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The relationship between body mass and elemental composition in nymphs of the grasshopper *Schistocerca americana*

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Abstract

Grasshoppers, like all other organisms, are composed of a combination of elements, but little is known about the extent to which the elemental composition in grasshoppers changes over the course of nymphal development. In this study the grasshopper *Schistocerca americana* was reared on a diet of seedling wheat and wheat germ; nymphs of various mass were collected and analyzed for elemental composition. In total, 12 different elements, including the macroelements carbon, nitrogen, and phosphorus, were quantified. Results show that the amount of a given element increased linearly with increasing body mass, but that the rate of increase differed depending on the element. Results also show that the concentration of some elements dropped dramatically over the course of development. We discuss our results in the context of limiting nutrients and ecological stoichiometry.

Key words

stoichiometry, physiology, nitrogen, carbon, phosphorus

Introduction

All organisms are composed of a mixture of elements, but different organisms have different chemical compositions. This is certainly the case for grasshoppers and the plants on which they feed. A review of the literature suggests that most heterotrophs, such as grasshoppers, show a strong ability to maintain elemental homeostasis via physiological control, and they do this despite changes in the chemical composition of the environment, including their food (Sterner & Elser 2002). However, it is also the case that individual organisms often show differences in elemental composition over the course of their life. For example, elemental composition may vary with sex, instar, and between pre- and postreproductive adults (Sterner & Elser 2002).

The study of elemental balance in organisms is called ecological stoichiometry (ES), and much of what we know about ES in organisms comes from work in aquatic systems. Recently however, a growing number of ES studies have focused on terrestrial organisms, especially insects (*e.g.*, Fagan *et al.* 2002; Schade *et al.* 2002, 2003; Woods *et al.* 2004). Generally speaking, adult insects have been the focus of these studies, with an emphasis on comparing elemental profiles across a number of different species and body sizes. To our knowledge no studies exist comparing elemental composition across a range of sizes and developmental stages for a single species.

In this study, we investigate the relationship between body mass and elemental composition in an immature grasshopper. We used nymphs of the American bird grasshopper, *Schistocerca americana*, reared on a constant natural diet throughout their lifetime, and originating from parents that had also been reared on the same diet. Whole-body nymphs were analyzed for a total of 12 elements, including carbon, nitrogen and phosphorus. We discuss the results from these analyses in the context of limiting nutrients and ecological stoichiometry.

Methods

Experimental insects.—American bird grasshoppers, *S. americana*, came from a culture maintained in the Department of Entomology, Texas A&M University, since 2006. The grasshoppers were fed a diet of greenhouse-grown seedling wheat and wheat germ, and maintained at 29-31°C under a L12:D12 photo-regime. Out of this culture two groups of 15 immature individuals were collected for body elemental composition analysis, and within each group a mixture of instars and body mass was represented. All individuals were transferred separately to vials and frozen for 24 h. Next they were dried in an oven at 40° C for 72 h, and then weighed to the nearest 0.1 mg. They were then returned to the drying oven for an additional 24 h, and individuals reweighed to confirm they had reached a constant dry mass.

The first group of insects was prepared for nitrogen (N) and carbon (C) analysis. Here a small magnetic stir bar was placed inside each vial, the vial was capped, and then vortexed for one minute to completely homogenize the dry sample. Each sample was then weighed to the nearest 0.1 mg, wrapped in a small sheet of tin foil, and placed individually into stainless steel crucibles. The samples were next placed in an Elementar vario MAX CN high temperature carbon-nitrogen analyzer set at 950° C and analyzed using methods similar to those discussed by McGeehen and Naylor (1988).

The second set of individuals was prepared for phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) analysis. The samples were ground as described above, weighed to the nearest 0.1 mg, and then transferred to polypropylene digestion tubes. The samples were digested using trace-metal grade nitric acid on a 105°C graphite block. Following digestion, samples were brought to volume and analyzed using a Spectro axial CIROS inductively coupled plasma [Atomic Emission Spectrometry] (Havlin & Soltanpour 1980).

