Arthropod prey vary among orders in their nutrient and exoskeleton content

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Abstract

Insectivores gain macronutrients and elements from consuming arthropod prey, but must also deal with indigestible components (i.e., exoskeleton) of prey. For example, avian chicks (e.g. northern bobwhites; *Colinus virginianus*) have limited gut space, and ingesting prey with relatively higher proportions of indigestible components may impact assimilation efficiency, growth, and survival. The ability of insectivores to choose higher quality prey would depend on prey taxa varying consistently in nutritional content. We tested if there were consistent differences among taxonomic orders of arthropod prey in their macronutrient (protein and lipid), elemental (C and N), and exoskeleton content. We used northern bobwhite chicks as our focal insectivore and focused on their potential prey as a case study. We also tested the influence of indigestible exoskeleton on the measurement of macronutrient content and the ability of elemental content to predict macronutrients. We found large and consistent variation in macronutrient and elemental content within and between arthropod orders. Some orders had consistently high protein content and low exoskeleton content (i.e., Araneae) and are likely higher quality prey for insectivores. Abundant orders common in the diets of insectivores, like Hymenoptera and Coleoptera, had high exoskeleton content and low protein content. We also found support for the ability of elements to predict macronutrients, and found that metabolizable (i.e. exoskeleton removed) elemental content better predicted macronutrient content. A better understanding of arthropod nutrient content is critical for elucidating the role of spatial and temporal variation in prey communities in shaping the growth and survival of insectivores.

Key words: insectivore, prey quality, indigestible components, protein, lipid

Introduction

Arthropods are an essential food source for a wide variety of invertebrates and vertebrates (Uetz et al. 1992; Kaspari and Joern 1993; Durst et al. 2008; Butler et al. 2012). Generalist predators often consume a diversity of prey that can vary widely in quality. Arthropod prey provide bulk nutrients such as carbohydrates, lipids, and protein that are important as a source of energy and for building body mass (Nestler et al. 1942; Eubanks and Dimmick 1974; Giuliano et al. 1996; Harveson et al. 2004). While prey can vary in a variety of nutrients including macronutrients and micronutrients, variation in macronutrient content of prey may be of particular interest because macronutrients are required in large quantities by consumers and can vary widely among species. For example, arthropod bodies can be composed of 10-85% protein and 5-32% lipids by dry mass (Wilder et al. 2013). Past studies have identified particular prey species that are high or low quality due to their nutritional or defensive compound content (Lase and Wolf 2010; Lease and Wolf 2011; Wilder 2011, Razeng and Watson 2014). Yet, less is known about consistency or variation within and among arthropod orders in their nutritional content. Consistency of nutritional content within orders of arthropods could form an evolutionary basis through which predators could base prey choice decisions, and may allow better understanding of how spatial and temporal variation in prey communities affect the distribution of nutrients across the landscape and their availability to opportunistic predators.

In addition to macronutrients, exoskeleton may also be an important dietary consideration for insectivores. Exoskeleton is often a large component of arthropod bodies and can vary among taxa, with exoskeleton comprising 18-60% of dry mass (Lease and Wolf 2010). Arthropod exoskeleton consists largely of chitin (20-50%), but can have considerable amounts of protein locked within the chitinous matrix during sclerotization (Lease and Wolf 2010). Hence,

exoskeleton can contain significant amounts of both carbon and nitrogen. Yet, exoskeletal protein, carbon, and nitrogen are largely unavailable to most consumers since they are unable to digest chitin (Bell 1990; Weiser et al. 1997). In addition to affecting digestibility of prey, exoskeleton can affect the measurement of prey nutrient content. For example, a common measure of arthropod nutrient content (i.e., crude protein = 6.25 x total nitrogen) assumes that all nitrogen is available to consumers (Jones 1941; Peoples 1992; Peoples et al. 1994; Razeng and Watson 2014). Use of different measures of nutrients can lead to different conclusions. For example, measures of the crude protein content of beetles have suggested that they have high protein content (Razeng and Watson 2014) while colorimetric assays of protein have suggested that beetles have low metabolizable protein content (Wilder et al. 2013). Because many insectivores feed opportunistically and changes in prey abundance influence consumption, is important to consider how the relative proportions of digestible and indigestible arthropod tissues influence prey quality, nutrient availability for predators, and the way that nutrients in prey are measured (Lease and Wolf 2010; Wilder et al. 2013; Wilder et al. 2019).

A variety of vertebrate and invertebrate insectivores rely on arthropods for most or all of their diet. For example, northern bobwhites (*Colinus virginianus*; hereafter bobwhite) are seasonally insectivorous, and the proportion of arthropods in the bobwhite diet depends on sex and life stage (Eubanks and Dimmick 1974; Doxon and Carroll 2010; Butler et al. 2012; Foye et al. 2015). Brooding hens require large amounts of arthropod-derived protein and energy in order to produce high quality eggs (Giuliano et al. 1996; Harveson et al. 2004), and chicks require a high-protein (~28%), arthropod-based diet (94.1% up to two weeks post-hatch) to quickly accumulate mass and develop feathers necessary for locomotion and predator avoidance (Nestler et al. 1942; Eubanks and Dimmick 1974; Giuliano et al. 1996; Harveson et al. 2004; Foye et al.

2015). Yet, like many insectivores, quail cannot digest exoskeleton (only 6.7% digestibility; Weiser et al. 1997).

We collected potential arthropod prey of northern bobwhites to: 1) test if different taxonomic orders of arthropods vary consistently in nutrient content in terms of macronutrients (lipid and protein), exoskeleton, and elements (C and N), and 2) test the strength of correlation between elements (i.e., C and N) and macronutrients (lipids and protein) in potential prey. Additionally, we partitioned the elements into those in the exoskeleton (i.e., indigestible) and those in the rest of the body (i.e., metabolizable) to test if total or metabolizable elemental content was more closely related to macronutrient content of prey. We chose bobwhite quail as a focal insectivore because they feed on a defined portion of the arthropod community, mostly ground-dwelling arthropods. It also allowed us to focus our invertebrate sampling on a defined habitat (i.e., shrubland of western Oklahoma) and explore variation in prey quality in the context of a natural community of prey available to a focal insectivore species.

Methods

Study Site

The arthropods used in this study were collected at Packsaddle Wildlife Management Area in Ellis County, Oklahoma during the months of May, June, and July 2019. Annual rainfall is 63.5 cm on average. Packsaddle WMA contains a wide variety of soil types including fine sandy loams, loam fine sands, and fine sands (Oklahoma Dept. of Wildlife Conservation and the United States Department of Agriculture). The 6,475-ha WMA is managed with prescribed fire, strip disking, and cattle grazing primarily for the production of game birds such as bobwhites and

common turkey (*Meleagris gallopavo*), but many other vertebrate and invertebrate insectivores inhabit the area for a significant portion of the year. Common vegetation present at sites includes grasses such as big bluestem (*Andropogon gerardii*), Indian grass (*Sorghastrum nutans*), little bluestem (*Schizachyrium scoparium*), side-oats grama (*Bouteloua curtipendula*), and buffalo grass (*Bouteloua dactyloides*), as well as shrubs like shinnery-oak (*Quercus havardii*), sand sagebrush (*Artemisia filifolia*), and sandplum (*Prunus angustifolia*; Oklahoma Dept. of Wildlife Conservation).

