



Nutritional composition of the preferred prey of insectivorous birds: popularity reflects quality

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Food availability is emerging as a key determinant of avian occurrence and habitat use in a variety of systems, but insectivores have received less attention than other groups and the potential influence of nutritional quality has rarely been considered. Rather than a uniform food source, arthropods vary greatly in terms of nutritional composition, but does this variation translate into differential consumption? Building on previous work that demonstrated clear preference for some arthropod groups by 13 species of ground-foraging insectivores, we compare the nutritional composition of these arthropod groups with other groups commonly encountered but seldom consumed in the same habitat types. Using samples of arthropods collected from a eucalypt woodland in southern Australia, we found the high frequency prey groups (Coleoptera, Lepidoptera, Orthoptera and Araneae) consistently contained higher fractions of crude protein and total fat than the low frequency groups (Diptera, Hymenoptera and Odonata). Even more clear-cut differences were noted in terms of micronutrients; high frequency prey containing significantly greater concentrations of seven elements than low frequency prey and significantly greater amounts per individual arthropod for all eleven elements measured. These results indicate that the nutritional quality plays an important role in prey selection in insectivores and suggests that micronutrients may be more important determinants of prey choice than previously recognized. Integrating these findings with previous work suggesting food limitation may constrain distribution patterns of birds in fragmented landscapes, we contend that variation in nutritional quality helps explain observed patterns in insectivore diets and occurrence. In addition to explaining why smaller and more disturbed habitats are unable to support resident insectivore populations, this bottom-up mechanism may underlie the disproportionate sensitivity of insectivores to land-use intensification.

Insectivores are a prominent component of many biomes: dominating diversity patterns, regulating food webs and performing a variety of ecosystem functions. While the habitat preferences, migratory patterns and landscape-scale determinants of diversity of insectivores inter alia have motivated substantial research, the mechanisms underlying these patterns are seldom considered (Bennett and Watson 2011). Unlike frugivores, nectarivores or granivores for which food resources are relatively easy to define and measure, relationships between insectivore occurrence and food availability have rarely been explored (Walters et al. 1999, Wilson et al. 1999). Those few studies which have examined insect availability have invariably found it to be a critical determinant of insectivore occurrence (Zanette et al. 2000, see also Kaspari and Joern 1993) and changes to food availability are now considered to underlie many observed changes in insectivore distribution (Robinson 1998, Watson 2011). Population decline in insectivorous birds is now a well-documented, global phenomenon. Declines in this physiologically and ecologically diverse group of birds can often be attributed to changes in their arthropod prey base, caused by factors such as habitat loss (Zanette et al. 2000, Paquette et al. 2014), changes to farming practices (Britschgi et al. 2006), and chemical pesticides such as imidacloprid or bacterial agents

such as *Bacillus thuringiensis israelensis* (Poulin et al. 2010, Hallmann et al. 2014).

Arthropods vary greatly in nutritional value, with studies of commercially raised (Studier et al. 1991, Bukkens 1997, Barker et al. 1998, Finke 2002) and wild-caught arthropods (Robel et al. 1995, Oyarzun et al. 1996, Arnold et al. 2010, Eeva et al. 2010) documenting major differences in macronutrient composition. The nutrient profile of arthropods depends on multiple factors, including diet, life phase, soil type and season (Studier et al. 1991, Chen et al. 2004, Deblauwe and Janssens 2008, Arnold et al. 2010). Insectivorous birds can adjust for this variability in prey quality, preferring certain prey types to maximise energy intake (Kaspari and Joern 1993, McCarty and Winkler 1999), and switching prey when specific nutrients are required (Graveland and van Gijzen 1994, Graveland and Van Der Wal 1996, Naef-Daenzer et al. 2000). Although differing concentrations of amino acids (Ramsay and Houston 2003, Arnold et al. 2007) and fatty acids (McWilliams et al. 2002) among prey types have previously been shown to influence prey choice in insectivorous passerines, variation in micronutrients between arthropod groups and the associated influence on insectivore dietary composition has been overlooked.

