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Dietary composition and nutritional outcomes in two marsupials, *Sminthopsis macroura* and *S. crassicaudata*

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Little is known about the specific dietary preferences of many marsupials. We undertook digestion studies in 2 species of insectivorous marsupials, the stripe-faced dunnart (*Sminthopsis macroura*) and the fat-tailed dunnart (*Sminthopsis crassicaudata*), because these are 2 species that are regularly kept in captivity, although nothing is known about their nutritional requirements in the wild. Morphology and dimensions of the gastrointestinal tract of both species also were assessed. The test diets included 2 laboratory-type diets: cat formulation and Wombaroo small carnivore mix; and natural insect diets: adult crickets (*Acheta domesticus*), Australian wood cockroaches (*Panesthia australis*), and mealworm larvae (*Tenebrio molitor*). Composition of the test diets on a dry-matter basis varied considerably; ranges included gross energy 16–27 kJ/g, crude protein 38–64%, lipids 9–51%, calcium (Ca) 340–17,800 mg/kg, and phosphorus 6,600–16,000 mg/kg. Depending on the diet, the digestible energy intake ranged from 359 to 816 kJ kg^{−0.75} day^{−1} for stripe-faced dunnarts and digestible energy intake ranged from 542 to 990 kJ kg^{−0.75} day^{−1} for fat-tailed dunnarts. No single diet was appropriate if fed alone, notably the insect diets, which required Ca supplementation. The morphology of the gastrointestinal tracts of both species was simple and consisted of a unilocular stomach and relatively uniform intestine. The morphometrics of the gastrointestinal tracts of fat-tailed dunnarts were proportionally larger than for stripe-faced dunnarts. Fat-tailed dunnarts also needed to consume more nutrients per unit of body mass for maintenance in captivity compared to stripe-faced dunnarts.

Key words: dasyurid, insectivore, maintenance energy requirement, mineral assimilation, nutrition

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Australia is a geomorphologically ancient continent, and it is well recognized that the soil is nutrient impoverished (Morton et al. 2011). Despite this nutrient deficiency, the continent is home to a wide range of plants, vertebrates, and a significant diversity of invertebrates (Morton et al. 2011; Mucina and Wardell-Johnson 2011). The bulk of the world's marsupials live in Australia, and there are 58 species of carnivorous marsupials, from the family Dasyuridae, in Australia (and a further 17 species in Papua New Guinea), and almost half of the Dasyuridae species live in the arid zone (Geiser and Pavey 2007). The stripe-faced dunnart (*Sminthopsis macroura*) is a small (20–27 g) insectivorous marsupial that inhabits arid zones of central and northern Australia (Morton and Dickman 2008b). In contrast, the fat-tailed dunnart (*Sminthopsis crassicaudata*) is a smaller (13–20 g) insectivorous marsupial, but inhabits more diverse habitats, including arid, semiarid, and mesic environments in southern and central Australia (Morton

1978a; Morton and Dickman 2008a). Both species prey on arthropods, small mammals, and small reptiles, although little is known about their nutritional requirements. Captive diets often consist of cat formulations, dry dog food, and other commercially available pet foods (Morton et al. 1983; Selwood and Cui 2006; Morton and Dickman 2008a; Munn et al. 2010). Both species store fat in their tails and are capable of using torpor, which is a temporary, controlled reduction in metabolic rate and associated drop in body temperature (Morton 1978b; Körtner and Geiser 2009; Warnecke and Geiser 2010).

Both species of dunnart are listed as “least concern” on the IUCN Red List of Threatened Species (Burbidge et al. 2011; Woinarski and Dickman 2011). The stripe-faced dunnart,

however, is listed as “vulnerable” in New South Wales under the Threatened Species Conservation Act 1995. The status of the stripe-faced dunnart is considered vulnerable because of localized population declines within the state of New South Wales (Frank and Soderquist 2005). The stripe-faced and fat-tailed dunnarts provide suitable models for other endangered dunnart species because of their overlap in body size, habitat use, and insectivory. The stripe-faced dunnart and fat-tailed dunnart are readily maintained in captive environments, and are frequently held for display, research, and education purposes. Knowledge of nutritional requirements of common dunnart species contributes to maintaining threatened species more appropriately.

Nutrition has been studied more extensively in the fat-tailed dunnart than in the stripe-faced dunnart. Previous research on the fat-tailed dunnart has shown food intake is influenced by opioid peptides, leptin, sex, photoperiod, and macronutrient composition of food (Hope et al. 1997a, 1997b, 1999; Ng et al. 1999). The role of food availability in torpor and activity rhythms, and nutrient uptake by embryos has been studied in the stripe-faced dunnart (Coleman et al. 1989; Gardner et al. 1996; Kennedy et al. 1996; Song and Geiser 1997). The previous work has been based on studies using commercial food products. To our knowledge no studies have previously looked at nutritional composition of diets in relation to nutritional requirements for maintenance of these species in captivity.

Uptake and digestion of nutrients is related to choice of diet, and gastrointestinal tract morphology and histology. Generally, dasyurids have a simple gastrointestinal tract that lacks a caecum (Hume 1999). Gastrointestinal morphology has been described in larger dasyurid species such as spotted-tailed quoll (*Dasyurus maculatus*), eastern quoll (*D. viverrinus*), kowari (*Dasyuroides byrnei*), brush-tailed phascogale (*Phascogale tapoatafa*), kultarr (*Antechinomys laniger*), and fat-tailed false antechinus (*Pseudantechinus macdonnellensis*—Mitchell 1905; Beddard 1908; Stannard and Old 2013). Previously, descriptions of the dunnart gastrointestinal tract have been limited to gross morphology and gut-associated lymphoid tissues (Mitchell 1916; Schultz 1976; Old et al. 2003, 2004). Gross morphology and dimensions were described for the largest species in the *Sminthopsis* genus, the Julia Creek dunnart (*Sminthopsis douglasi*—Hume et al. 2000). All these studies, however, lack detailed morphological measurements of the gastrointestinal tract.

Because of the paucity of knowledge about gut morphology and appropriate diet intake in dunnarts, we aimed to determine the nutrient composition of a current captive diet, commercially available diet, and live food items that are similar to that available in the wild for both the fat-tailed and stripe-faced dunnarts; determine the apparent retention of nutrients for each of the diet items; and determine morphology and dimensions of the gastrointestinal tract of both species.

MATERIALS AND METHODS

Animals.—Eleven adult male stripe-faced dunnarts and 8 adult male fat-tailed dunnarts were used for this study. Males

were used because a previous study demonstrated that they robustly adapt physiologically and behaviorally to variable amounts of food (Munn et al. 2010). The animals were from a captive colony based at the University of Sydney, Sydney, New South Wales, Australia. The *S. macroura* originated from a colony at LaTrobe University and the *S. crassicaudata* originated from wild-caught animals from southern Queensland. Each animal was housed individually in a plastic enclosure (20 × 45 × 25 cm) with a mesh lid. Animals were provided with a wood-shaving substrate, cardboard nest box, and a toy for behavioral enrichment. Animals were held under photocycles of 12L:12D and room temperature was maintained at 22°C ± 5°C. Water was available ad libitum, and fresh food, in excess of daily requirements, was provided in the late afternoon, before the animal became active. This study was undertaken following guidelines of the American Society of Mammalogists (Sikes et al. 2011) and with the approval of the University of Western Sydney’s Animal Care and Ethics Committee (A7982) and the University of Sydney’s Animal Ethics Committee (K22/11–2010/3/5444).

