MATERIALS AND METHODS

Experimental design and diet preparation

We used a modified geometric analysis design (Simpson & Raubenheimer 1995) that orthogonally varied both diet quality (proportion of plant to animal tissue in the diet) and diet quantity (proportion of crab body weight offered). This design incorporated 5 levels of diet quality with different amounts of plant to animal material (100:0, 75:25, 50:50, 25:75, and 0:100% by weight) and 4 diet quantities (2, 3, 5, and 11% of crab dry body weight per day). Crabs generally consume between 1.5 and 3% of their dry body weight per day (e.g. Olafsson et al. 2002), so these diet quantities spanned a range from limited to abundant food conditions. Each combination of diet quality and quantity was replicated twice for a total sample size of 40 individuals. We were interested in the actual diet consumed, which cannot be controlled or replicated (i.e. individual diet choice would cause 10 crabs with identical food choices to consume 10 unique diets over the 6 wk experiment, leading to 10 unique diet treatments rather than 10 replicates of the same treatment). However, the diet offered can be controlled and replicated, and can be used to maximize diet variation. We therefore elected to use a re gression-type experimental design to maximize diet variation with our experimental treatments rather than increase replication of a more limited range of offered diets. The analyses described hereafter also reflect this strategy.

Red mangrove leaves, which account for the majority of the natural diet of Aratus pisonii (Erickson et al. 2003), served as the plant material in our study. House crickets Achetus domesticus were offered as animal tissue. A. pisonii feed on insects, including crickets, in the field (Beever et al. 1979) and prefer A. domesticus to fresh plant and algal material in laboratory feeding assays (Erickson et al. 2008). In order to ensure that crabs consumed the desired proportion of plant to animal tissue, the 2 food types were mixed and embedded in an agar substrate in the 5 proportions of plant to animal material described previously. Freshly picked red mangrove leaves were collected from Avalon State Park in St. Lucie County, Florida, in June 2012. Live A. domesticus were ob - tained from commercial sources and euthanized by freezing. Both food types were then placed in a drying oven at 68°C until they reached a constant dry weight. They were ground separately into a fine powder using a Coffee-mate® coffee grinder and mixed to produce the 5 ratios of plant to animal tissue described above. They were then incorporated into an artificial agar diet by combining 2.8% agar, 4.6% food powder mixture, and 92.6% boiling deionised water by weight. This mix ture was poured into 2.05 cm3 plastic molds, which were placed in a drying oven at 68°C until they reached a constant weight and formed dried agar cubes.

Experimental setup and maintenance

Adult female Aratus pisonii (carapace width, CW:17 to 24 mm) were collected from Avalon State Park in June 2012 and transported to the wet lab at the Smithsonian Marine Station at Fort Pierce, Florida. Each

crab was placed into an individual plastic aquarium (22.8 cm length × 15.2 cm width × 16.5 cm height) with a 400 ml glass finger bowl containing approximately 200 to 300 ml of filtered seawater (salinity ~31 ppt). They were maintained for 3 to 4 d without food to standardize hunger levels and clear their guts prior to the start of the experiment. Total dry weight of experimental crabs was estimated based on initial CW at the time of collection using a previously established relationship between CW and dry weight (adj $R^2 = 0.822$, $p < 0.001$, $n = 20$, M. Riley unpubl. data). This estimated dry weight was subsequently used to determine the precise amount of food to offer each crab. The experiment consisted of a 3 d diet cycle that was repeated 14 times for a total experimental duration of 6 wk. Diet cubes of the appropriate diet quality were shaved down to within ±0.005 g of the assigned weight for each crab, and then offered to crabs for 48 h. After that time, any remaining uneaten food was removed and seawater in finger bowls was refilled to its original volume to account for evaporative water loss. Crabs were then maintained without food for 24 h to allow for digestion and fecal production, after which the contents of each experimental chamber were poured into a flask attached to a vacuum pump and rinsed through Whatman® qualitative filter paper (Grade 1, 11 µm particle size retention) to collect feces. Aratus pisonii were then offered fresh diet cubes, and a new feeding cycle was initiated. Uneaten food was dried at 68°C until it reached a constant dry weight to remove any humidity or water that the cubes may have ab - sorbed from the finger bowls. Filter papers with collected feces were also dried at 68°C and weighed to quantify fecal production.