To explore the role that macronutrients and micronutrients play in prey choice of insectivorous birds, we evaluated the nutritional composition of various arthropod groups relative to their popularity in the recorded diets of ground-foraging insectivores. These birds belong to a group of 26 'Declining Woodland Birds' of southern Australia, all formerly abundant and widespread species that have undergone recent declines in abundance and distribution, especially in agriculture-dominated regions in southern and eastern Australia (Bennett and Watson 2011). As insectivory and ground-foraging behavior are major factors linking these species, nutrient limitation due to changing availability of preferred prey has been proposed as a possible contributor to their decline (Watson 2011). Rather than a uniquely Australian phenomenon, declines in ground-foraging insectivores have been noted in many other regions (Böhning-Gaese et al. 1993, Donald et al. 2001, Sekercioglu et al. 2002), hence the influence of prey availability (and specifically nutritional quality) is of wider relevance (Bennett and Watson 2011).

Building on previous work which established dietary preferences for 13 species of ground-foraging insectivores (Razeng and Watson 2012) we ask: 1) do frequently recorded prey groups differ nutritionally from those groups recorded less frequently? 2) Are macronutrients (fats and proteins) or micronutrients (trace minerals) more important in determining prey choice?

This paper is not intended to provide definitive nutritional properties of different arthropod groups or explore the interacting effects of sex, age and site-based differences on nutritional composition. Rather, our objective is to estimate variation in prey quality within a woodland habitat and to evaluate whether observed patterns of prey selection reflect nutritional value.

Methods

Study species

The arthropod taxa used in this study are derived from those defined by Razeng and Watson (2012) as occurring in the diet of ground-foraging, insectivorous passerines. These 13 bird species are a subset of the 'Declining Woodland Birds'

of southern Australia, a group of considerable conservation concern (Watson 2011 and references therein; Table 1). Of 21 prey groups identified by Razeng and Watson, we collected sufficient biomass to conduct nutritional analysis on 11. All of these groups are orders except for Formicidae which are considered separately to other members of the Hymenoptera (Razeng and Watson 2012).

Study site

Collection of arthropods for nutritional analysis was undertaken from November 2010 to February 2011 at a private property in SE New South Wales, Australia. Now managed as a conservation property, the property has a history of sheep and cattle grazing since ca 1892 (Miller 2010), representative of most woodland remnants in southern Australia (Watson 2011). Eleven of the thirteen declining insectivores studied by Razeng and Watson (2012) have been recorded within the study site and an adjoining travelling stock route (Table 1; Razeng 2011). The vegetation in the study site is broadly classed as box-gum grassy woodland, with an overstorey of *Eucalyptus* species, a sparse shrub layer of occasional *Acacia* spp, and a diverse ground layer comprising herbs, forbs and native grasses with patches of bare ground and leaf litter. The study period coincided with a strong La Niña weather pattern, with higher than average rainfall for several months preceding this study (average annual rainfall = 701 mm; rainfall for the year 2010 = 1050 mm (Bureau of Meteorology 2014). Within the collection period, 142 mm of rainfall was measured on-site, average daily maximum temperature was 33.1°C, and average minimum was 15.5°C.

Collection methods

The methods used for arthropod collection were selected to target those arthropods accessible to declining insectivores (Table 1, after Antos and Bennett 2006, Antos et al. 2008). Seventeen trap arrays, each containing 4 pitfall traps were deployed throughout the site (n = 68). Each pitfall trap consisted of a 110 mm diameter polypipe sunk 150 mm into the soil, with a collection container at the base of the trap. To avoid contamination of arthropod material, no killing agents or preserving chemicals were used. Trapping arrays were positioned to sample a range of microhabitat

Table 1. Sampling technique in relation to foraging substrate sampled and foraging actions employed by birds utilising these substrates.

| Sampling technique | Foraging substrate sampled | Foraging actions represented | Species employing these actions/substrates |
|------------------------------|--|-------------------------------|--|
| Pitfall trapping (dry traps) | Ground layer, including leaf litter, bare ground, grassy patches, fallen timber, etc | Probe, pounce, sally variants | White-browed babbler, grey-crowned babbler, brown treecreeper, chestnut-rumped thornbill, red-capped robin, hooded robin, eastern yellow robin, jacky winter, restless flycatcher |
| Active search | Leaf litter, coarse woody debris, under bark, logs | Probe, pounce, glean | White-browed babbler, grey-crowned babbler, crested bellbird, brown treecreeper, white-browed treecreeper |
| Sweep netting | Low foliage, bare ground | Glean, pounce, sally variants | Red-capped robin, hooded robin, jacky winter, speckled warbler, southern whiteface, restless flycatcher |