Invertebrate Collection and Identification

The goal of invertebrate collection for this study was to collect as diverse of a sample of potential prey of bobwhite quail as possible. Invertebrates common in the bobwhite diet based on crop analyses includes members of the orders *Hymenoptera, Coleoptera*, *Hemiptera*, *Orthoptera, Araneae*, and *Lepidoptera* (Eubanks and Dimmick 1974, Doxon and Carroll 2010, and Butler et al. 2012). Invertebrates were collected in three, 5-day sampling periods in May, June, and July 2019 using sweep net, dry pitfall, coverboard, and hand collection techniques. Sweep net samples were collected in burned, strip-disked, and unmanaged areas using 40-m transects, and a total of 20 sweep net samples were collected per sampling period. Collection locations were not evenly distributed across the landscape but were collected in areas of diverse topography and vegetative cover in order to maximize the diversity of potential bobwhite prey collected. Four 1-m square coverboards were deployed in one burned, one disked, and two unmanaged areas. Transects of five dry pitfall traps were placed in one burned, one disked, and two unmanaged areas. Coverboard and dry pitfall trap samples were collected twice daily (morning and evening),

and one hour was spent searching for and hand collecting invertebrates daily. All samples were stored in plastic bags and frozen until sorting.

Individual invertebrates were sorted out of plant matter and other debris and were initially sorted based on taxonomic order. Individuals were then given a morphospecies label based on differences in appearance, and representatives of each morphospecies were pinned in a reference collection. The number of morphospecies per order used in this study was related to sample availability and an attempt to avoid over or underrepresentation of taxa relative to their known biodiversity. In total, we measured the nutrient content of the following morphospecies: 23 Coleoptera, 22 Hemiptera, 3 Hymenoptera (all ants), 14 Orthoptera, 5 larval Lepidoptera, and 5 Araneae.

Nutrient Analyses

Two identical sets of 72 samples (i.e., same morphospecies) were prepared for exoskeleton and nutrient analysis, respectively, by drying samples for 24 hours at 60°C and measuring their dry mass. We sorted 15-30 mg of dry mass for each sample, with the number of individuals per sample varying based on the body size of the arthropods. For example, some Orthoptera samples were only 1-2 individuals, but ant samples contained as many as 30 individuals. Macronutrient and exoskeleton content was measured according to established protocols (Cuff et al. *in press*), which are summarized here. We measured lipid content of arthropods using a gravimetric method with chloroform as a solvent. All dried samples were soaked in chloroform for 72 hours (Wilder et al. 2013). Chloroform was removed and new chloroform was added every 24 hours, and samples were then dried for 24 hours at 60°C and reweighed (Wilder et al. 2013). Exoskeleton was removed from one set of samples by soaking in

0.1M NaOH to dissolve soft tissue (Lease and Wolf 2010). Samples were first sonicated at 80°C in 0.1M NaOH for 30 min and then allowed to soak for 24 hours. After 24 hours, samples were centrifuged at 10,000 RPM, the NaOH was removed, and fresh NaOH was added. After another 24 hours, samples were centrifuged again and the NaOH was removed, and samples were washed with water and dried at 60°C for 24 hours. The dry weight after soft tissue removal was used as a measure of exoskeleton.

Samples of 2-3 mg of ground, lipid-free arthropod tissue, as well as one sample of exoskeleton for each order of arthropods, were also prepared for elemental C and N content analysis. Samples were weighed on a microbalance and packaged in tin capsules to be combusted in an Elementar. Metabolizable elemental content was considered to be the elements in the part of the body that was not exoskeleton.

Protein content of samples was also measured using colorimetric assays on each morphospecies in which there was sufficient biomass remaining. Protein was extracted from arthropods by grinding lipid-free samples with a 3 mm steel ball bearing using a mixer mill at 30 hz for 3 minutes. Then, approximately 5 mg of ground arthropod material was soaked in 1 mL of 0.1M NaOH and sonicated at 80°C for 30 min. The supernatant was then used to conduct the Lowry assay and the Bradford Assay according to the kit instructions for microplate assays. Bovine IgG standard solutions were used to create standard curves.

Data Analysis

Statistical program R ver 3.4.2 (R Core Team 2013) was used to conduct one-way ANOVAs and Tukey's HSD post-hoc analysis to detect differences in lipid, exoskeleton, protein, and elemental content between and within orders of arthropods. Levene's test was used to test for

homogeneity of variance. When the assumption of homogeneity of variance was not met, we performed Welch's ANOVA and the Games-Howell posthoc test. Linear regression was used to test the relationship between elemental content and macronutrient content, and Aikake's Information Criterion for small sample sizes (AICc) was used to compare the predictive ability of total and metabolizable measures of elemental content. Nutrient content of arthropods is expressed as mg/100 mg dry mass to use units that are independent of body size.

Results

Among- and Within-Order Variation in Content

Exoskeleton Content. We observed wide variation in exoskeleton content across all orders; the lowest average exoskeleton content was 6.2 mg/100mg dry mass (Araneae) and the highest was 37.5 mg/100mg by dry mass (Coleoptera; Figure 1). Coleoptera exoskeleton content was more variable than any other order (Levene's test, $p = 0.05$). Welch's ANOVA, which we conducted due to unequal variances among groups, indicated that exoskeleton content differed significantly between orders of arthropods ($p < 0.001$). Araneae had the lowest mean exoskeleton content (6.2 $\pm$ 0.8 mg/100mg; mean $\pm$ 1 SE), and Coleoptera (37.5 $\pm$ 3.0 mg/100mg; $p < 0.05$) and ants (37.4 $\pm$ 6.1 mg/100mg) had the highest, although ants did not differ significantly from any order likely due to the small sample size of this group (Figure 1). The mean exoskeleton contents of ants and Coleoptera were ~6 times higher than Araneae. Orthoptera (10.6 $\pm$ 1.6 mg/100mg) and Lepidoptera (13.1 $\pm$ 5.0 mg/100mg) did not differ significantly from Araneae, and Hemiptera (21.7 $\pm$ 2.9 mg/100mg) had intermediate exoskeleton content (Figure 1).

