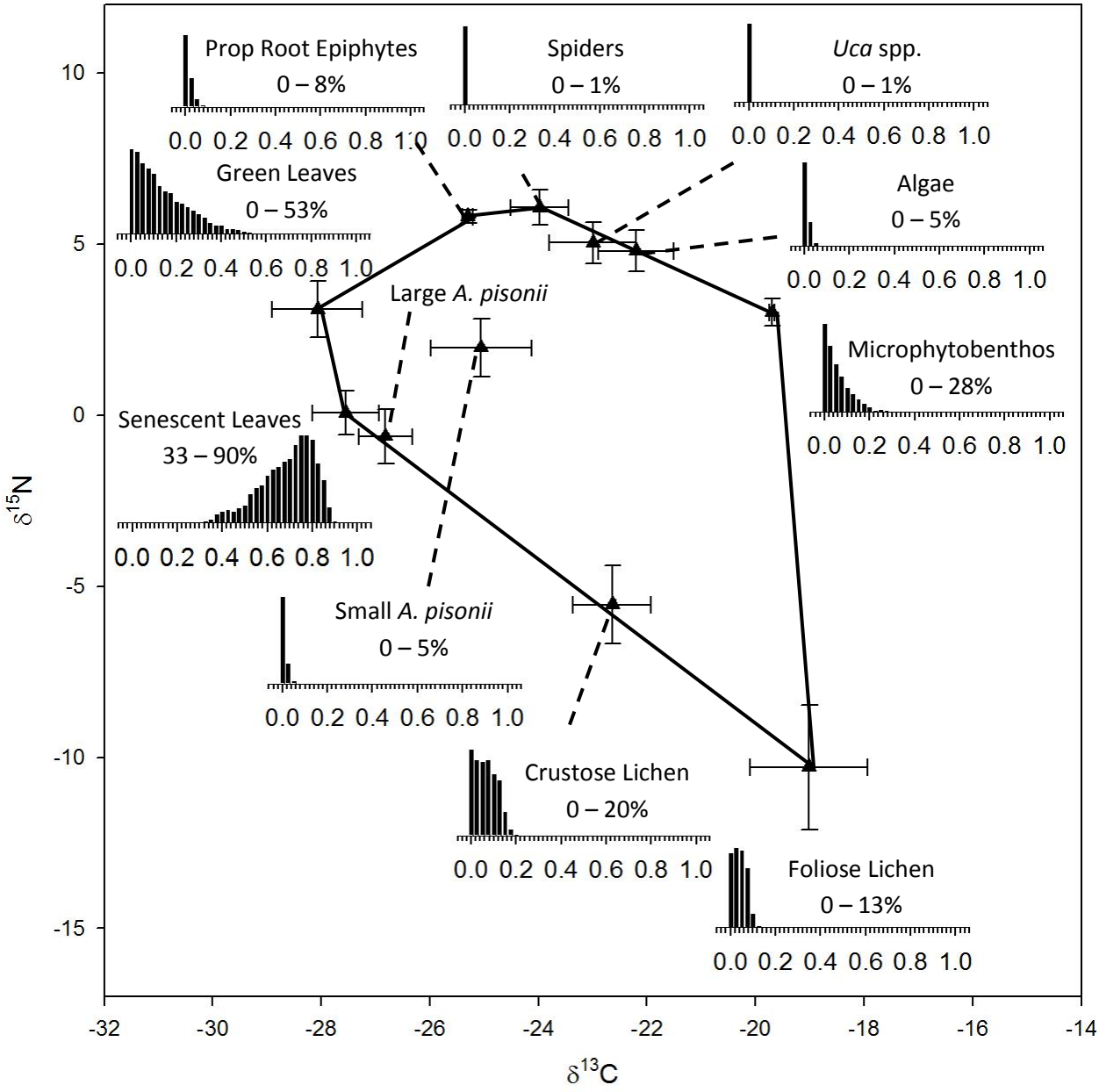

Figure 6 Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ signatures of 10 potential food sources for large *A. pisonii* using the mass balance method. Histograms show the distribution of feasible contributions from each source for the large *A. pisonii* diet generated in IsoSource using $\delta^{13}C_{\Delta}$ and $\delta^{15}N_{\Delta}$ values displayed in Table 5. Dashed lines indicate which histograms correspond to their appropriate data points when not positioned with obvious proximity. Values shown in boxes are 1-99 percentile ranges for these components.