Bird species are those 13 ground-foraging insectivores for which dietary preferences were established based on published dietary records (Razeng and Watson 2012). Bold denotes those eleven species previously recorded in the study site.

conditions and elevations within the site, in an effort to maximise catches of a range of arthropod taxa. Traps were emptied daily for a period of 20 consecutive days. Active searches under rotting logs and through coarse woody debris was an important method of capture for Coleoptera and Blattodea specimens. We acknowledge that ground-foraging insectivores may not be able to access arthropods in all locations used in active searches, but most species captured by this method were also collected in pitfall traps, so it was assumed that these taxa were surface-active, thus potentially accessible to birds. Ants were also collected actively from 5 nests located throughout the site. Sweep netting was conducted during the morning (08:00–11:00) and evening (17:00–19:00) to avoid the heat of the day and mimic peak foraging times. A 500 mm diameter sweep net, with 1 mm fine gauze netting was used, with sweeps performed at a height of < 1 m through potential foraging substrates. Upon collection, arthropods were placed on ice in an insulated container, in order to subdue aggressive behaviour and slow digestion of stomach contents. Once returned to the laboratory, arthropods were frozen at approximately -21°C .

Nutritional analysis

This study aims to discern differences in prey groups based on the most common level of classification in the literature, taxonomic order (Razeng and Watson 2012), and is intended as a baseline for finer-scale analyses in the future. We acknowledge that adults and larvae; males and females; individuals from high and low productivity sites of the same arthropod species may exhibit compositional differences. Since our overarching objective is to evaluate whether nutritional factors can explain documented patterns of preference in insectivores, and preferential consumption of arthropods based on sex, age or site-based differences has not been established (either for this group of insectivores or more generally) adults of both sexes, larvae and all instars of each prey group were combined for nutritional analysis. Arthropods were classified into the prey groups identified by Razeng and Watson (2012) using identification keys (CSIRO 2011) and a dissecting microscope (Olympus SZ51 \times 400). Frozen arthropod specimens were sorted using a series of sieves and a paintbrush to remove fine debris. To measure average biomass, 50 individuals from each prey group were randomly selected, with the exception of Araneae and Coleoptera, for which 100 individuals were used due to the large discrepancy in sizes, and Mantodea for which only 28 individuals were collected. Selected individuals from each order (whole arthropods with no body parts removed) were weighed together on an analytic balance (Sartorius Analytic A200S) to 0.0001 g. These were then dried in an oven at 60°C for 48–72 h, until a constant weight was obtained. Samples were then removed from the oven and re-weighed. The average dry weight (biomass) of individuals for each order was then determined to 0.0001 g. This process was repeated for the remaining individuals from each prey group, and the results were averaged to achieve the final result. After samples were dried, they were ground using a mortar and pestle until a particle size of < 1 mm was achieved.

Ash content was determined firstly by weighing sub-samples of approximately 0.5 g. Duplicate sub-samples

were created for those prey groups where sufficient arthropod material was available. Weighed samples were placed into crucibles and burned in a muffle furnace at 550°C over night (as per Bukkens 1997, Barker et al. 1998); remaining ash was then weighed and its fraction of the original weight calculated. Micronutrient content was determined via Inductively Coupled Plasma (ICP) using a Varian 710-ES ICP Optical Emission Spectrometer. Protein content was analysed using the standard method for determination of Total Kjeldahl Nitrogen (TKN), as per the American Public Health Association (APHA) (1998) standard 4500-N_{org} B, 4500-NH₃ C (Eaton and Franson 2005). 0.2 g of organic matter from each arthropod order was used in this process, duplicated where possible. TKN for each prey group was then multiplied by 6.25 (the standard conversion factor; Finke 2007) in order to determine crude protein content. Fat content was determined using the Soxhlet Extraction method, using 1 g samples of each prey group, replicated where possible, as per the APHA standard 5520 A & D (Eaton and Franson 2005).