Lipid Content. There was also wide variation in lipid content across all orders, with average values ranging from 7.1 mg/100mg dry mass (Orthoptera) to 20.1 mg/100mg dry mass (ants; Figure 1). Hemiptera lipid content was more variable than any other order (Levene's test; $p < 0.001$). Welch's ANOVA indicated that lipid content differed significantly between orders of arthropods ($p < 0.001$). Ants (20.1 $\pm$ 4.5 mg/100mg) and Hemiptera (19.5 $\pm$ 1.5 mg/100mg) had the highest average lipid content (Figure 1). The average lipid content of ants and Hemiptera were at least double Araneae, Lepidoptera, and Orthoptera lipid content (Figure 1). Coleopterans were intermediate (14.5 $\pm$ 1.9 mg/100mg), with significantly higher lipid content than Orthoptera ($p < 0.05$). Araneae (10.0 $\pm$ 1.7 mg/100mg), Lepidoptera (9.3 $\pm$ 1.0 mg/100mg), and Orthoptera (7.1 $\pm$ 0.80 mg/100mg) had the lowest average lipid content (Figure 1).

Protein Content. The Lowry assay suggested that there was large variation in protein content within and among orders of arthropods, with average values ranging from 20.3 mg/100mg (ants) to 53.4 mg/100 mg (Araneae; Figure 1). Levene's test indicated that there were no differences among taxa in variance of protein content measured by the Lowry assay ($p > 0.05$). Araneae had the highest protein content (53.4 $\pm$ 4.2 mg/100mg), and Coleoptera (26.5 $\pm$ 1.2 mg/100mg) and ants had the lowest (20.3 $\pm$ 2.9 mg/100mg; Figure 1). Orthoptera (43.4 $\pm$ 1.1 mg/100mg), Lepidoptera (38.7 $\pm$ 2.3 mg/100mg), and Hemiptera (33.2 $\pm$ 1.3 mg/100mg) had intermediate protein content, though Lepidoptera protein content did not differ from Orthoptera or Hemiptera (Figure 1).

The Bradford assay also suggested that there was large variation in protein content within and among orders of arthropods, with average values ranging from 14.4 mg/100mg (Lepidoptera) to 60.9 mg/100mg (Araneae; Figure 1). However, where the Lowry assay

produced distinct differences between intermediate and low-protein orders, the Bradford assay placed Orthoptera lower in rank and grouped Orthoptera, Lepidoptera, Coleoptera, and ants as the lowest in protein content. Levene's test indicated that there were no differences among taxa in variance of protein content measured by the Bradford assay ($p > 0.05$). Araneae had the highest protein content (60.9 $\pm$ 1.9 mg/100mg), and Orthoptera (26.6 $\pm$ 3.1 mg/100mg), Lepidoptera (14.4 $\pm$ 3.0 mg/100mg), Coleoptera (26.4 $\pm$ 2.0 mg/100mg), and ants had the lowest (23.8 $\pm$ 5.5 mg/100mg; Figure 1). Hemiptera had intermediate protein content (39.4 $\pm$ 1.4 mg/100mg), though it was not significantly different from ants (Figure 1).

Total Elemental Content. C and N content also varied within and between orders of arthropods. C content was somewhat less variable than N content. The lowest average total C content observed was 40.9 mg/100mg by dry mass and the highest was 48.5 mg/100mg by dry mass (Figure 2). Levene's test indicated variances in total C did not differ between orders ($p > 0.05$; Figure 3). However, total C content differed significantly between orders ($p < 0.001$). Lepidoptera had the lowest average total C content (40.9 $\pm$ 0.70 mg/100mg), and Hemiptera (48.1 $\pm$ 0.39 mg/100mg), Coleoptera adults (48.5 $\pm$ 0.35 mg/100mg), and ants (47.4 $\pm$ 0.075 mg/100mg) had the highest (Figure 2). Orthoptera had intermediate total C content (44.6 $\pm$ 0.53 mg/100mg), and Araneae total C content (43.9 $\pm$ 1.1 mg/100mg) was not significantly different from any other order (Figure 2).

The lowest average total N observed was 7.5 mg/100mg by dry mass and the highest was 10.7 mg/100mg by dry mass (Figure 3). Levene's test indicated that there were no differences among taxa in variance of total N ($p > 0.05$). Lepidoptera had the lowest average total N content (7.5 $\pm$ 0.46 mg/100mg) and Araneae had the highest (10.7 $\pm$ 0.44 mg/100mg; Figure 3). There

was a gradient in total N among taxa, with taxa ranked highest to lowest as Araneae, Orthoptera (9.6 ± 0.26 mg/100mg), Hemiptera (9.0 ± 0.20 mg/100mg), Coleoptera adults (8.9 ± 0.19 mg/100mg), ants (8.5 ± 0.98 mg/100mg), and Lepidoptera (Figure 3).

Metabolizable Elemental Content. Patterns in metabolizable C content were different than total C, particularly for orders with high exoskeleton content (i.e. Coleoptera adults; Figure 2). The lowest average metabolizable C was 31.2 mg/100mg by dry mass and the highest was 41.2 mg/100mg (Figure 2). Levene's test indicated that variance in metabolizable C differed between orders ($p < 0.05$), and Coleoptera adults had the most variable metabolizable C content (Figure 2). Welch's ANOVA indicated that metabolizable C differed between orders (Figure 2). Araneae (41.2 ± 0.94 mg/100mg), Orthoptera (40.1 ± 0.88 mg/100mg), Hemiptera (38.1 ± 1.5 mg/100mg), and Lepidoptera (36.3 ± 2.0 mg/100mg) had the highest average metabolizable C content, and Coleoptera adults (31.2 ± 1.5 mg/100mg) had the lowest (Figure 2). Ants (33.5 ± 2.3 mg/100mg) did not significantly differ from any other order (Figure 2).

When we analyzed metabolizable N (i.e., total N with exoskeleton N removed), the rank of some orders changed relative to the results for total N (Figure 3). The lowest average metabolizable N observed was 4.8 mg/100mg by dry mass and the highest was 9.98 mg/100mg by dry mass (Figure 3). Levene's test indicated that there were no differences among taxa in variance of metabolizable N ($p > 0.05$). Araneae had the highest average metabolizable N content (9.98 ± 0.38 mg/100mg; Figure 3). The mean metabolizable N content of Araneae was approximately double that of the lowest two orders: Coleoptera adults and ants (Figure 3). Orthoptera had similar metabolizable N content (8.9 ± 0.25 mg/100mg) to Araneae, but was only significantly higher than Coleoptera and Hymenoptera ($p < 0.05$; Figure 3). Hemiptera was

intermediate (7.1 ± 0.27 mg/100mg), but was only significantly lower than Araneae and higher than Coleoptera ($p < 0.05$; Figure 3). Lepidoptera (6.8 ± 0.60 mg/100mg), Coleoptera adults (5.4 ± 0.26 mg/100mg), and ants (4.8 ± 0.77 mg/100mg) had the lowest metabolizable N content (Figure 3).