Digestibility trials: diets.—On day 1 animals were moved into the experiment room and allowed to adjust to the room for 5 days prior to starting the nutrition trials. The food items for each of the 5 trials were given in the order as follows: 1st, cat formulation—lamb and kidney loaf style (Whiskas; Mars Petcare, Wodonga, Victoria, Australia); 2nd, adult crickets (*Acheta domesticus*); 3rd, Australian wood cockroaches (*Panesthia australis*); 4th, mealworm larvae (*Tenebrio molitor*); and 5th, Wombaroo marsupial formulation (Wombaroo Food Products, Glen Osmond, South Australia, Australia). Wombaroo marsupial formulation is a commercial diet for marsupial formulation (Wombaroo Food Products 2011). A 2-week rest period was provided between trials, and during this time animals were maintained on their usual cat formulation diet (Whiskas, lamb and kidney loaf style). Each of these dietary items was chosen for the trials because they are commonly used in captive diets for dunnart species.

Digestibility trials: procedure.—The digestibility trials investigated 5 dietary protocols. The order in which the diets were fed was chosen as usual food first, followed by insects, which were readily accepted, whereas marsupial formulation was left to last, because some dasyurids, such as captive red-tailed phascogales (*Phascogale calura*) have refused to eat this diet (Stannard and Old 2011). During the digestibility trials the animals were fed 1 food type, in excess of daily requirements, at 1500 h daily for a total of 10 days. The first 5 days of the trial were an adjustment period from the previous dietary regime, and during the last 5 days all fecal matter, uneaten food, and a sample of food were collected daily, weighed (± 0.1 g), and stored at –20°C until analysis. During the trials animals were provided with a paper substrate that absorbed urine and allowed for the easy collection of feces directly from the paper. Animals were weighed (± 0.1 g) and tail width (mm) was recorded at the start and end of each of the food trials. Tail width was taken at the base of the tail using vernier

TABLE 1.—Composition of food items on a dry-matter basis ($n = 2$; all data are mean ± 1 SD). Superscript capital letters within a row denote significant differences ($P < 0.01$) between diets.

	Crickets (<i>Acheta domesticus</i>)	Cockroaches (<i>Panesthia australis</i>)	Mealworm larvae (<i>Tenebrio molitor</i>)	Cat formulation	Marsupial formulation
Dry matter (%) ^a	28.9 \pm 1.0	33.9 \pm 0.4	42.6 \pm 0.5	14.4 \pm 0.4 ^A	54.1 \pm 0.9
Gross energy (kJ/g)	15.8 \pm 0.4	21.5 \pm 0.6	24.5 \pm 0.4	27.1 \pm 0.6 ^A	20.5 \pm 0.6
Crude protein (%)	63.5 \pm 0.9	59.1 \pm 0.5	45.9 \pm 0.8	38.7 \pm 0.7 ^A	37.7 \pm 0.2 ^A
Lipids (%)	9.3 \pm 0.1	22.3 \pm 0.3	20.0 \pm 0.1	51.3 \pm 1.0 ^A	13.3 \pm 0
Calcium (mg/kg)	2,000	2,000	340 ^A	8,200 ^A	17,800 ^A
Phosphorus (mg/kg)	11,000	8,000 ^A	6,600 ^A	12,700	16,000
Iron (mg/kg)	56	81	61	220 ^A	340 ^A
Sodium (mg/kg)	5,200	4,900	1,480 ^A	8,400	9,500
Potassium (mg/kg)	14,400	11,400	8,300 ^A	14,300	11,000
Magnesium (mg/kg)	1,630	1,400	1,800	640 ^A	1,590

^a Based on fresh mass.

calipers. Tail width was recorded as an indicator of fat storage and general health of the animal.

Dunnarts were weighed twice during the 2-week rest periods to determine if they had returned to their pretrial body mass. On completion of the nutrition trials 8 stripe-faced dunnarts and 8 fat-tailed dunnarts were euthanized, organs were removed, and gastrointestinal lengths were measured (± 0.1 mm). On the day prior to euthanasia animals were fed cat formulation. Because euthanasia of animals occurred before their usual feeding time, they were not fed on the day of euthanasia, and all stomachs were empty prior to measurement. Measurements of gastrointestinal tracts from dunnarts (4 of each species) also were obtained opportunistically from animals euthanized for another independent experiment where the diet of the dunnarts was the same as their usual cat formulation diet, and therefore should have no impact on gastrointestinal dimensions. The euthanized individuals were healthy.

Sample analysis.—Food samples, fecal matter and uneaten food were oven dried and analyzed for protein, energy, lipid, and mineral (iron [Fe], calcium [Ca], phosphorous [P], sodium [Na], potassium [K], and magnesium [Mg]) composition. Crude protein was determined using the Kjeldahl method, calculated as nitrogen $\times 6.25$ (Willits et al. 1949; Jones 1991). Gross energy was determined using an oxygen bomb calorimeter (Parr 6200; Parr Instrument Company, Moline, Illinois) with a benzoic acid standard (Cowan et al. 1974). Total lipids were extracted with a chloroform:methanol (1:1 volume:volume) mixture (Folch et al. 1957). Mineral analysis was conducted by Waite Analytical Services, Glen Osmond, South Australia, Australia. Samples were analyzed in duplicate for each analysis and a 5% precision value was observed. Digestible energy intake data were expressed on the basis of metabolic body mass, body mass as kilograms to the power of 0.75 ($M^{0.75}$), to compare species of differing body mass. The scale of 0.75 has been used previously to scale energy intake data for marsupials (Green and Eberhard 1979; Moyle et al. 1995; Hume 1999; Gibson and Hume 2000).

Statistical analysis.—A 1-way analysis of variance (ANOVA) and Tukey's post hoc tests were used to compare nutrient composition of the 5 diets. A repeated-measures ANOVA and least significant difference post hoc tests were

used to examine changes in mass and tail widths within each species. One-way analysis of covariance was used to compare the relationship between nutrient intake and output of each diet for each species. Bonferroni post hoc tests were used to determine differences between retention of nutrients for each diet consumed by each species. Unpaired Student's *t*-tests were used to compare retention values (intake minus excretion) between stripe-faced and fat-tailed dunnarts on the same diet and at specific time points. All statistical analyses were performed using the SPSS statistical analysis package (IBM Corp. 2012).

RESULTS

Dietary analysis.—Composition of the diets varied considerably (Table 1); cat formulation had the lowest dry matter ($F_{4,5} = 1,436.9$; $P = 0.001$) and highest energy ($F_{4,5} = 138.4$; $P = 0.001$) of the diets provided. The insect diets all had significantly higher crude protein than both the cat formulation and marsupial formulation diets ($F_{4,5} = 1,003.9$; $P = 0.001$; cat formulation $P < 0.01$; marsupial formulation $P < 0.01$), and although lipid content varied between the insect diets, all had significantly less lipid content than the cat formulation ($F_{4,5} = 2,864.5$; $P = 0.001$). Mineral and salt ion content varied considerably between diets, although these were more consistent for the insect diets than for the commercial diets (Table 1). Marsupial formulation has the highest levels of dry matter, Ca, P, Fe, and Na. Crickets had the lowest energy and highest protein composition of the diets provided.

Body condition.—There was no significant difference in body mass for the stripe-faced dunnart between the pretrial and start measurements for each trial (Fig. 1a). The body mass of the stripe-faced dunnart varied across the course of the feeding trials ($F_{16,10} = 8.9$; $P < 0.01$; Fig. 1a). Tail widths of the stripe-faced dunnart also varied across the course of the feeding trials. There was a significant increase in tail width during the mealworm trial ($F_{16,10} = 8.9$; $P < 0.01$; Fig. 1b).