Due to the nature of the information collected and the fact that the minimum sample mass required for reliable analysis far exceeds the mass of individual arthropods, quantitative analyses were not considered appropriate for macronutrients (after Studier et al. 1991, Robel et al. 1995, Oyarzun et al. 1996, Deblauwe and Janssens 2008). Duplicate samples were tested where possible, but insufficient arthropod material was collected to allow for further replication (Razeng 2011). The material analysed for each prey group represented the combined tissue particles of multiple individuals, therefore our results are average values for all individuals collected in each group. For micronutrients, two groups of arthropods were defined based on their frequency in the described diet of declining woodland birds (Razeng and Watson 2012): the four most frequently consumed orders (Coleoptera, Lepidoptera, Orthoptera, Araneae; excluding Formicidae and Hemiptera) and the three least frequently consumed orders (Diptera, Hymenoptera, Odonata; excluding Blattodea). Although frequently reported as prey, many records of ants relate solely to one bird species (brown tree creeper *Climacteris picumnus*) and their occasional presence in the gut contents of other species may result from inadvertent consumption while preening or feeding on the ground (Razeng and Watson 2012). Hemiptera were excluded as most individuals collected belonged to the family Miridae, which did not appear in diet records. Cockroaches were excluded from the latter group since they are primarily nocturnal, hence their relative rarity in bird diets may relate more to accessibility than to actual preference. To evaluate whether these two groups differ in nutritional value, the mean quantities of 11 elements (expressed in micrograms per individual arthropod) for the three most frequently consumed groups were compared with means from the three least frequently consumed orders using independent sample t-tests (having satisfied Levene's test for heteroscedasticity). These quantities were calculated by multiplying the overall content derived from bulked samples by the mean dry biomass, representing the quantum of nutrient obtained by ingesting an individual arthropod of each group. Finally, to assess relationships between macronutrients and micronutrients, correlations (Pearson) were run between concentrations of all eleven micronutrients and crude fat and protein content for all 10 arthropod groups.

Results

Summary of collected arthropod material

A total of 134.85 g of arthropod biomass was collected during the November–February collection period. Arthropods from a total of 11 prey groups were collected (10 insect groups and 1 arachnid group) in quantities sufficient for performing nutritional analysis (>2 g). The arthropod samples collected contained sufficient biomass to perform analysis of water content for all 11 prey groups, micronutrient composition and crude protein for 10 groups, and crude fat and ash content for 8 groups.

Macronutrient composition

Crude protein content varied from 76.25% dry mass for Araneae to 58.62% for Formicidae (overall mean of $66.93\% \pm 5.97$; Table 2). Crude fat content was between 7 and 12% for all prey groups analysed, except for Hemipterans for which the crude fat content was 16.5%. Araneae had the highest wet weight (195.49 mg/individual) and Blattodea the highest dry weight (biomass) which, at 66.45 mg/individual, was almost double that of the prey group with the second highest biomass; Orthoptera (37.68 mg/individual). The Formicidae group had both the lowest wet weight and biomass (11.16 and 4.09 mg/individual respectively).

The top five of the 6 most consumed prey groups (Coleoptera, Lepidoptera, Hemiptera, Orthoptera and Araneae) had high quantities of either crude fat or crude protein, however, whilst being the second most frequently recorded prey type, Formicidae had relatively low quantities of both macronutrients. The least recorded prey groups (Diptera, Hymenoptera, Blattodea, Mantodea and Odonata) contained either low or moderate amounts of these nutrients (where measured). Coleoptera consistently appeared within the top three values for all macronutrient factors examined except mean wet weight (Table 2).

Micronutrient composition

In general, relative proportions of micronutrients varied widely between prey types on a weight-by-weight basis (Table 3) with Araneae the most consistently rich across all elements examined. The largest ranges in values were that of sulphur (S) content, which ranged from 3023 mg kg^{-1} in Coleoptera, to 9077 mg kg^{-1} in Araneae; and potassium (K), which ranged from 6237 mg kg^{-1} in Formicidae, to 9790 mg kg^{-1} in Lepidoptera. Selenium (Se) had the smallest range, from 0.2 mg kg^{-1} in Orthoptera, to 1.6 mg kg^{-1} in Odonata. All prey groups had low calcium (Ca) content (mean = $1073 \text{ mg kg}^{-1} \pm 321$; Graveland and van Gijzen 1994) ranging from 5371 mg kg^{-1} in Coleoptera to 627 mg kg^{-1} in Odonata, and all prey types examined had Ca:P ratios < 0.31.