Elemental and Macronutrient Relationships

Relationships Between C and C-containing Compounds. Total C content was positively related to lipid content ($R^2 = 0.3$; $p < 0.0001$; Figure 4). However, the low R^2 value suggests there is much unexplained variation (Figure 4). Metabolizable C also displayed a positive linear relationship with lipid content ($R^2 = 0.06$; $p = 0.02$; Figure 4). Lipid content was better predicted by total C content than metabolizable C (Table 1). The model with total C as the predictor was the top model (delta AICc = 0.00) and received more support in its ability to predict lipid content than the metabolizable C model (delta AICc = 22.20; Table 1).

Total C content was also positively related to exoskeleton content ($R^2 = 0.1$; $p = 0.004$; Figure 4). However, total C poorly accounted for variation in the exoskeletal data (Figure 4). Metabolizable C displayed a negative linear relationship with exoskeletal content ($R^2 = 0.9$; $p < 0.0001$; Figure 4). AICc model selection indicated that metabolizable C was a much better predictor of exoskeleton content than total C (Table 1). Metabolizable C was the top model (delta AICc = 0.00) and total C received considerably less support in its predictive ability of exoskeleton content (delta AICc = 136.24; Table 1).

Relationships Between N and Protein. Metabolizable N and the Lowry assay displayed the strongest correlation ($R^2 = 0.5$; $p < 0.0001$; Figure 5). The Lowry assay displayed a weaker

correlation with total N (R^2 = 0.2; p = 0.0002; Figure 5). Metabolizable N content was a better predictor of the Lowry assay than total N (Table 1). The model containing metabolizable N as the predictor for Lowry protein content received considerably more support (delta AICc = 0.00) than the model using total N as the predictor (delta AICc = 35.35; Table 1).

The Bradford assay displayed similar correlations between metabolizable N (R^2 = 0.3; p < 0.0001) and total N (R^2 = 0.2; p < 0.0001; Figure 5). AICc model selection indicated that metabolizable N predicted Bradford protein content better than total N (Table 1). The top model contained metabolizable N as the predictor (delta AICc = 0.00), and the total N model received less support (delta AICc = 5.21).

Discussion

We observed substantial variation in elemental and macronutrient content within and between orders of common arthropods. Overall, our results support the hypothesis that arthropod taxa are consistently different from each other in nutrient content. Although, some taxa are more variable in nutrient content than others. Araneae had the highest protein content and lowest exoskeleton content, although Orthoptera also had high protein content and Lepidoptera also had low exoskeleton content. In contrast, Coleoptera adults and ants had the highest exoskeleton content and lowest protein content. Ants and Hemiptera had the highest lipid content, although they were also the most variable. Large, consistent variation in macronutrient content within and between orders of arthropods underscores how the frequency of individual orders in the diets of predators may affect their nutrient intake (Bell 1990; Weiser et al. 1997; Wilder et al. 2019).

Variation in nutrient content within orders of arthropods may also have important consequences for consumers. Adult beetles are extremely variable in body form and nutritional

298 composition (Sloggett 2008; McCullough et al. 2015), and we found that Coleoptera exoskeleton
299 content was the most variable of any order. Coleoptera also exhibited large within-order
300 variation in lipid content. Hemiptera also displayed consistently large within-order variation in
301 exoskeleton and lipid content. Thus, it appears that some orders are highly variable in nutrient
302 content, particularly in nutrients contributing to pools of C, where other orders (i.e. Araneae,
303 Orthoptera, Lepidoptera) and macronutrient/elemental pools (i.e. protein/N) remained fairly
304 consistent.

305 Our results show mixed support for the relationships between elements and
306 macronutrients. Total C was a better predictor of lipid content, and metabolizable C was a better
307 predictor of exoskeleton content, although the relationship was inverse (i.e., arthropods with
308 higher metabolizable C had less exoskeleton). The relationship between nitrogen and nutrients
309 was better supported. Metabolizable N predicted protein content measured by both the Bradford
310 and Lowry assays better than total N. These results suggest that metabolizable N can be a
311 predictor of macronutrient content and potential nutrient intake of insectivores, likely because
312 metabolizable N excludes indigestible exoskeletal content (Bell 1990; Weiser et al. 1997; Wilder
313 et al. 2019). Other preliminary data suggest that metabolizable N may be highly correlated with
314 metabolizable amino acid content of samples, which is considered to be one of the most accurate
315 but most expensive measures of protein content (Wilder et al. Unpublished results).

316 Many consumers cannot digest exoskeleton in meaningful quantities, and it is therefore
317 essential to consider how indigestible components of prey influence potential nutrient intake
318 (Weiser et al. 1997; Wilder et al. 2019). For example, two of the most common arthropod orders,
319 Coleoptera (37.5 $\pm$ 3.0 mg/100mg) and ants (37.4 $\pm$ 6.1 mg/100mg) had the highest mean
320 exoskeleton content of all orders (Doxon and Carroll 2010; Butler et al. 2012). Thus, over a third

of the dry mass of these prey is likely indigestible. Measures that do not account for elements or macronutrients contained in indigestible tissues will result in different conclusions about variation in nutrient content within and between arthropod orders than ones that account for it (i.e. metabolizable N, Lowry and Bradford assays; Wilder et al. *In preparation*).

These results suggest that variation in the relative abundance of high (e.g., Coleoptera and ants) versus low (e.g., Araneae) exoskeleton prey could have important impacts on overall nutrient intake by insectivores, including bobwhite chicks (Weiser et al. 1997; Butler et al. 2012; Foye 2015; Morrow et al. 2015). Individuals likely gain greater nutritional benefits when consuming prey low in exoskeleton due to increases in assimilation efficiency (Nestler 1942; Peoples et al. 1994; Weiser et al. 1997), but we found that some common prey in the bobwhite diet (ants and Coleoptera) had the lowest metabolizable N/protein content and the highest exoskeleton content (Eubanks and Dimmick 1974; Butler et al. 2012). While some insectivores are able to modulate expenditures related to handling indigestible components (i.e. extraoral digestion in spiders avoids ingestion of exoskeleton; Cohen 1995), many consumers do not have these adaptations and cannot digest exoskeleton. It is thus critical to consider how ingestion of indigestible components impact consumers, as limitation in macro- and/or micronutrients may result in deficiencies in assimilation, growth, development, locomotor ability, reproduction, and ultimate survival (Gregg and Rogers 1986; Peoples 1992; Peoples 1994; Kuar and Ab 2015).