The body masses of fat-tailed dunnarts varied across the course of the feeding trials (Fig. 2a) and increased significantly ($F_{16,8} = 6.5$; $P = 0.003$) on the mealworm trial ($F_{16,8} = 6.5$; $P < 0.05$) and decreased significantly on the cat formulation trial

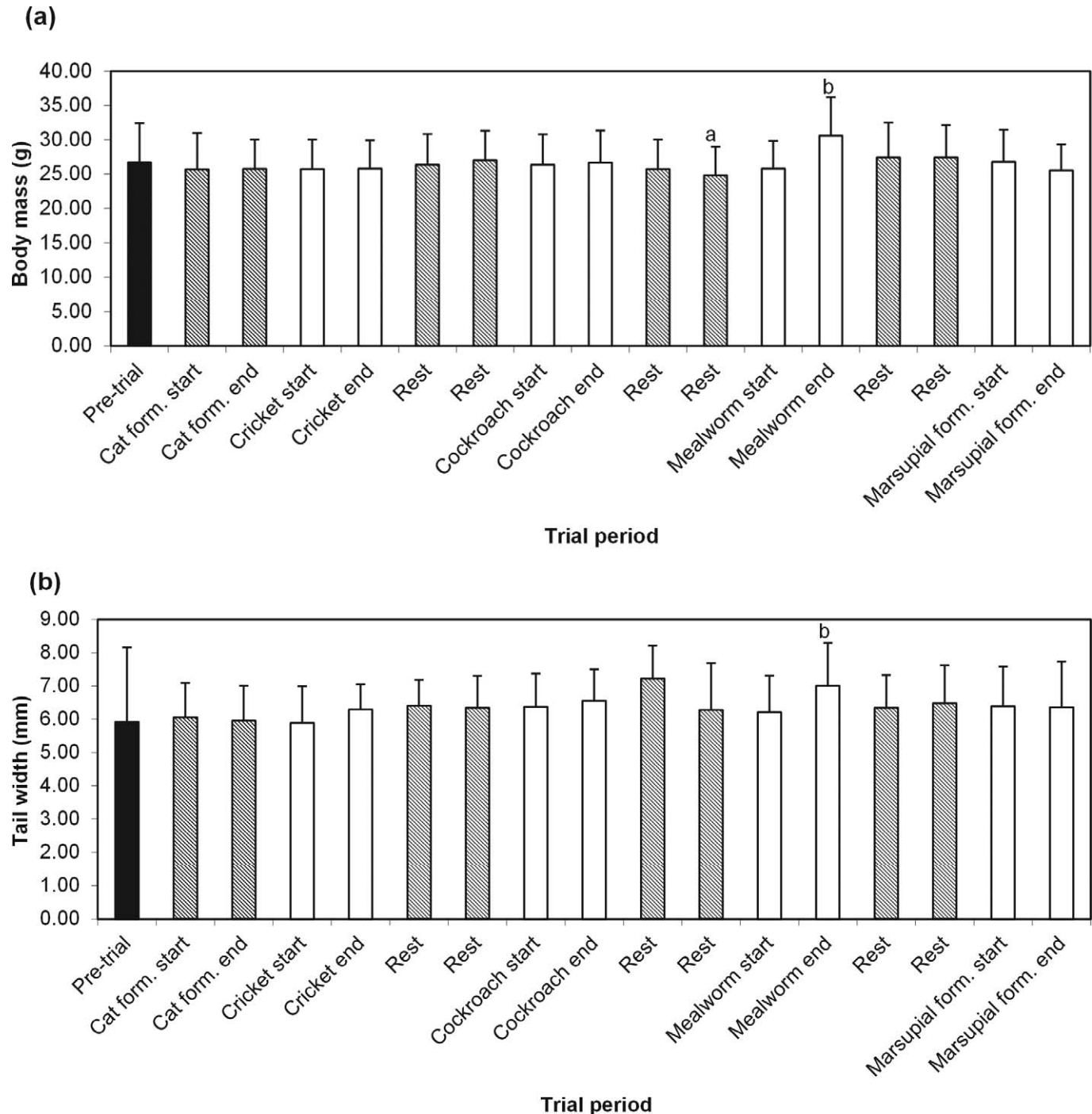


FIG. 1.—Changes in the a) body mass and b) tail width of *Sminthopsis macroura* over the course of the nutrition trials. During rest periods animals were fed cat formulation. All data are means ± 1 SD; “a” indicates significant change in body mass or tail width from pretrial value, $P < 0.05$; “b” indicates significant change in body mass or tail width during the course of a feeding trial, $P < 0.05$.

($F_{16,8} = 6.5$; $P < 0.01$). A significant ($F_{16,8} = 6.5$; $P < 0.05$) loss of tail width occurred during the cockroach trial. During the mealworm trial a significant gain ($F_{16,8} = 6.5$; $P < 0.05$) occurred in tail width of fat-tailed dunnarts (Fig. 2b).

Dietary trials.—Stripe-faced dunnarts consumed the equivalent of 17–50% of their body mass in wet matter per day and 9–15% on a dry-matter basis, and percentage consumption differed significantly between diets ($F_{4,11} =$

212.9; $P < 0.01$; Supporting Information S1, DOI: 10.1644/13-MAMM-A-071.S1). Stripe-faced dunnarts consumed a significantly greater percentage of food with respect to body mass when presented with the cat formulation diet ($P < 0.01$). Fat-tailed dunnarts consumed the equivalent of 27–81% of their body mass in wet matter per day and 5–9% on a dry-matter basis, and percentage consumption differed significantly between diets ($F_{4,8} = 116.5$; $P < 0.01$), with a significantly