Data analysis.—Two relationships were analyzed and plotted: 1) total element amount against body dry mass, and 2) element concentration against body dry mass. Additionally, the ratios of carbon:nitrogen (C:N), nitrogen:phosphorus (N:P), and carbon:

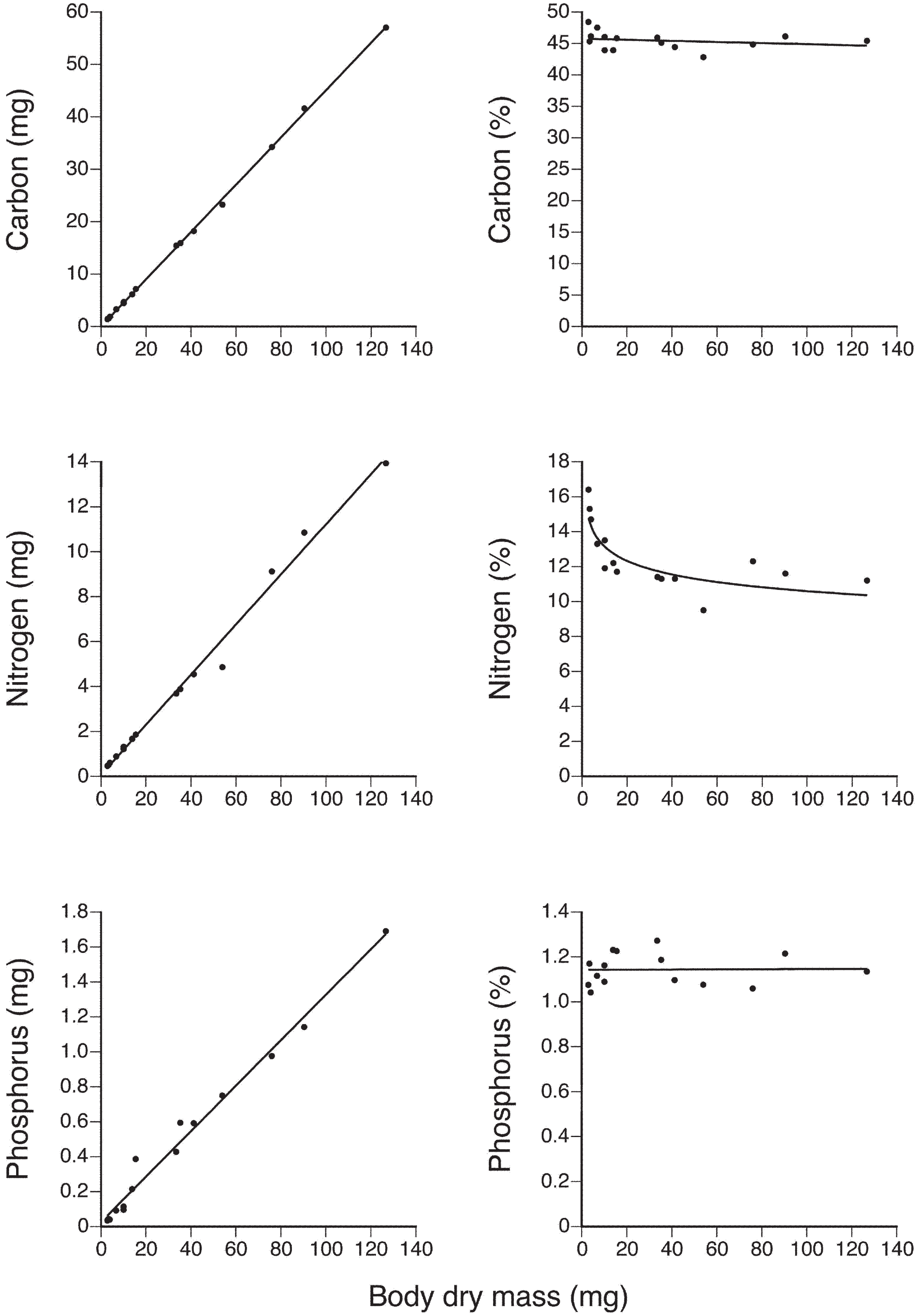

Fig. 1. Total amounts and concentrations of carbon (C), nitrogen (N) and phosphorus (P) plotted against body dry mass.

Table 1. Curve fits and regression formulae for each element plotted against body dry mass (mg). In all but one instance (Iron), linear curve fits provided the best fit.