Another finding of our comparison of nitrogen and protein was that the method of estimating protein content of arthropods had significant impacts on the results. Our results suggested that the Lowry assay and metabolizable N provided similar patterns of results in estimated nutrient content of arthropods. Total N has commonly been used to calculate crude protein, which is N x 6.25 (Jones 1941; Bukkens 1997; Finke 2013; Finke 2015). Yet, our results

suggested that there can be considerable differences between total N and metabolizable N for some taxa, especially Coleoptera and Hymenoptera. It is important to note this distinction because measures of N or protein content that do not account for exoskeleton can overestimate protein content available to consumers. Additionally, protein content from Lowry and Bradford assays correlated better with metabolizable N than total N, supporting the accuracy of metabolizable measures of nutrient content (Wilder et al. 2019; Wilder et al. *In preparation*). Interestingly, the two colorimetric protein assays, Bradford and Lowry, also resulted in different patterns of results, likely because each assay interacts with amino acids slightly differently (Winters and Minchin 2005; Ku et al. 2013). This suggests that N-based measures, such as metabolizable N, may be less prone to measurement variation caused by differences in amino acid or protein structure between samples.

Lipid content was also highly variable within and between orders of arthropods. It is likely that observed differences in lipid content and variation within and between orders is due in part to the diversity of trophic levels represented by taxa contained therein (Wilder et al. 2013). For example, Coleoptera and Hemiptera are extremely diverse orders that contain detritivores, herbivores, omnivores and carnivores, and these orders exhibited higher variation in lipid content than any other order. Groups that contain only predators, like Araneae, displayed lower and less variable lipid content, but some herbivorous arthropods, such as Lepidoptera and Orthoptera, also displayed low variation and low lipid content. Variation within orders could also result from variation among individuals in their feeding history, sex, developmental stage, and reproductive state (Lease and Wolf 2011). Unlike exoskeleton and protein, lipids are stored in large quantities and rapidly mobilized for energy (Canavoso et al. 2001), and it is likely that we observed

366 significant variation between individual arthropods based on their individual lipid storage
367 reserves.
368 Total C content grouped orders into three distinct levels, whereas metabolizable C
369 produced only two. However, there was much larger within-order variation in metabolizable C,
370 particularly in orders with high and variable exoskeleton content (i.e. Coleoptera). The ability of
371 C to predict macronutrients and indigestible components also differed between total and
372 metabolizable C content. Total C content predicted lipid content better than metabolizable C
373 content, though neither measure of C accounted well for variation in lipid content ($R^2 \leq 0.3$).
374 Elemental C may not be a good predictor of arthropod lipid content because variation in C
375 content stems from three pools in individual arthropods: lipid, exoskeleton, and all other organic
376 compounds, all of which contain C by definition (Lease and Wolf 2010; Lease and Wolf 2011).
377 For exoskeleton, total C was a poor predictor of exoskeleton content ($R^2 = 0.1$), but
378 metabolizable C was a strong inverse predictor of exoskeleton ($R^2 = 0.9$). Arthropods that had
379 low exoskeleton content had high metabolizable C content, suggesting that consumers of
380 arthropods gain more metabolizable carbon from prey low in exoskeleton content (Bell 1990;
381 Weiser et al. 1997).
382 Prey availability is important to consumers in that it influences nutrient intake. Yet, not
383 all prey are equal in their nutrient content. Common arthropod prey vary significantly in the
384 content of nutrients and indigestible components. Consuming prey high in indigestible content
385 likely decreases overall nutrient intake and could have consequences for growth or survival (Hejl
386 and Verner 1990; Miles 1990; Sakai and Noon 1990; Kaspari and Joern 1993; Morrow et al.
387 2015). Ongoing declines in grassland arthropods and birds necessitate increased understanding of
388 the interactions that determine the growth and survival of these species (Brennan 1991;

Hernandez et al. 2013; Sanchez-Bayo and Wyckhuys 2019). In addition, conditions that alter the community composition of arthropods in ways that shift the relative balance of high versus low exoskeleton prey could have consequences for insectivore growth, even if the overall abundance of prey does not change. For example, Reeves et al. (*In review)* found that prescribed burning significantly increased the abundance of ants, which have high exoskeleton and low protein content, at the current study site. Our results suggest that measures of food availability for animals that feed on arthropods should consider more than the abundance and diversity of these prey as different orders of arthropods can vary significantly in their nutrient availability and digestibility for consumers.

Declarations

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Consent to Participate: Not applicable.

Consent for Publication: Not applicable.

Authors' contributions: Author Contributions: All authors conceived and designed the experiments. JTR collected, performed nutrient extraction on, and analyzed the data. JTR and SMW wrote the manuscript; other authors provided editorial advice.

Data Accessibility: Arthropod nutrient content data: Dryad doi:10.5061/dryad.t76hdr81b

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Table 1. AICc model selection of linear models of exoskeleton, lipid, and protein content (Lowry and Bradford). Exoskeleton and lipid content were predicted using total and metabolizable C, and protein content was predicted using total and metabolizable N. Models within delta AICc < 2.00 are considered to receive equal support.

Model Response	Predictor	K	AICc	delta AICc	Model Weight	Cumulative Weight
Lipid	Total C	3	484.56	0.00	1	1
	Metabolizable C	3	506.76	22.20	0	1
	Null	2	510.10	25.54	0	1
Exoskeleton	Metabolizable C	3	468.05	0.00	1	1
	Total C	3	604.29	136.24	0	1
	Null	2	610.55	142.50	0	1
Lowry Protein	Metabolizable N	3	494.67	0.00	1	1
	Total N	3	530.01	35.35	0	1
	Null	2	541.76	47.10	0	1
Bradford Protein	Metabolizable N	3	547.98	0.00	0.93	0.93
	Total N	3	553.19	5.21	0.07	1
	Null	2	566.97	18.99	0	1

Figure Legends

Figure 1. Indigestible (exoskeleton) and macronutrient (lipid and protein) content of 72 arthropods as a proportion of total dry mass (mg/100mg dry mass). Protein content was measured by the Lowry and Bradford assays. Orders not connected by the same letter are significantly different ($p < 0.05$).

Figure 2. Total and metabolizable C content of 72 arthropods as a proportion of dry mass. Orders not connected by the same letter are significantly different ($p < 0.05$).

Figure 3. Total and metabolizable N content of 72 arthropods as a proportion of dry mass. Orders not connected by the same letter are significantly different ($p < 0.05$).

Figure 4. Linear models of total and metabolizable C content with lipid and exoskeleton content of 72 arthropods. All values are displayed in mg/100 mg dry mass.

Figure 5. Linear models of total N, metabolizable N, and protein content measured by the Lowry and Bradford assays. All values are displayed in mg/100 mg dry mass.