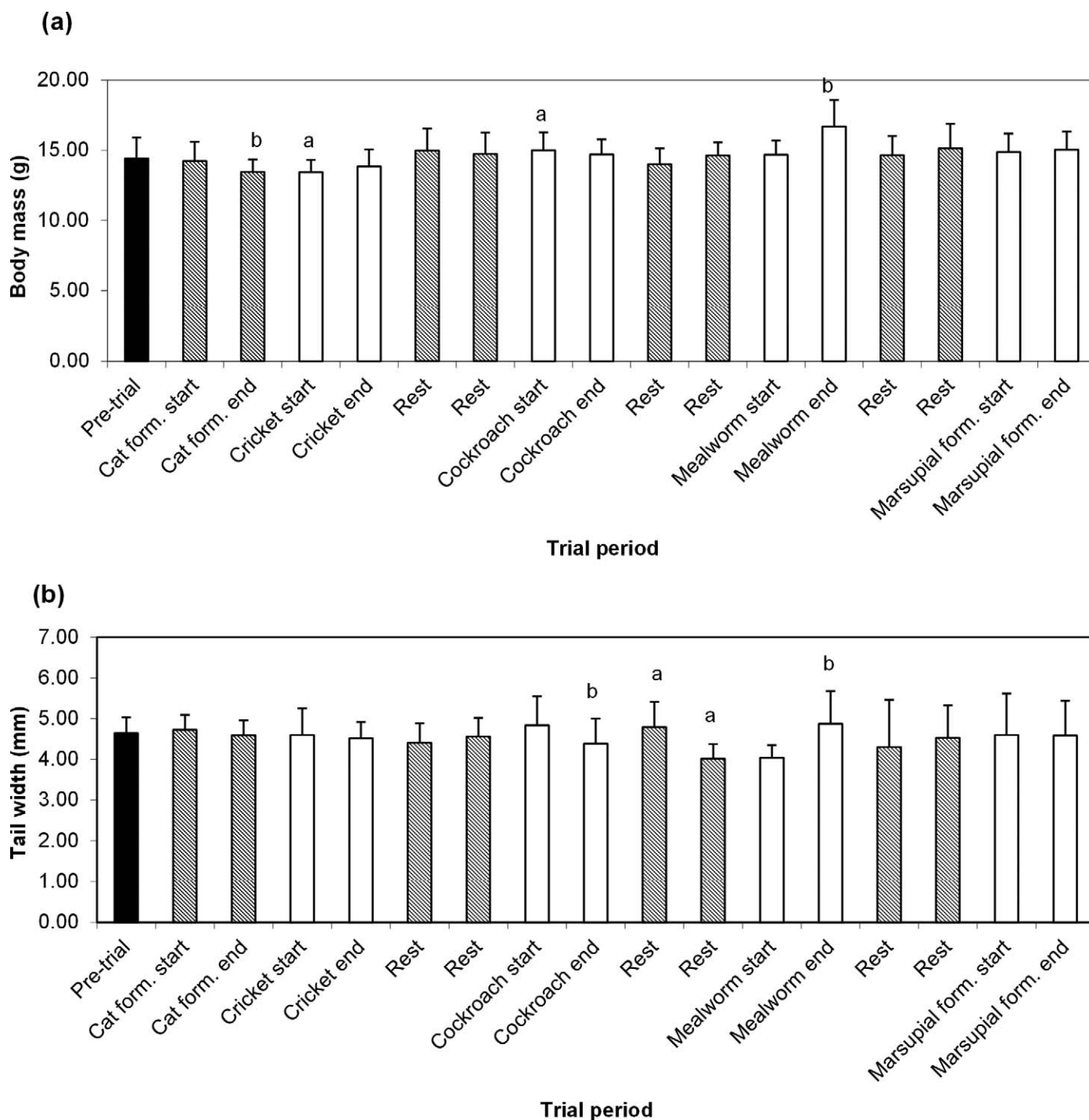


FIG. 2.—Changes in the a) body mass and b) tail width of *Sminthopsis crassicaudata* over the course of the nutrition trials. During rest periods animals were fed cat formulation. All data are means \pm 1 SD; “a” indicates significant change in body mass or tail width from pretrial value, $P < 0.05$; “b” indicates significant change in body mass or tail width during the course of a feeding trial, $P < 0.05$.

lower percentage ($P < 0.01$) on the marsupial formulation diet and significantly higher percentage on the cat formulation diet ($P < 0.01$; Supporting Information S2, DOI: 10.1644/13-MAMM-A-071.S2).

Homogeneity-of-regression analysis found a significant linear relationship between intake and excretion for dry matter ($F_{4,55} = 7.0$; $P < 0.01$), protein ($F_{4,55} = 5.1$; $P < 0.01$), gross

energy ($F_{4,55} = 9.0$; $P < 0.01$), and lipids ($F_{4,55} = 3.1$; $P = 0.03$) in stripe-faced dunnarts (Fig. 3). Mineral (Fe, Ca, Mg, Na, K, and P) intake, however, did not have a linear relationship with output (Fig. 3). The relationship between intake and output of dry matter ($F_{4,40} = 7.5$; $P = 0.02$), protein ($F_{4,40} = 5.6$; $P < 0.01$), energy ($F_{4,40} = 6.2$; $P < 0.01$), and Fe ($F_{4,40} = 3.8$; $P = 0.01$) was linear for fat-tailed dunnarts (Fig.

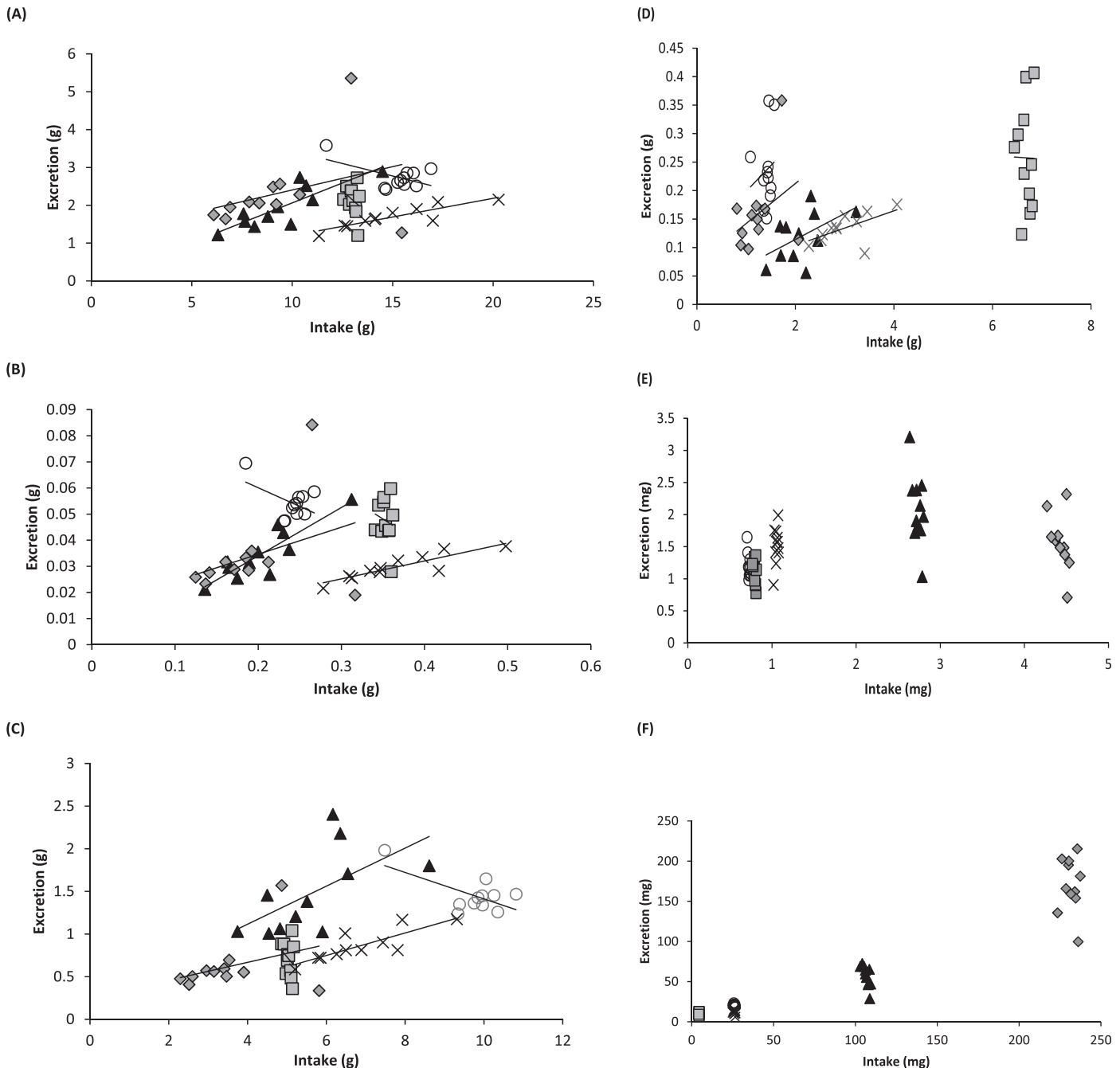


FIG. 3.—*Sminthopsis macroura* intake versus output data for each nutrient and mineral: A) dry matter; B) energy; C) protein; D) lipids; E) iron; F) calcium.

4). Lipid and mineral (Ca, Mg, Na, K, and P) intake and excretion had a nonlinear relationship in fat-tailed dunnarts (Fig. 4). Intake and excretion varied between diets for the same nutrient for both species of dunnart. Fat-tailed dunnarts consumed similar quantities of dry matter for each diet ($F_{4,40} = 2.3$; $P = 0.08$), retention also was similar across the diets with the exception of cat formulation, on which the fat-tailed dunnarts retained significantly ($F_{4,40} = 20.5$; $P < 0.01$) less dry matter compared with the other 4 diets.