When considered per unit biomass, ten significant differences between high frequency and low frequency prey groups were detected, greater concentrations associated with high frequency prey for seven elements and for the ratio of calcium to phosphorous (Table 4). These differences became more marked when expressed as quantities per individual arthropod, those arthropods consumed more frequently containing significantly greater quantities of all eleven elements examined (values for Cohen's $d > 0.5$; Table 4).

When quantities of micronutrients were compared with that of macronutrients, with the exception of Mn for which concentration varied independently of both crude fat and crude protein, all other correlations were significant (Pearson correlation coefficient > 0.74, $p < 0.04$); highly significant for K, Ca, Mg, P and Zn (Pearson correlation coefficient > 0.9; $p < 0.002$).

Discussion

Arthropods collected within the study site varied widely in mass, proportion of macronutrients and concentrations of micronutrients, indicating that some prey groups have much higher relative nutritional quality than others. With the

Table 2. Comparative composition of macronutrients, of 11 arthropod taxa collected from the Slate Hill study site, that are known to occur in the diet of ground-foraging insectivores in south-eastern Australia. Arthropod groups are arranged from most frequently consumed to least frequently consumed, after Razeng and Watson's (2012) quantitative synthesis of published dietary records. Values represent means from all individuals processed, with wet weight and dry weight relating to the mass of individual arthropods (mean of 50 randomly selected individuals; 100 for Araneae and Coleoptera). Moisture content is expressed on a wet matter (WM) basis, while crude protein (CP), crude fat (CF) and crude ash (CA) are expressed on a dry matter (DM) basis. Bold font indicates the 3 highest values for each factor examined. Insufficient quantities of Hymenoptera, Mantodea and Odonata were collected to enable full analysis of macronutrients.

| Prey group | Wet weight (mg) | Dry weight (mg) | Moisture (%WM) | Crude protein (%DM) | Crude fat (%DM) | Crude ash (%DM) |
|-------------|-----------------|-----------------|----------------|---------------------|-----------------|-----------------|
| Coleoptera | 101.17 | 34.15 | 66.24 | 73.75 | 12.00 | 8.21 |
| Formicidae | 11.16 | 4.09 | 63.37 | 58.62 | 10.60 | 6.29 |
| Lepidoptera | 98.98 | 33.29 | 70.95 | 64.38 | 12.00 | 4.52 |
| Hemiptera | 15.36 | 7.67 | 50.09 | 63.75 | 16.50 | 3.04 |
| Orthoptera | 109.46 | 37.68 | 65.57 | 68.75 | 7.36 | 3.49 |
| Araneae | 195.49 | 29.45 | 69.88 | 76.25 | 9.55 | 4.19 |
| Diptera | 16.03 | 6.73 | 58.03 | 61.81 | 9.55 | 3.20 |
| Hymenoptera | 27.79 | 12.97 | 53.35 | 67.50 | – | – |
| Blattodea | 188.90 | 66.45 | 64.82 | 68.13 | 10.90 | 8.43 |
| Mantodea | 18.35 | 13.89 | 57.63 | – | – | – |
| Odonata | 35.05 | 13.91 | 60.32 | 68.13 | – | – |
| Mean | 83.54 | 23.66 | 60.48 | 66.93 | 11.06 | 5.17 |
| SD | 79.3 | 18.7 | 7.2 | 5.97 | 2.7 | 2.2 |

Table 3. Comparative micronutrient composition of 10 arthropod taxa consumed by ground-foraging insectivores in south-eastern Australia (there was insufficient Mantodea material collected for elemental analysis). Arthropods were collected from the Slate Hill study site in southern NSW; bold font indicates 3 highest values for each element.