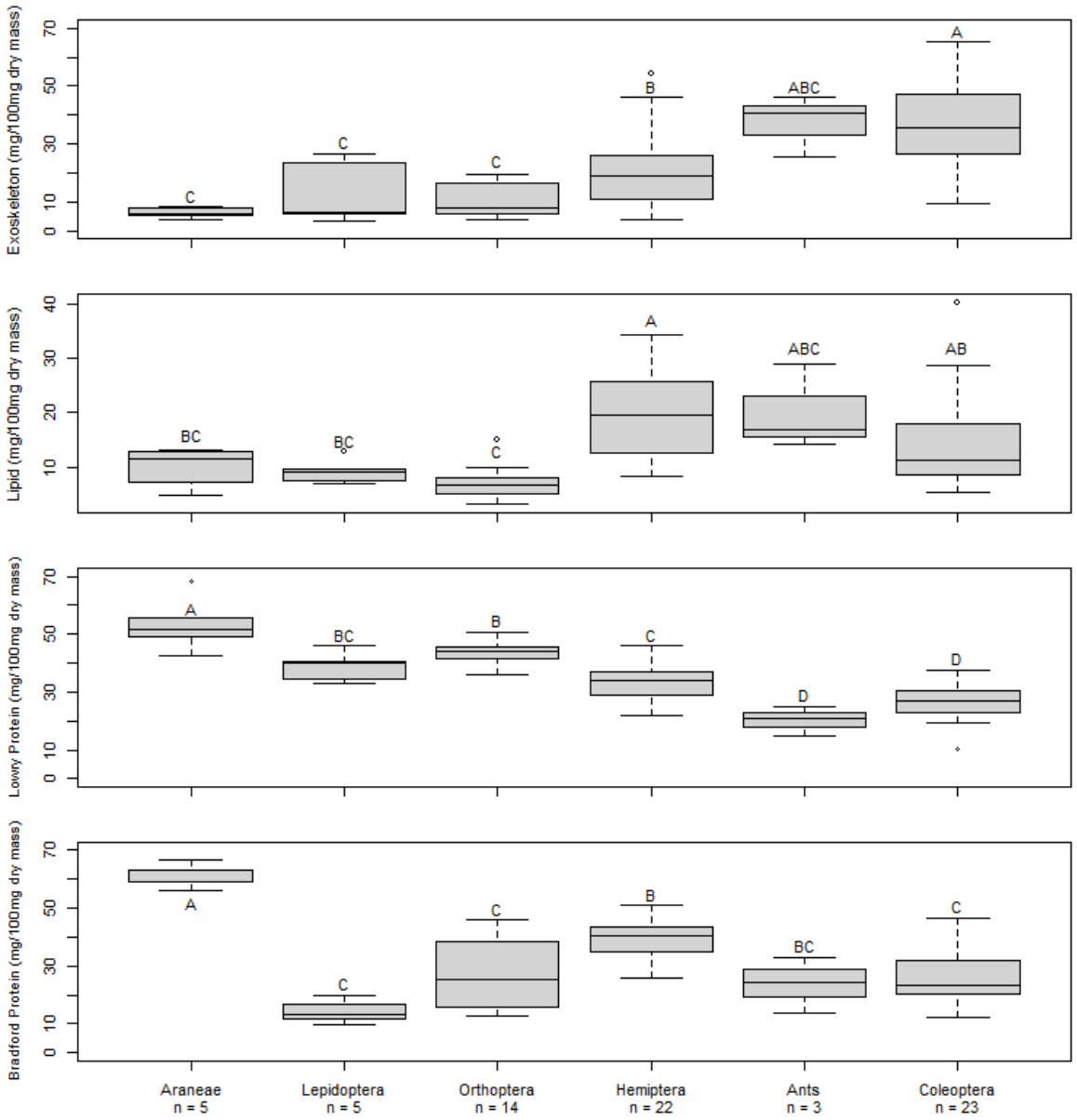
Exoskeleton (mg/100mg dry mass)
0
10
30
50
70
C
C
C
B
ABC
A
Lipid (mg/100mg dry mass)
10
20
30
40
BC
BC
C
A
ABC
AB
Lowry Protein (mg/100mg dry mass)
0
10
30
50
70
A
BC
B
C
D
D
Bradford Protein (mg/100mg dry mass)
0
10
30
50
70
A
C
C
B
BC
C
Araneae
n = 5
Lepidoptera
n = 5
Orthoptera
n = 14
Hemiptera
n = 22
Ants
n = 3
Coleoptera
n = 23

Figure 1.

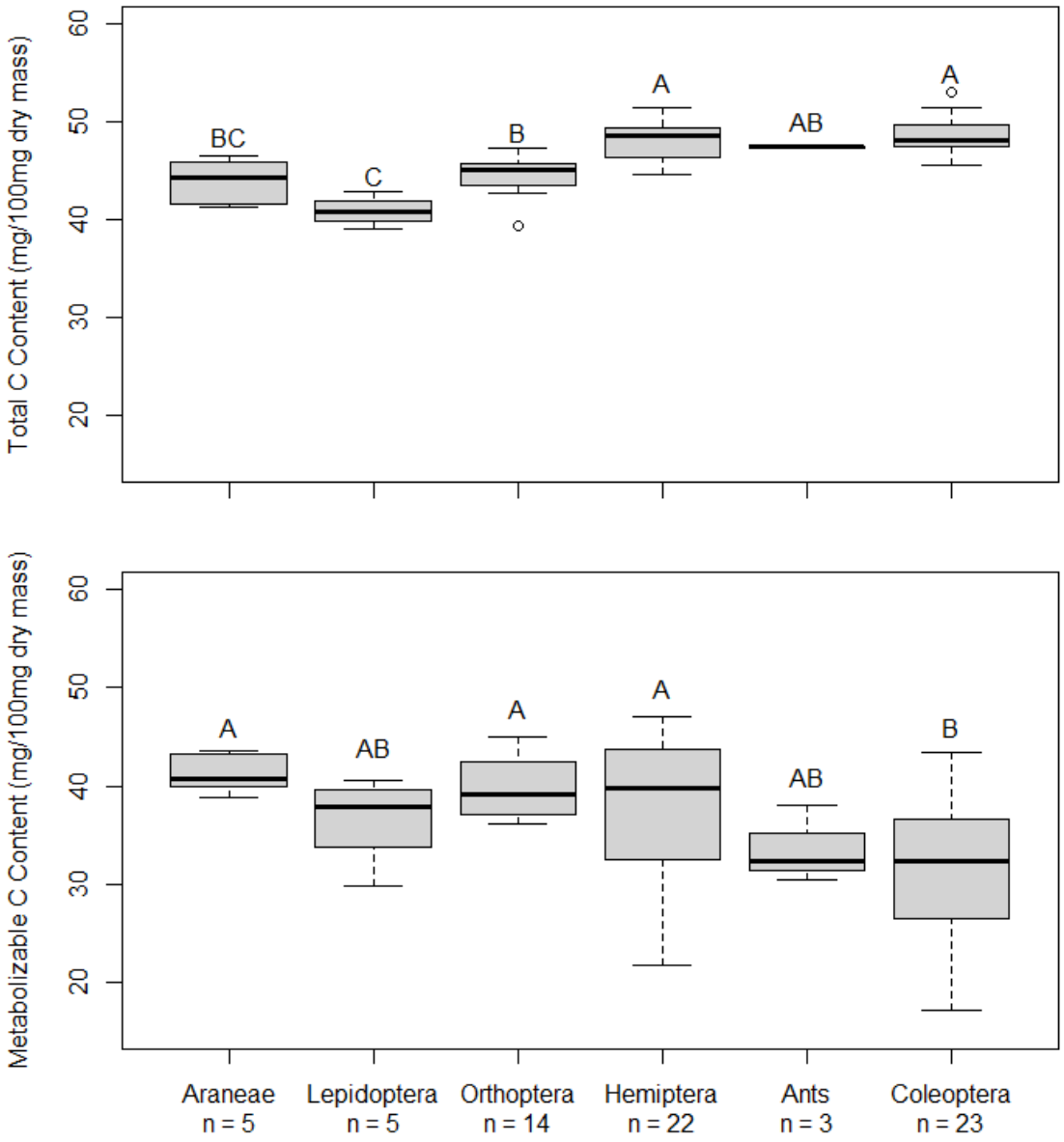
Total C Content (mg/100mg dry mass)
20
30
40
50
60
BC
C
B
A
AB
A
Metabolizable C Content (mg/100mg dry mass)
20
30
40
50
60
A
AB
A
A
AB
B
Araneae
n = 5
Lepidoptera
n = 5
Orthoptera
n = 14
Hemiptera
n = 22
Ants
n = 3
Coleoptera
n = 23