Stripe-faced and fat-tailed dunnarts, when maintained on the same diet, have similar retention of nutrients. The slope of the

linear relationships of macronutrient (dry matter, protein, energy, and lipids) uptake was the same for both species; except for gross energy of the cricket diet, stripe-faced dunnarts' slope was 1.3 whereas fat-tailed dunnarts' slope was 1.1. Stripe-faced dunnarts had significantly higher retention of dry matter than fat-tailed dunnarts when on the cricket ($t = 4.6$; $P < 0.01$) and cat formulation ($t = 39.4$; $P < 0.01$) diets. Similarly, stripe-faced dunnarts had significantly higher retention of protein on the cricket ($t = 5.1$; $P < 0.01$) and cat formulation ($t = 4.3$; $P < 0.01$) diets. However, retention of protein on the marsupial formulation ($t = -3.7$; P

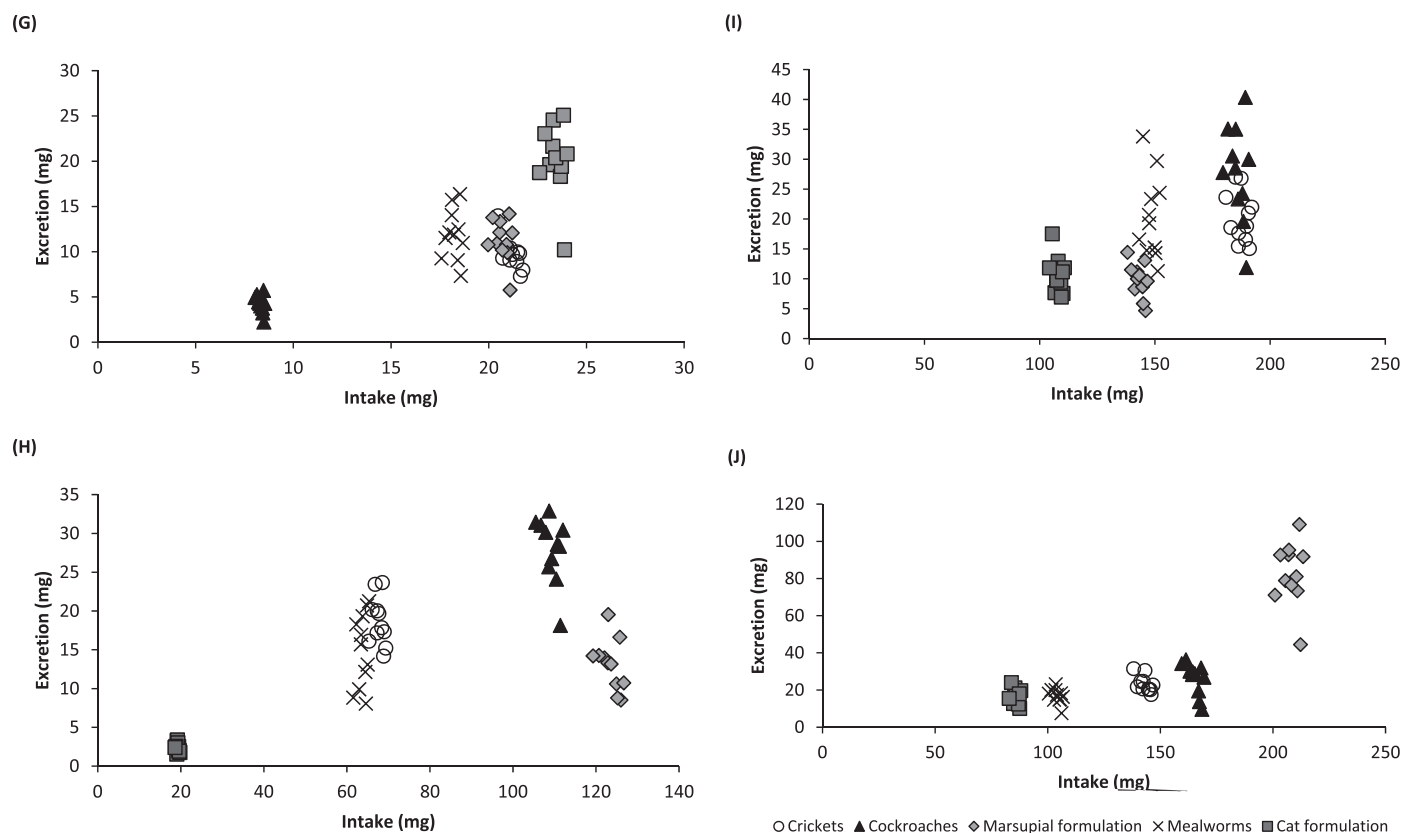


FIG. 3.—Continued. G) magnesium; H) sodium; I) potassium; and J) phosphorus. The linear relationship between intake and excretion is shown by the linear line of best fit.

< 0.01) was significantly lower than in fat-tailed dunnarts. Gross energy retention was significantly higher for cat formulation ($t = 4.5$; $P < 0.01$) and significantly lower for marsupial formulation ($t = -3.8$; $P < 0.01$) in stripe-faced dunnarts compared with fat-tailed dunnarts. Generally, stripe-faced dunnarts had lower intakes of lipids on all diets, cat formulation being the only exception, than fat-tailed dunnarts. Lipid retention in stripe-faced dunnarts, however, was significantly higher on the cricket ($t = 3.5$; $P < 0.01$) and cat formulation ($t = 4.7$; $P < 0.01$) diets and significantly lower on the marsupial formulation diet ($t = -3.2$; $P < 0.01$) compared to fat-tailed dunnarts. When maintained on the marsupial formulation, the smaller fat-tailed dunnart consumed and retained more nutrients and minerals (except P) than did the stripe-faced dunnart.

The apparent absorption of minerals was variable for both species. Mineral intake by stripe-faced dunnarts was similar across individuals when maintained on the same diet; however, excretion varied between individuals. For example, Ca intake on the cat formulation diet by stripe-faced dunnarts ranged from 103 to 109 mg, whereas excretion had a larger range of 29–73 mg. Compared with fat-tailed dunnarts, stripe-faced dunnarts had significantly higher retention of minerals on the cockroach diet (Fe [$t = 2.0$; $P = 0.04$], Ca [$t = 3.9$; $P = 0.00$], Na [$t = 2.8$; $P = 0.02$], K [$t = 3.5$; $P = 0.00$], and P [$t = 4.0$; $P = 0.00$]) and on the cat formulation diet (Ca [$t = 3.0$; $P = 0.01$],

Mg [$t = 3.8$; $P = 0.00$], Na [$t = 5.2$; $P = 0.00$], K [$t = 3.8$; $P = 0.00$], and P [$t = 5.5$; $P = 0.00$]). Retention of Ca on the mealworm diet differed significantly to the other diets, and between species ($t = -3.0$; $P = 0.01$). Ca excretion was higher than intake on the mealworm diet for both species. Negative retention values also were obtained for Fe for all 3 insect diets for both species of dunnart.

Digestible energy intake for stripe-faced dunnarts ranged from 359.1 ± 80.8 to 816.3 ± 95.3 kJ kg^{-0.75} day⁻¹ (mean \pm SD) and was significantly greater when they were provided with the mealworm and cat formulation diets, compared to the other insect diets and the marsupial formulation diet ($F_{4,11} = 33.5$; $P < 0.01$; cat formulation $P < 0.01$; mealworm $P < 0.01$). The digestible energy of the mealworm diet also was significantly higher than that of the cat formulation diet for the stripe-faced dunnart ($P < 0.01$). Digestible energy intake for fat-tailed dunnarts ranged between 542.4 ± 84.4 and 989.9 ± 136.9 kJ kg^{-0.75} day⁻¹ depending on diet, and was significantly higher for the mealworm and commercial diets compared to the other insect diets ($F_{4,8} = 13.2$; $P < 0.01$; mealworm $P < 0.01$; Table 2).