Element	Curve fit	R^2	Units	Regression equation
Nitrogen (N)	linear	0.990	mg	y = 0.112*x + 0.064
Carbon (C)	linear	0.999	mg	y = 0.451*x + 0.024
Phosphorus (P)	linear	0.984	mg	y = 0.013*x + 0.025
Sulfur (S)	linear	0.997	µg	y = 5.731*x + 7.496
Potassium (K)	linear	0.987	µg	y = 9.730*x + 0.000
Sodium (Na)	linear	0.967	µg	y = 1.437*x + 175.710
Calcium (Ca)	linear	0.878	µg	y = 0.737*x + 58.862
Magnesium (Mg)	linear	0.964	µg	y = 2.540*x + 0.957
Zinc (Zn)	linear	0.986	µg	y = 0.141*x + 0.198
Iron (Fe)	power	0.861	µg	$y = -3.99 * x*^{1.34}$
Manganese (Mn)	linear	0.983	µg	y = 0.026*x + 0.443
Copper (Cu)	linear	0.798	µg	y = 0.027*x + 0.338

phosphorus (C:P) were plotted. For each relationship a regression equation and R^2-value is presented, both of which were obtained using the statistical package JMP 7.0.1 (SAS Institute, Inc.).

Results

Carbon, nitrogen and phosphorus.—The relationships of carbon, nitrogen and phosphorus with body dry mass are shown in Figure 1, and each increases linearly with body dry mass. Carbon and phosphorous constitute approximately 45% and 1%, respectively, of the total dry mass of *S. americana* (Table 1), and their proportion remains fairly constant throughout the grasshoppers' entire nymphal development. In contrast, nitrogen concentrations are much higher in first (15-17%) and second instars (13-15%), compared to later instars (10-12%).

The ratio of these elements to one another was also explored (Fig. 2). In the first two instars, when the grasshoppers are still quite small, the C:N ratio increases, the C:P ratio remains constant, and the N:P ratio decreases. During the latter instars, the ratios remain rather constant (C:N = 4, C:P = 40, and N:P = 10).

The other elements.—The amount of sulfur increases linearly as grasshoppers grow, adding 5.7 µg of sulfur for every 1 mg increase in dry body mass (Table 1). However, sulfur concentration remains reasonably steady throughout development (Fig. 3).

The alkali metals sodium and potassium, and the alkaline earth metals calcium and magnesium, also increase linearly as grasshoppers grow. Interestingly, the sodium and calcium concentrations, and to a lesser extent magnesium concentrations, are greatly elevated in the first couple of instars compared to the later and much larger instars. In contrast, potassium levels are slightly lower in early instars compared to later instars, but as grasshoppers become larger the tissue concentrations of potassium seem to stabilize between 8000-10000 ppm (Fig. 4). It is also worth noting here that the y-intercept for sodium and calcium are very high. This is in contrast to all the other elements, which tend to have a y-intercept that approximates zero (Table 1).

Lastly, the transition elements zinc, iron, manganese and copper, are shown in Figure 5. All increase linearly as dry body mass increases, except for iron, which increases exponentially, but very weakly (Table 1). In all instances these increases are very minor: for example 0.140 µg of Zn, 0.026 µg of manganese, and 0.027 µg of copper for every 1 mg increase in body dry mass (Table 1). However, the concentrations of these metals in the body differ depending