Figure 2.

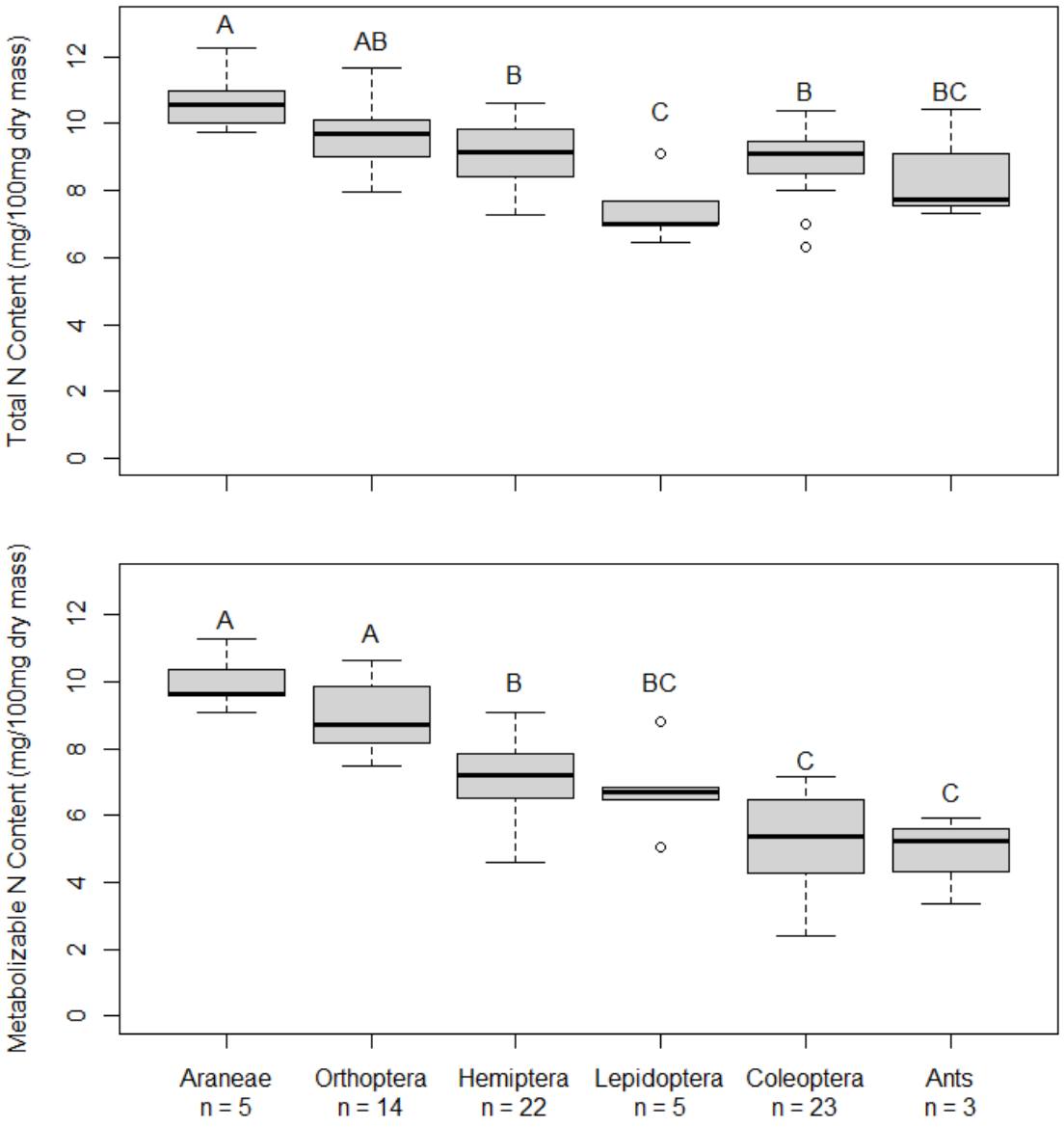
Total N Content (mg/100mg dry mass)
0
2
4
6
8
10
12
A
AB
B
C
B
BC
Metabolizable N Content (mg/100mg dry mass)
0
2
4
6
8
10
12
A
A
B
BC
C
C
Araneae
n = 5
Orthoptera
n = 14
Hemiptera
n = 22
Lepidoptera
n = 5
Coleoptera
n = 23
Ants
n = 3

Figure 3.