The gastrointestinal tracts of stripe-faced and fat-tailed dunnarts were similar; both had a unilocular stomach (Fig. 5). There was no external differentiation between the small and large intestine, which was fairly uniform along the tract (Fig. 5). No caecum was present in either species. The liver had 3 or 4 lobes, and was a dark reddish color. The liver was located close to the

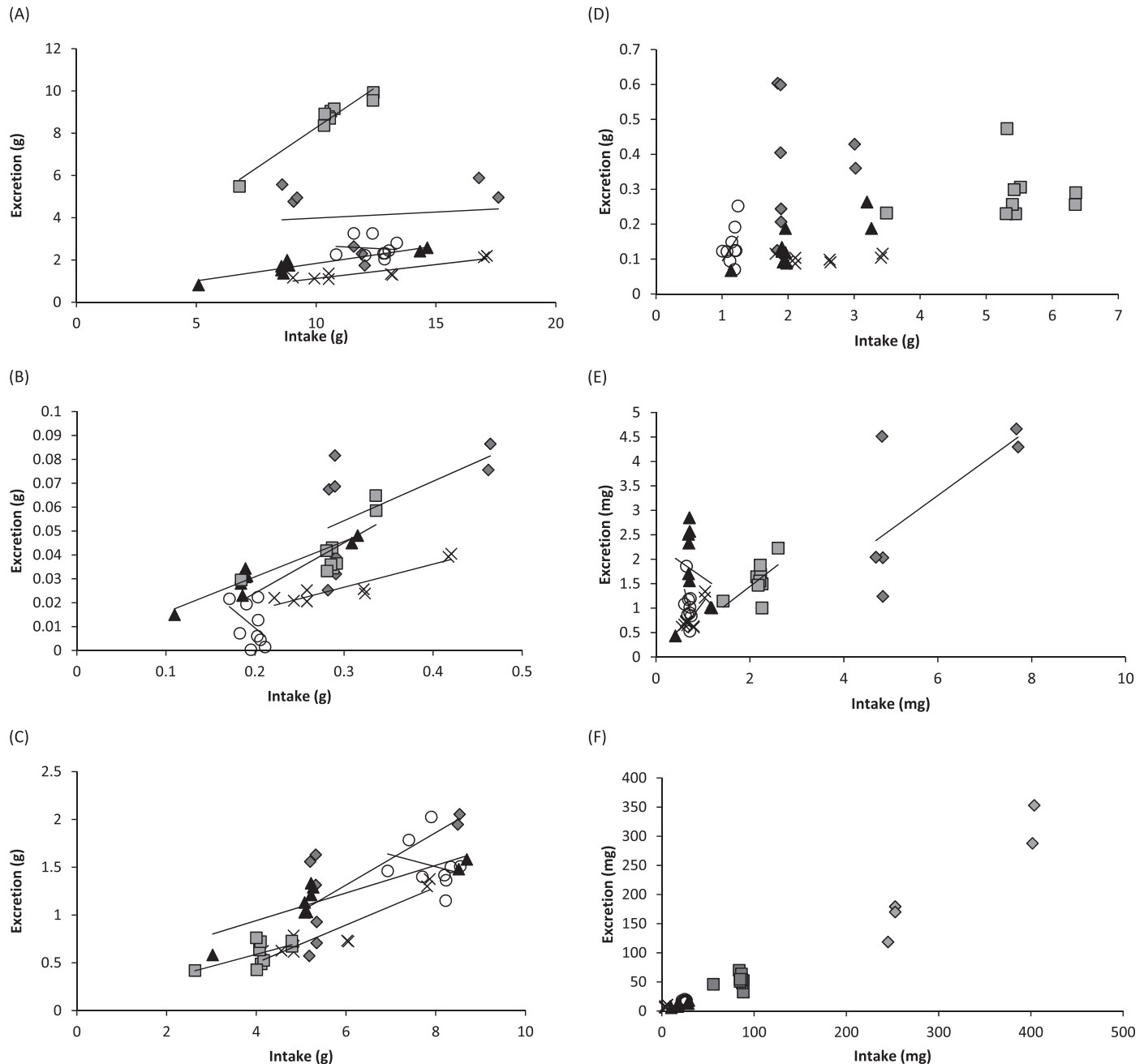


FIG. 4.—*Sminthopsis crassicaudata* intake versus output data for each nutrient and mineral: A) dry matter; B) energy; C) protein; D) lipids; E) iron; F) calcium.

stomach and the gallbladder was underneath the liver. The Y-shaped spleen was located in the upper left portion of the abdomen proximal to the stomach and was a dark purple-red color. There were no significant differences in the absolute measurements of both stomach ($t = -0.7$; $P = 0.22$ stomach length; $t = -0.4$; $P = 0.920$ stomach width) and intestine ($t = 0.2$; $P = 0.220$) between the stripe-faced and fat-tailed dunnarts (Table 3). There also was no significant difference in absolute intestine (stripe-faced dunnart $t = 1.0$; $P = 0.49$; fat-tailed dunnart $t = 2.2$; $P = 0.14$) and stomach lengths (stripe-faced dunnart $t = -0.1$; $P = 0.88$ stomach length; $t = 2.2$; $P = 0.78$; fat-tailed dunnart $t = 0.8$;

$P = 0.7$ stomach length; $t = -1.8$; $P = 0.13$ stomach width) between dunnarts euthanized at the end of this experiment compared to those euthanized for a previous experiment. However, when adjusted for body mass the intestinal length and stomach dimensions were significantly proportionally larger for fat-tailed dunnarts than for stripe-faced dunnarts (stomach length $t = 5.2$; $P < 0.01$; intestine $t = 6.56$; $P < 0.01$).

DISCUSSION

The study demonstrates that the 2 dunnart species have different responses to the dietary challenges presented to them.