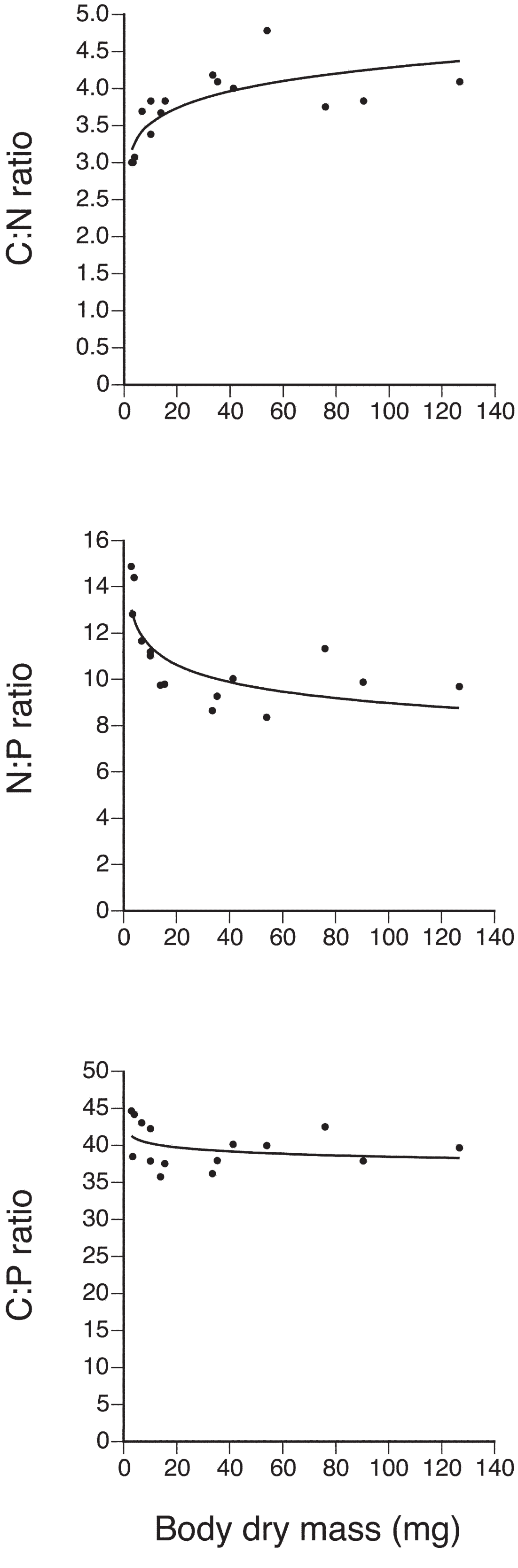

Fig. 2. Ratios of C:N, N:P, and C:P plotted against body dry mass.

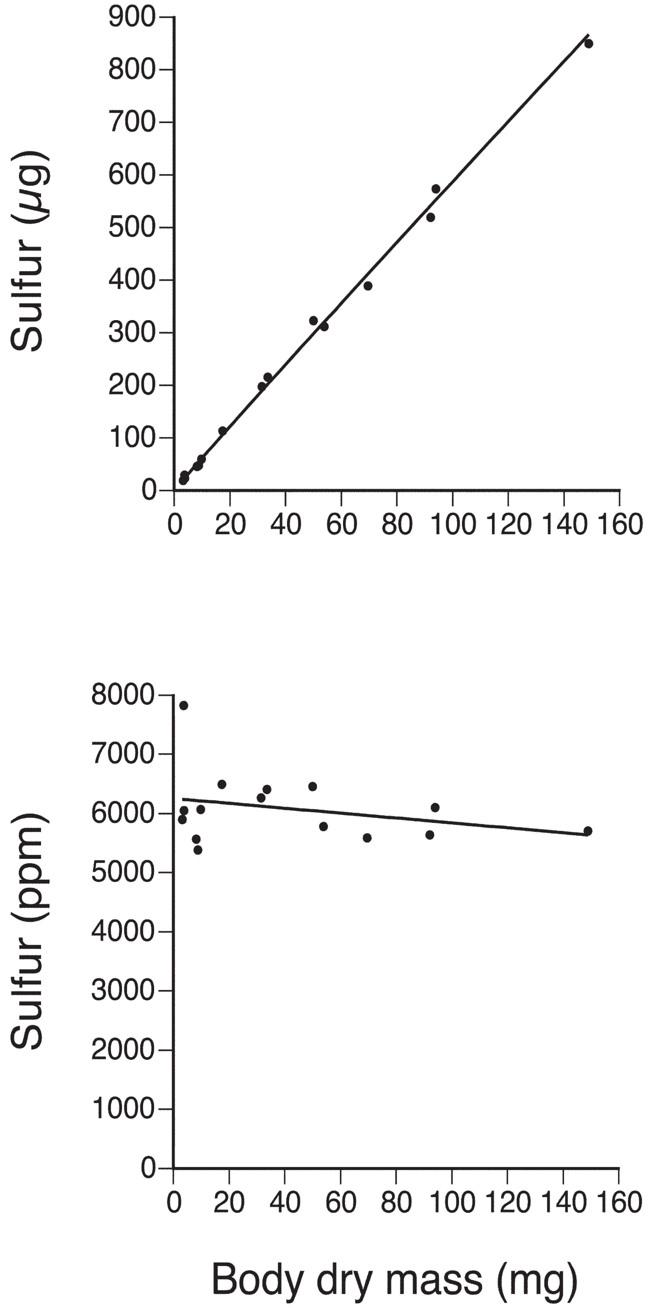

Fig. 3. Total amount and concentration of sulfur (S) plotted against body dry mass.