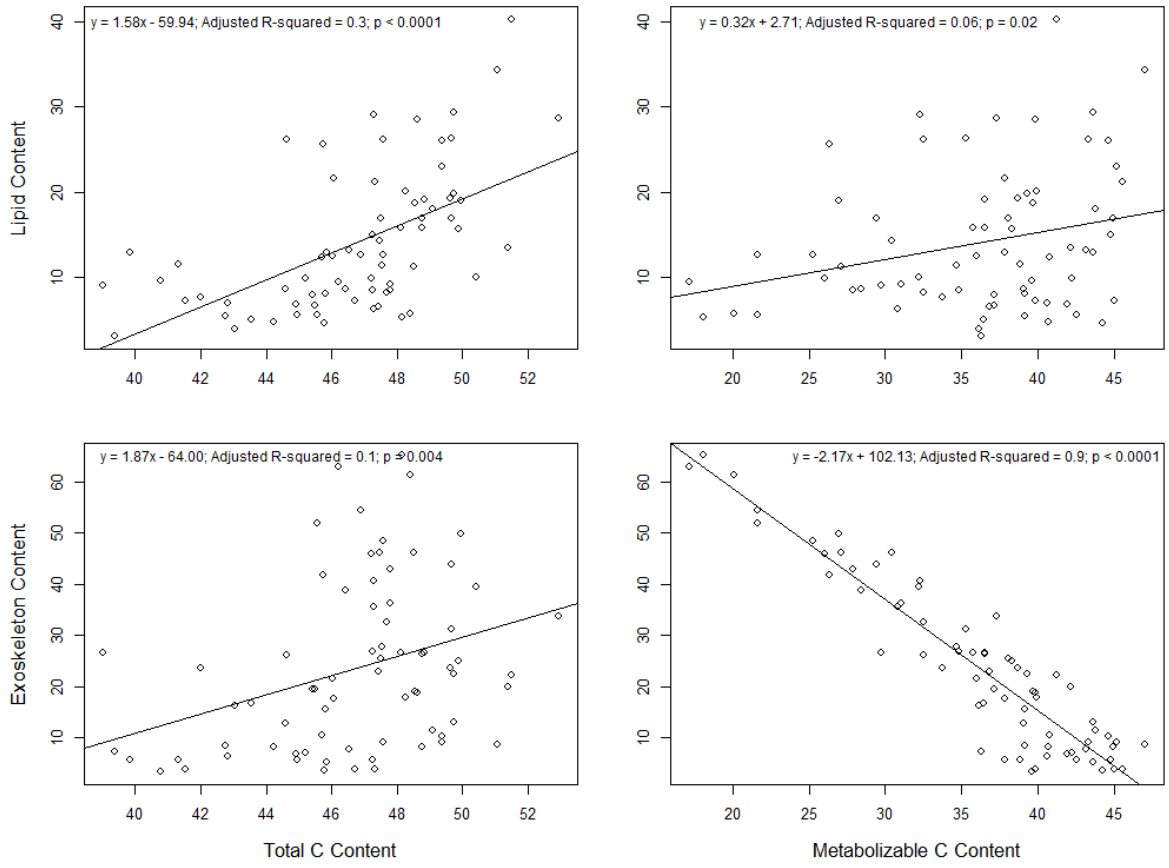
y = 1.58x - 59.94; Adjusted R-squared = 0.3; p < 0.0001
y = 0.32x + 2.71; Adjusted R-squared = 0.06; p = 0.02
y = 1.87x - 64.00; Adjusted R-squared = 0.1; p = 0.004
y = -2.17x + 102.13; Adjusted R-squared = 0.9; p < 0.0001
Lipid Content
Exoskeleton Content
Total C Content
Metabolizable C Content

Figure 4.

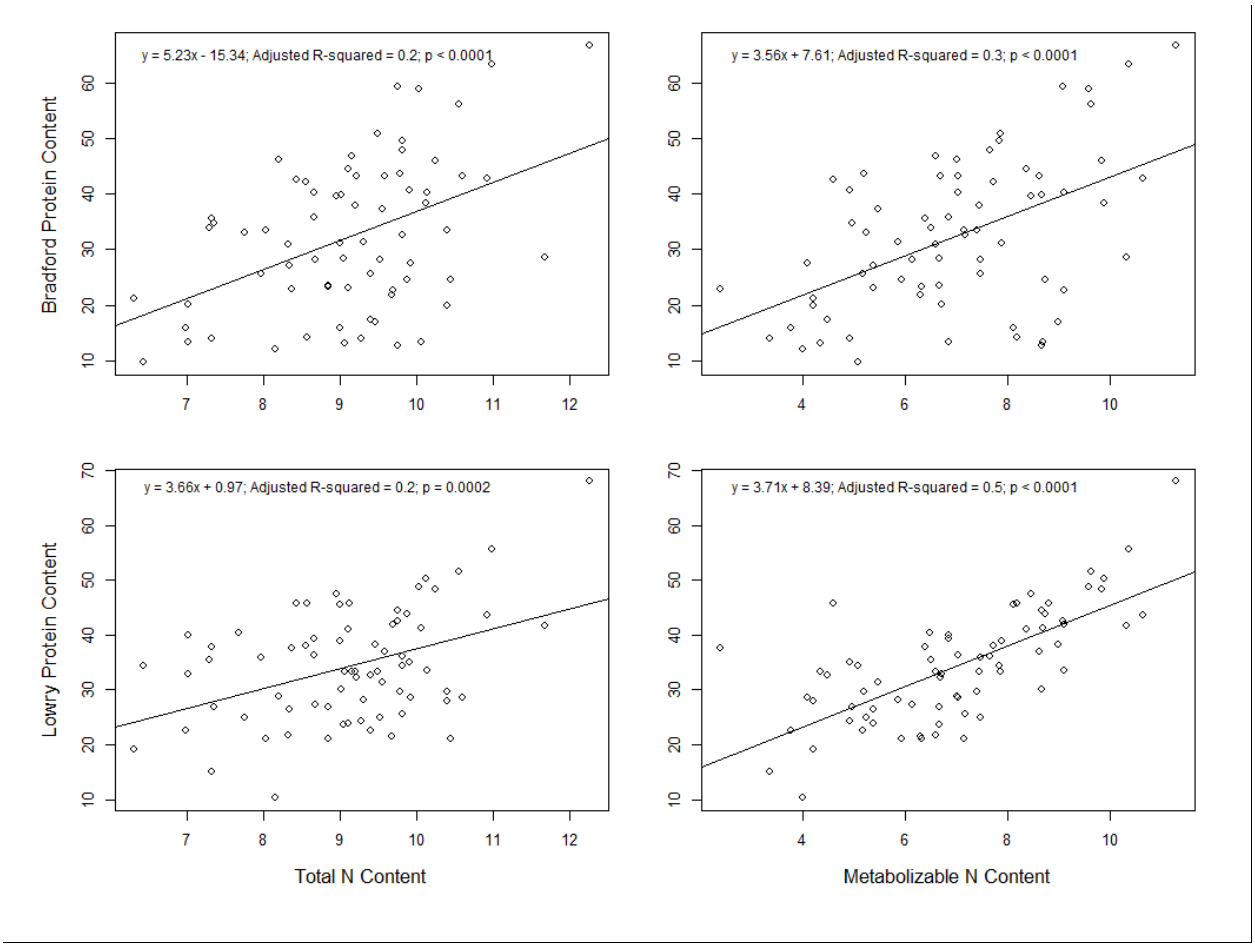
y = 5.23x - 15.34; Adjusted R-squared = 0.2; p < 0.0001
y = 3.56x + 7.61; Adjusted R-squared = 0.3; p < 0.0001
y = 3.66x + 0.97; Adjusted R-squared = 0.2; p = 0.0002
y = 3.71x + 8.39; Adjusted R-squared = 0.5; p < 0.0001
Bradford Protein Content
Lowry Protein Content
Total N Content
Metabolizable N Content

Figure 5.