| Prey group | Na (mg kg ⁻¹) | K (mg kg ⁻¹) | Ca (mg kg ⁻¹) | Mg (mg kg ⁻¹) | P (mg kg ⁻¹) | S (mg kg ⁻¹) | Se (mg kg ⁻¹) | Fe (mg kg ⁻¹) | Zn (mg kg ⁻¹) | Mn (mg kg ⁻¹) | Cu (mg kg ⁻¹) | Ca/P |
|-------------|---------------------------|--------------------------|---------------------------|---------------------------|--------------------------|--------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|-------------|
| Coleoptera | 1108 | 8949 | 1657 | 1720 | 5371 | 3203 | 0.3 | 1117 | 83 | 113 | 16 | 0.31 |
| Formicidae | 2257 | 6237 | 1263 | 1224 | 5747 | 4184 | 0.7 | 565 | 133 | 97 | 9 | 0.22 |
| Lepidoptera | 422 | 9790 | 667 | 1035 | 6478 | 4319 | 0.8 | 173 | 151 | 28 | 16 | 0.10 |
| Hemiptera | 422 | 6584 | 833 | 1137 | 6630 | 5414 | 0.8 | 182 | 114 | 261 | 40 | 0.13 |
| Orthoptera | 952 | 8743 | 916 | 916 | 7421 | 4873 | 0.2 | 82 | 133 | 220 | 38 | 0.12 |
| Araneae | 3170 | 7786 | 1263 | 1338 | 8443 | 9077 | 0.9 | 162 | 237 | 46 | 66 | 0.15 |
| Diptera | 1956 | 7232 | 966 | 953 | 7526 | 6270 | 0.8 | 151 | 131 | 51 | 20 | 0.13 |
| Hymenoptera | 1079 | 7165 | 910 | 912 | 6995 | 6292 | 0.6 | 163 | 137 | 122 | 24 | 0.13 |
| Blattodea | 1802 | 8367 | 1628 | 1137 | 6361 | 3953 | 0.8 | 1050 | 156 | 40 | 34 | 0.26 |
| Odonata | 2262 | 6879 | 627 | 781 | 7064 | 7953 | 1.6 | 161 | 92 | 14 | 20 | 0.09 |
| Mean (SD) | 1543 (993) | 7773 (1143) | 1073 (321) | 1115 (176) | 6804 (784) | 5554 (1770) | 0.7 (04) | 381 (314) | 137 (40) | 99 (88) | 28 (17) | 0.2 (0.1) |

Table 4. Comparison of nutrient composition in terms of both amount per unit mass (mg kg⁻¹) and amount per individual (µg) between high frequency prey groups (HF; Coleoptera, Lepidoptera, Orthoptera and Araneae) and low frequency prey groups (Diptera, Hymenoptera and Odonata); bold font denotes the group with the highest mean. In the one case where Levene's test determined variances were unequal (Cu per individual), adjusted t statistics are given. In terms of composition, those arthropods eaten more frequently contained significantly greater concentrations of 7 of 11 elements examined as well as a higher ratio of calcium to phosphorus. In terms of total amount per individual, those arthropods eaten more frequently contained significantly greater quantities of all eleven elements examined. *Denotes medium effect sizes, 0.50 < d < 0.80; **denotes large effect sizes, d > 0.80.