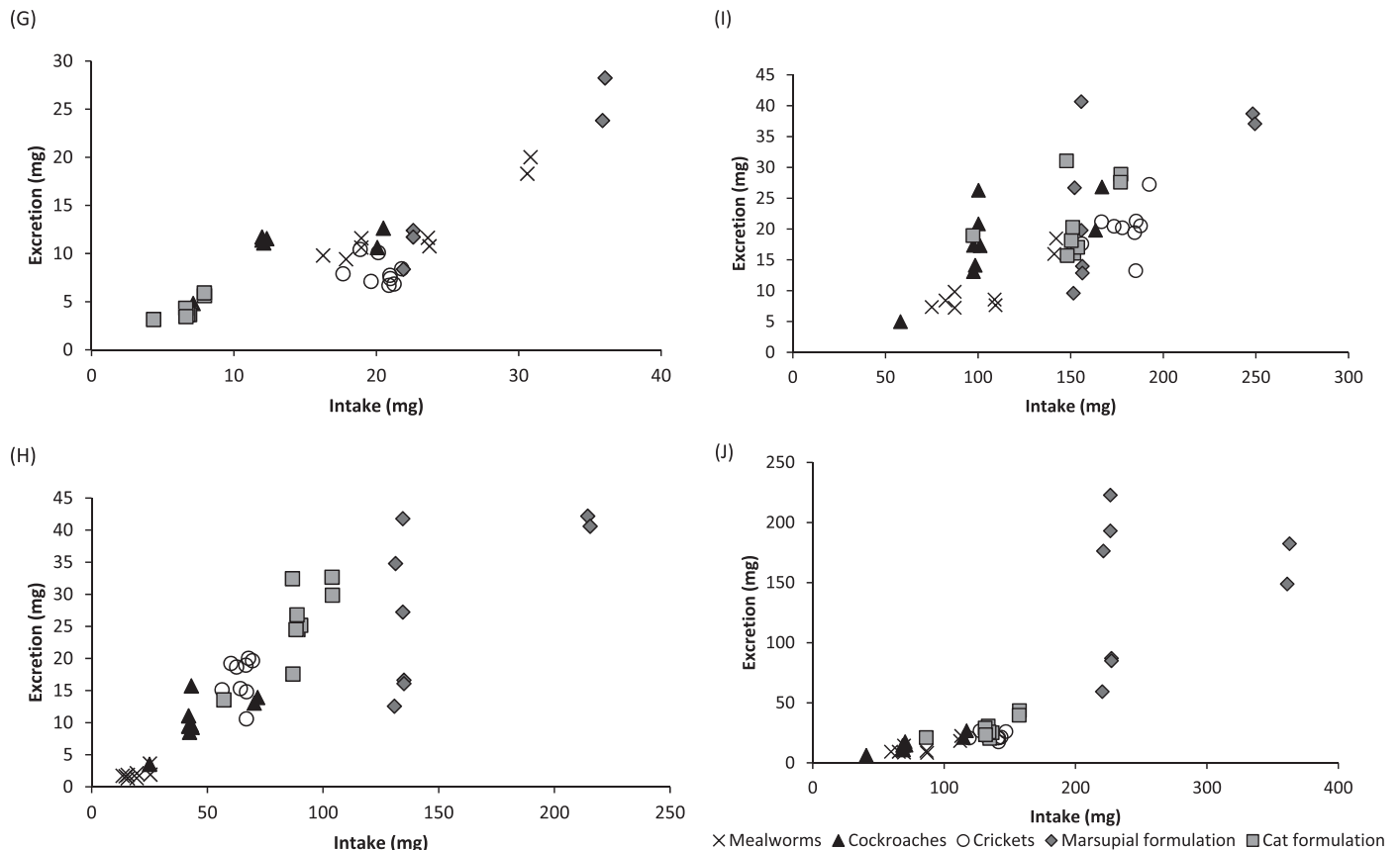


FIG. 4.—Continued. G) magnesium; H) sodium; I) potassium; and J) phosphorus. The linear relationship between intake and excretion is shown by the linear line of best fit.

We found that body mass and tail width change with exposure to diets of different digestibility and lipid and protein content. The digestibility of the diets was generally high, as would be expected for diets for carnivores. Minerals, however, were variably absorbed, depending on composition and availability of the mineral in the diet. In some cases the minerals present in particular diets appeared not to be absorbed by the animals.

Stripe-faced dunnarts consumed up to 50% and fat-tailed dunnarts up to 81% of their body mass equivalent in food per day. The highest consumption rate was for the cat formulation diet for both species. Cat formulation has a high water composition, and likely accounts for the high percentage of food relative to body mass consumed. When the cat formulation was excluded, stripe-faced dunnarts consumed 17–30% and fat-tailed dunnarts 27–53% of food relative to their body mass, similar to that observed in other dasyurids. Findings from other dasyurids in captivity for food consumption were 31% in dusky antechinus (*Antechinus swainsonii*), 37% in the brown antechinus (*A. stuartii*; now *A. agilis*), and 17–39% in the red-tailed phascogale and the kultarr (Cowan et al. 1974; Nagy et al. 1978; Stannard and Old 2011).

Tail fat accounts for 25% of total body fat in fat-tailed dunnarts and it plays an important role in fat storage in this species (Hope et al. 1997b). A significant loss of tail width occurred in the fat-tailed dunnart during the cockroach trial;

body mass loss, however, was not significant between the start and end of the cockroach trial. It appears the physiological response of fat-tailed dunnarts was to use some of their stored fat during this trial, because cockroaches are lower in fat and higher in protein than their usual cat formulation diet. The loss in tail width also suggests fat-tailed dunnarts have a higher daily energy requirement than was offered in the cockroach diet. During the mealworm trial a significant gain in tail width and gain in body mass was observed in the fat-tailed dunnart. Similar results have been shown with fat-tailed dunnarts increasing tail width on high-fat, low-carbohydrate diets (Ng et al. 1999). We acknowledge that carbohydrates could play a significant role in providing energy to the dunnarts during these trials. Insects in particular can provide substantial quantities of dietary energy in the form of carbohydrates (Raubenheimer and Rothman 2013). Insects such as mealworm larvae and crickets contain between 11% and 19% carbohydrates (measured as neutral detergent fiber—Barker et al. 1998; Finke 2002). However, our samples were very small (range 1–3 g) and we were only able to conduct a limited number of total analyses for each scat sample, hence carbohydrates were not analyzed.

Unlike fat-tailed dunnarts, stripe-faced dunnarts did not have a significant change in tail width on the cockroach diet, suggesting their lipid and energy requirements were being met during this trial. The stripe-faced dunnart showed a significant

TABLE 2.—Digestible energy intakes ($\text{kJ kg}^{-0.75} \text{ day}^{-1}$) for each diet for *Sminthopsis macroura* and *S. crassicaudata*. Superscript capital letters within a row denote significant differences ($P < 0.01$) between digestible energy intakes.

	Crickets	Cockroaches	Mealworms	Cat formulation	Marsupial formulation
<i>S. macroura</i> ($n = 11$)	359.1 ± 80.8	396.3 ± 78.3	816.3 ± 95.3^A	595.2 ± 87.0^A	392.7 ± 180.1
<i>S. crassicaudata</i> ($n = 8$)	542.4 ± 84.4	620 ± 98.0	989.9 ± 136.9^A	765.4 ± 52.3^A	894.2 ± 207.1

increase in tail width on the mealworm diet. Mealworms have a moderately high fat and high dry-matter content and it is likely the dunnarts were storing excess energy as fat in their tail during this trial to be used when physiologically challenged at a later time, if required (King et al. 2011).

Digestible energy intake is equivalent to daily maintenance energy requirement (MER) when an animal is maintaining body mass (Hume 1999), and we found the MER of the stripe-faced dunnart is $359 \text{ kJ kg}^{-0.75} \text{ day}^{-1}$ and of the fat-tailed dunnart is $542 \text{ kJ kg}^{-0.75} \text{ day}^{-1}$. These MER values were determined from the diet trial that had a zero body mass change. Energy requirements above $595 \text{ kJ kg}^{-0.75} \text{ day}^{-1}$ were determined for the cat formulation diet. The cat formulation diet provided relatively low levels of protein and high lipid and water composition, possibly accounting for the body mass loss on higher energy intakes in fat-tailed dunnarts. In larger dasyurids a higher MER has been observed compared to dunnarts (Tasmanian devil [*Sarcophilus harrisii*] and eastern quoll $545 \text{ kJ kg}^{-0.75} \text{ day}^{-1}$ [Green and Eberhard 1979]). The dunnarts showed lower energy requirements than do other small dasyurids (kultarr $695 \text{ kJ kg}^{-0.75} \text{ day}^{-1}$; red-tailed phascogale $954 \text{ kJ kg}^{-0.75} \text{ day}^{-1}$; dusky antechinus $933 \text{ kJ kg}^{-0.75} \text{ day}^{-1}$ [Cowan et al. 1974; Stannard and Old 2011]). In marsupials, generally the MER increases with decreased body mass (Hume 1999). As expected, the smaller fat-tailed dunnart has a higher MER than the stripe-faced dunnart. Compared to other small dasyurids, however, the stripe-faced dunnart has much lower MER than could be expected. It is possible that energy use is different in these species due to activity levels and species differences in the use of torpor during the experiment period.

The MER of the stripe-faced dunnart ($359 \text{ kJ kg}^{-0.75} \text{ day}^{-1}$) is similar to that found for smaller eutherian shrews (body mass 9.5–16.2 g) where MER ranges from 252 to $371 \text{ kJ kg}^{-0.75} \text{ day}^{-1}$ (Genoud and Vogel 1990). The African giant shrew (*Crocidura oliveri*), which is $\sim 30\%$ larger (38 g) than the stripe-faced dunnart, has an MER of $721 \text{ kJ kg}^{-0.75} \text{ day}^{-1}$, approximately double that found for the stripe-faced dunnart (Genoud and Vogel 1990). The MER of the stripe-faced dunnart is approximately 50% lower than found for eutherian counterparts. This is expected because it has been seen previously that marsupial requirements are approximately 30% lower than those of their eutherian counterparts (Hume 1974; Green and Eberhard 1979). In contrast, however, we found in the present study that the fat-tailed dunnart had a much higher MER than similar-sized eutherian counterparts.