Sample processing

At the conclusion of the experiment, crabs were frozen at -80°C and dissected within 3 mo. In order to determine the relative physiological condition of individual crabs, the main energy storage organ, the hepatopancreas (O'Connor & Gilbert 1968, Parvathy 1971), was removed from the crab body. Similarly, the ovaries were isolated from the remainder of the crab body in order to determine reproductive in - vestment of individual crabs. The hepatopancreas, ovaries, and remainder of the body were dried to a constant weight at 68°C. Dry weight of the hepato - pancreas as a proportion of the total dry body weight (i.e. the hepatosomatic index, HSI) was used as a mass-specific metric of investment in the main energy storage organ. Similarly, the dry weight of the ovaries as a proportion of the total dry body weight (i.e. the gonadosomatic index, GSI) was used as a mass-specific metric of reproductive investment.

Statistical analyses

Mean food consumption (g) was during a 3 d feeding cycle calculated by averaging the dry weight of the food consumed during each of the 14 feeding cycles (determined using the difference between initial and final food weight in each feeding period). To eliminate any potential influence of starvation on diet consumption (see Cronin & Hay 1996) during the first feeding cycle, consumption values from the first feeding cycle were not included in this average. To standardize mean food consumption by crab body weight, the amount of food consumed during each feeding cycle was divided by the dry weight of the crab's body (g). Additionally, standardized mean food consumption was divided by 3 (due to the 3 d feeding cycle) to calculate standardized mean daily food consumption. Although feeding was limited to 2 d within the feeding cycle, we calculated daily consumption based on the entire length of the 3 d

feeding cycle. Standardized mean daily plant and animal consumption were calculated by multiplying the standardized mean daily food consumption by the percentage of the diet (0, 25, 50, 75, or 100%) that was plant or animal material, respectively. Assimilation efficiency was calculated using the amount of feces produced during each feeding period in the formula: [total weight of food consumed (g) - total weight of feces produced (g)] / total weight of food consumed (g). The resulting values were averaged and converted to percentages to calculate mean assimilation efficiency (% of food consumed that was assimilated) by individual crabs. Four of the experimental crabs consistently placed food pellets into the finger bowls of water, breaking down the food pellets and making it difficult to distinguish uneaten food from disassociated feces. To avoid biasing our results by inadvertently mistaking food and feces, we eliminated these 4 crabs from the analyses. The influence of diet quality and diet quantity treatments on standardized mean daily food consumption was determined using a general linear model with percentage of plant material in the diet and percentage of dry crab body weight offered per day as predictor variables. The influence of diet on assimilation efficiency was determined using a general linear model with mean daily plant consumption and mean daily animal consumption as continuous predictor variables.

The influence of diet on survivorship was determined using a logistic regression; occurrence of death was the response variable, and mean daily plant consumption and mean daily animal consumption were used as predictor variables. Although all crabs were included in our analyses of survivorship (n = 36), our analyses of physiological condition and reproductive effort were restricted to crabs that survived the duration of the experiment (n = 29). The influence of diet on physiological condition (approximated by the HSI) was determined using a general linear model with mean daily plant consumption and mean daily animal consumption as continuous predictor variables. Due to the relatively high molting frequency of this species (every 53 d, Warner 1967), molting was also included as a categorical factor in this linear model. Similarly, the influence of diet on reproductive effort (approximated by the GSI) was determined using a general linear model with mean daily plant consumption and mean daily animal consumption as continuous predictor variables. Zoeal release was included as a categorical factor in the model; due to year-round reproductive output in this species (Warner 1967), 17 crabs were gravid at the start of the experiment and released zoeae 2 to 26 d after the experiment began (mean ± SD = 10 ± 6 d, n = 17). Two models were developed and are presented for the GSI: one that included all surviving crabs (n = 29), and one in which the single crab in the experiment with late-stage developing ovaries was re moved (n = 28). This particular crab was maintained on a mixed diet of 25:75% plant to animal material and offered 8% of its body weight per day, and as a result of the advanced stage of its ovary devel opment, its GSI was very high relative to the re mainder of the experimental crabs (i.e. it was in late-stage vitellogenesis at the conclusion of the experiment).

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