| Measure | Statistic | Na | K | Ca | Mg | P | S | Se | Fe | Zn | Mn | Cu | Ca/P |
|---------------------|-------------------------|--------------------|---------------------|--------------------|--------------------|---------------------|---------------------|---------------------|--------------------|------------------|------------------|------------------|-------------------|
| mg kg ⁻¹ | Mean ^{HF} (SD) | 1413 (1207) | 8817 (823) | 1126 (430) | 1252 (359) | 6928 (1312) | 5368 (2568) | 0.54 (0.38) | 383 (491) | 151 (64) | 102 (87) | 34 (24) | 0.17 (0.1) |
| | Mean ^{LF} (SD) | 1765 (614) | 7092 (187) | 834 (182) | 882 (90) | 7195 (288) | 6838 (965) | 0.98 (0.51) | 158 (6) | 120 (25) | 62 (55) | 21(3) | 0.12 (0.02) |
| | Levene's F (Sig.) | 1.07 (0.35) | 1.76 (0.24) | 2.81 (0.16) | 4.55 (0.086) | 4.55 (0.086) | 1.70 (0.25) | 0.44 (0.54) | 6.09 (0.057) | 0.99 (0.37) | 0.75 (0.43) | 5.43 (0.067) | 2.96 (0.15) |
| | t-test t (Sig.) | -0.46 (0.67) | 3.5 (0.02) | 1.08 (0.33) | 1.71 (0.15) | 1.71 (0.15) | -0.93 (0.40) | -1.32 (0.24) | 0.775 (0.047) | 0.79 (0.49) | 0.68 (0.53) | 0.90 (0.41) | 0.92 (0.35) |
| | Cohen's d | 0.37 | 2.9** | 0.88** | 1.41** | 0.28 | 0.76* | 0.98** | 0.65* | 0.64* | 0.55* | 0.75* | 0.77* |
| µg per individual | Mean ^{HF} (SD) | 45.3 (33.8) | 297.6 (46.7) | 37.6 (14.2) | 41.8 (11.5) | 231.8 (41.5) | 176.0 (68.0) | 0.017 (0.01) | 12.9 (16.8) | 5.0 (1.7) | 3.6 (3.4) | 1.1 (0.7) | |
| | Mean ^{LF} (SD) | 19.5 (10.3) | 79.1 (26.4) | 9.0 (2.7) | 9.7 (2.9) | 79.9 (25.6) | 78.1 (34.3) | 0.011 (0.02) | 1.8 (0.7) | 1.3 (0.4) | 0.7 (0.8) | 0.24 (0.1) | |
| | Levene's F (Sig.) | 1.94 (0.22) | 0.81 (0.41) | 1.98 (0.22) | 2.97 (0.15) | 1.20 (0.32) | 1.18 (0.33) | 2.28 (0.19) | 5.81 (0.061) | 1.16 (0.33) | 3.04 (0.14) | 15.73 (0.011) | |
| | t-test t (Sig.) | 1.25 (0.27) | 7.18 (0.001) | 3.36 (0.02) | 4.6 (0.006) | 5.5 (0.003) | 2.25 (0.074) | 0.72 (0.05) | 1.12 (0.31) | 3.58 (0.016) | 1.43 (0.21) | 2.49 (0.084) | |
| | Cohen's d | 1.03** | 5.76** | 2.80** | 3.81** | 4.41** | 1.82** | 0.59* | 0.94** | 2.96** | 1.18** | 1.77** | |

exception of ants, the most popular prey types are high in either crude fat, crude protein or, in the case of beetles (the most frequently recorded prey group), both. These patterns were even more clear-cut in terms of micronutrients – the three most popular prey groups having consistently higher amounts of key elements than the three least popular prey groups. Indeed, variation in macronutrients and micronutrients were closely inter-related, those arthropod groups with the highest fat and protein contents also enriched in most of the elements studied. Collectively, these findings provide strong support for the idea that nutritional quality is a key determinant of prey choice for ground-foraging insectivores.

Coleoptera (beetles) stood out in terms of macronutrients, having high levels of crude protein and crude fat. The protein estimate obtained here for beetles is higher than that found in other studies, however previous nutritional analyses of beetles have been performed on larval phases of commercially-raised species (Bukkens 1997, Finke 2002). The only example of nutritional analysis we found on adult, wild-caught, terrestrial beetles was that of Robel et al. (1995), which found individuals from the family Coccinellidae had a protein content of 62.4%. Most beetles collected for this study were adults and, as beetle larvae are generally soft-bodied with few sclerotinised parts, it is possible that the difference in protein content between our study and others is associated with these structures. While some protein is likely to be tied up in indigestible compounds, Finke (2002, 2007) has argued that a large percentage of nitrogen in insects can be recovered as amino acids, suggesting that the contribution of chitin to total nitrogen content is relatively small. Thus $N \times 6.25$ can be considered a reasonable estimate of crude protein and the digestible protein of Coleoptera is still likely to be greater than that of the other arthropods analysed here.

Spiders also had particularly high protein content and were higher in moisture than beetles, which may be an important factor for birds which obtain much of their water from food. No other study was found which quantified macronutrient content of spiders, but many studies have noted preferences for spiders, especially during the breeding season (reviewed by Razeng and Watson 2011). Fat content for all arthropods examined was relatively low when compared to studies examining commercially raised insect larvae, but was similar to values for adult arthropods (Bukkens 1997). Hemiptera had the highest proportion of fat (16.5%), which was similar to estimates of fat content in periodical cicadas found by Brown and Chippendale (1973).