Nutrient retention levels were generally higher for stripe-faced dunnarts compared to fat-tailed dunnarts when maintained on the same diet. Gross energy, however, is the exception, with fat-

tailed dunnarts having a higher retention on 3 of the 5 diets. This is highlighted by the MER, with stripe-faced dunnarts having 18–56% lower energy intake compared to fat-tailed dunnarts when maintained on the same diet. Therefore, fat-tailed dunnarts need to consume more energy per unit of body mass for maintenance in captivity than do stripe-faced dunnarts. Higher retention rates on the marsupial formulation by fat-tailed dunnarts is likely due to on average consuming 2.8 g of food (on a dry-matter basis) more than stripe-faced dunnarts. This suggests that fat-tailed dunnarts prefer the marsupial formulation more so than do stripe-faced dunnarts.

Excretion values of minerals varied more so than the intake values and thus had a nonlinear relationship in stripe-faced dunnarts. The variation could be due to interactions with other minerals, individual demand for specific minerals, individual metabolism, or age. For both species, negative values were obtained for apparent retention of Fe when animals were consuming insect diets and also for Ca when both species were on the mealworm diet. Ca and Fe were only available in small quantities in those food items. It could be that the dunnarts were not able to extract the minerals presented in this type of food, or the abrasive nature of the chitin caused endogenous losses from the gut. It is also possible endogenous losses of Fe were related to exposure to the previous diet, where Fe transporters were down-regulated due to significant amounts of absorbable Fe

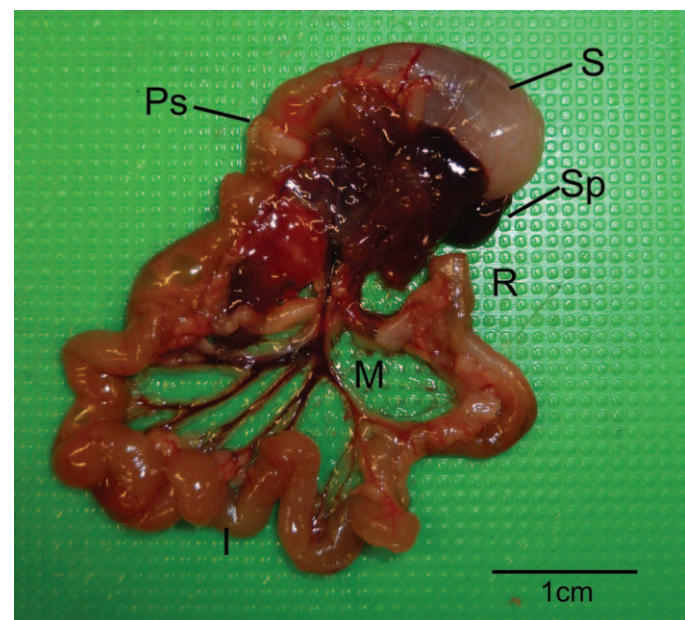


FIG. 5.—The gastrointestinal tract of *Sminthopsis macroura*, where S = stomach, Ps = pyloric sphincter, I = intestine, M = mesenteric tissue, R = rectum, and Sp = spleen.

TABLE 3.—Gastrointestinal tract measurements of *Sminthopsis macroura* and *S. crassicaudata*. Data are presented as means \pm 1 SD.

<i>n</i>	<i>S. macroura</i>		<i>S. crassicaudata</i>	
	8	4	8	4
Body mass (g)	25.4 \pm 2.7	20.8 \pm 3.3	14.8 \pm 1.3	11.8 \pm 1.9
Stomach length (mm) ^a	12.4 \pm 1.5	10.2 \pm 1.7	11.7 \pm 1.7	10.8 \pm 2.2
Stomach width (mm) ^a	6.5 \pm 0.8	6.6 \pm 0.8	5.7 \pm 0.9	7.2 \pm 2.0
Total intestine length (mm)	102.2 \pm 15.0	94 \pm 11.0	108.5 \pm 16.4	85.7 \pm 7.3

^a Measurement taken at widest point.

present in the earlier diets of the animals, such that Fe transport was not necessary, and the absorption was related to current diet, as has been noted in rats with zinc (Zn) digestibility (Johnson et al. 1988). Apparent absorption of Fe from the cat formulation and marsupial formulation diets showed positive values, suggesting the animals were able to digest and absorb Fe from these diets. Another possibility is that Fe is protein-bound in some nonabsorbable way such that it cannot be absorbed readily from the insect diets. The negative values could represent normal shedding of cells from the gut, and thus presenting more Fe in the feces than was in the food.

Dunnarts have relatively short and simple digestive tracts. They have the smallest tract lengths of the small dasyurids studied, which is expected because they are the 2 smallest species studied. Intestinal length of the Julia Creek dunnart was 170–220 mm and of the kultarr was 122–219 mm (Hume et al. 2000; Stannard and Old 2013). On average, the ratio of body mass (g) to intestine length (mm) is 1:4 for the stripe-faced dunnart and the Julia Creek dunnart (Hume et al. 2000), and 1:7 for the fat-tailed dunnart and the kultarr (Stannard and Old 2013). The short and simple digestive tract allows food to pass quickly through the tract and is ideal for handling an insect diet. Arthropods are generally high in protein and lipids, which are easily digested and absorbed by animals (Barker et al. 1998; Finke 2002). In didelphid marsupials including *Caluromys philander*, *Didelphis aurita*, and *Philander frenatus*, gastrointestinal tract morphology and length have been related to diet type (Santori et al. 2004). Didelphids show a large amount of dietary variation within the family, eating foods from insects and fruits to vertebrates. Didelphids that are generally frugivorous have longer gastrointestinal tracts, whereas the more insectivorous species have shorter, simpler digestive tracts (Santori et al. 2004). Similarly, gastrointestinal tract morphology and length has been related to diet and phylogeny in African mole-rats (Bathergidae spp.—Kotzé et al. 2010). It is likely diet has influenced gastrointestinal tract morphology and dimensions of stripe-faced and fat-tailed dunnarts, although we did not test long-term outcomes of diets on morphology in the present study.

The biggest difference between the dunnarts' gastrointestinal measurements in this study was the length of intestine. The smaller fat-tailed dunnart had a relatively longer intestine length than the stripe-faced dunnart. When comparing this with diet digestibility, digestive tract morphology was impacted most by the marsupial formulation diet. Stripe-faced dunnarts had a lower dry-matter intake and higher digestibility values

(of dry matter, gross energy, protein, and lipids) compared to fat-tailed dunnarts. Differences in digestibility values would likely be due to a larger stomach capacity and lower dry-matter intake. Fat-tailed dunnarts ate more on a dry-matter basis and even though they had a relatively longer intestine for their body size than stripe-faced dunnarts, it did not compensate for the large volume of food travelling through the digestive tract.

From the animals' responses (body-mass and tail-width changes, food intake, and digestibility values) presented here it can be seen that no single diet used in this study is appropriate for feeding captive dunnarts if fed alone. For example, mealworms had a low Ca and high digestible energy composition, which caused a large increase in body mass. Maintaining dunnarts on this diet alone would lead to obesity or Ca and Fe deficiency-related illnesses, or both. Ideally, a captive diet could provide a combination of the diets presented in this study to provide nutrients in a range of absorbable availabilities to adequately meet the nutrient requirements of captive dunnarts, both for these species and other endangered dunnarts. Our study also suggests that the arid-restricted stripe-faced dunnart may be more efficient at extracting nutritional requirements from its diet than the more widespread fat-tailed dunnart.

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SUPPORTING INFORMATION

SUPPORTING INFORMATION S1.—Body mass, tail widths, food intake, and apparent digestibility of the diets (AD) for the stripe-faced dunnart (*Sminthopsis macroura*).

Found at DOI: 10.1644/13-MAMM-A-071.S1

SUPPORTING INFORMATION S2.—Body mass, tail widths, food intake, and apparent digestibility of the diets (AD) for the fat-tailed dunnart (*Sminthopsis crassicaudata*).

Found at DOI: 10.1644/13-MAMM-A-071.S2

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