Although the second most frequently consumed prey type, ants had the lowest levels of crude protein and the lowest dry weight, both of which were within range of estimates obtained by Deblauwe and Janssens (2008) for various wild ant species in Cameroon. In addition, due to their small size, ants have a high surface area to volume ratio, and thus higher relative chitin content than larger insects, which means they may be difficult to digest and of little energetic reward. There are a number of possible explanations for the frequent occurrence of ants in the diet of ground-foraging insectivores. Ants are common in the environment, and are prevalent in fragmented habitats (Gibb and Hochuli 2002). It is possible that they are consumed by these birds purely

opportunistically, or accidentally whilst gleaning surfaces for more rewarding prey. As ants have year-round presence in the habitat with conspicuous nests and trails, they may also be exploited in times of food shortage, when other prey types are unavailable. Of the thirteen ground-foraging insectivores studied here, ants were only found to dominate the stomach contents of brown treecreepers (Noske 1982), and 30% of ant records were from this species. With these possibilities considered, combined with their poor nutritional quality, the high frequency of occurrence of ants in the diet records may not be indicative of their dietary value.

Micronutrient requirements have only been assessed for commercially important poultry species (NRC 1994) so, although unlikely to be comparable with insectivorous passerines, these benchmarks can serve as a starting point for evaluating the biological significance of the variation in nutrients recorded between arthropod groups. Proportions of most micronutrients examined either met or exceeded maintenance requirements for poultry (where known). A major exception was sodium, in which only Araneae (spiders) met known requirements for poultry, whilst others such as Lepidoptera, Hemiptera and Orthoptera were found to be depauperate in this nutrient. Manganese was another exception, as five prey groups exceeded known requirements (Coleoptera, Formicidae, Hemiptera, Orthoptera and Hymenoptera). When considered in terms of proportion of micronutrients per individual biomass, it becomes apparent that preferred prey groups contain significantly higher proportions of important micronutrients than less preferred groups, those micronutrients that showed significant differences (K, Ca, Mg, P and Zn) integral for normal functioning and reproduction (Klasing 1998).

To prevent calcium leaching from the bones, calcium must be consumed in amounts equal to or greater than the consumed amount of phosphorous, thereby giving a ratio of at least 1:1 (Klasing 1998). None of the arthropod taxa examined in this study met this requirement, and arthropods in general are known to be low in calcium (Bukkens 1997). Although Coleoptera had the greatest proportion of calcium, this amount is not sufficient to prevent calcium leaching. Insectivorous birds are generally known to be reliant on the ingestion of non-food items (such as snail shells and calcareous grit) to acquire sufficient calcium (Graveland and van Gijzen 1994). Calcium limitation has been proposed as contributing to declines of insectivorous forest birds in Europe, as increasingly acidified soils lead to reduced abundance of snails, and therefore reduced access to biologically-available calcium sources (Graveland and Van Der Wal 1996). However, as the dietary literature pertaining to Australian ground-foraging insectivores contained only a single record of a mollusc being consumed (Razeng 2011), snails do not appear to be an important source of calcium for these woodland birds, and it is likely they are more dependent on ingestion of grit or soil to meet calcium requirements.

Integrating our findings with previous work on arthropod biomass, we suggest that nutritional factors may be a critical determinant of insectivore occurrence, helping to explain occurrence patterns at the patch and landscape scale. In Australian woodlands, arthropod communities have been shown to have greater abundance and diversity on richer soils (Recher et al. 1996), and larger, less disturbed patches,

with greater heterogeneity and situated on richer soils support diverse arthropod communities and a greater abundance of higher quality arthropods (Bromham et al. 1999, Taylor 2008). Thus, insectivores occurring in these larger patches have access to more abundant prey (Zanette et al. 2000) and spend less time foraging (Walters et al. 1999). Many of the remaining woodland habitats in south-eastern Australia are located on rocky ridges and other upper-catchment areas with impoverished soils which are unsuited to production agriculture (Pressey and Tully 1994, Watson 2011). Thus, in addition to lower prey abundances, most of the remaining woodlands in southern Australia may be unable to provide the balance of amino acids, fatty acids, trace minerals and other nutrients required by insectivores. Changes in diet composition have been implicated in declines of insectivorous birds elsewhere (Britschgi et al. 2006), with dietary composition during the breeding season especially critical (Ramsay and Houston 2003, Arnold et al. 2010, Eeva et al. 2010). By considering habitat quality in nutritional terms, particular groups of species, parts of the landscape and seasons can be distinguished as especially vulnerable, informing targeted and efficient management actions to boost limiting resources.

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