on the age of the grasshoppers. Manganese and copper are both initially high in 1st-instars, and then drop rapidly in the 2nd instar before leveling-off in later instars. In contrast, the concentrations of zinc and iron gradually drop and increase, respectively, as body dry mass increases.

Discussion

To our knowledge this is the first paper that provides a detailed report on elemental body composition for a range of mass in an immature grasshopper reared on a constant diet — plus having had parents that were reared on the same diet. Our results show that in almost all cases the absolute amounts of individual elements in the body increase linearly as body mass increases, but that the absolute amounts of any particular element and its rate of increase, differ depending upon the specific element. The results also indicate that the elemental body composition, expressed as a percentage of the body mass, does one of two things as grasshopper nymphs develop to adulthood. It either: 1) remains relatively constant, or 2) decreases substantially over the course of the first few instars.

Carbon, nitrogen and phosphorus are three of the main constituents in biological structural molecules, including proteins, nucleic acids, lipids, energetic nucleotides, carbohydrates, and pigments (Sterner & Elser 2002). Carbon comprises a significant portion of all of these compounds, and in our grasshoppers carbon consistently comprised almost half of the total body mass over the course of development. The Texas field cricket, *Gryllus texensis*, has shown a similar carbon body profile (Bertram *et al.* 2008).

Phosphorus, which occurs primarily in nucleic acids and energetic nucleotides (*e.g.*, ATP), also remained at a constant level (≈1%) throughout immature development. This result contrasts with other studies on adult terrestrial insects that show P is inversely related to body size (Fagan *et al.* 2002, Woods *et al.* 2004).

With respect to nitrogen body content, we found that values were much higher in early instars (1st through 3rd) compared to later instar grasshoppers (4th through 6th). This contrasts with Fagan *et al.* (2003), who found no differences in body nitrogen levels when comparing three species of immature and adult grasshoppers (*S. americana, Melanoplus packardii, and M. angustipennis*). It is not clear what age classes of immatures were analyzed in the Fagan *et al.* study, but a comparison with our results suggests their analysis was likely restricted to later instar grasshoppers. Nitrogen is often considered a limiting nutrient for grasshoppers (Joern & Behmer 1997, 1998, White 1993); but a significant nitrogen investment by females into eggs may provide a nitrogen reserve to be drawn upon during early development, a time when small size may limit young grasshoppers to explore and select foods that best match nitrogen needs. Such high allocation of nitrogen to eggs also may help explain why adult female grasshoppers resorb eggs when reared on nitrogen-poor food sources (Chapman 1998).

We also examined C, N, and P, within an ecological stoichiometry framework (Sterner & Elser 2002). The most frequently examined elemental relationship is C:N, and we found that C:N ratios increased in a curvilinear fashion as immature grasshoppers increased in mass. This changing relationship was a function of body nitrogen levels decreasing as grasshoppers moved from early instars to later instars. In contrast, the N:P ratio decreased as grasshoppers grew, which is in contrast to current predictions concerning growth in invertebrates (Sterner & Elser 2002). As for the C:P ratio, no significant trend was observed as grasshoppers developed through successive immature stages. It is worth noting again here that these ratios were obtained from grasshoppers that had been eating a constant diet of seedling-wheat and wheat germ. It would be interesting to observe the extent to which these ratios change when grasshoppers are reared on a range of different diets, for example a forb-only diet, or one that contains a mix of grasses and forbs.

The remaining elements analyzed in this study include the macro-element sulfur, four major ions that are essential in the function of cells (K, Na, Ca, and Mg), and four trace metals that provide catalytic function (Zn, Fe, Mn, Cu). The amino acids methionine (essential) and cysteine (nonessential) are the main source of sulfur in our grasshoppers, and tissue levels of sulfur remained constant throughout development.

In contrast, none of the major ions remained constant through-

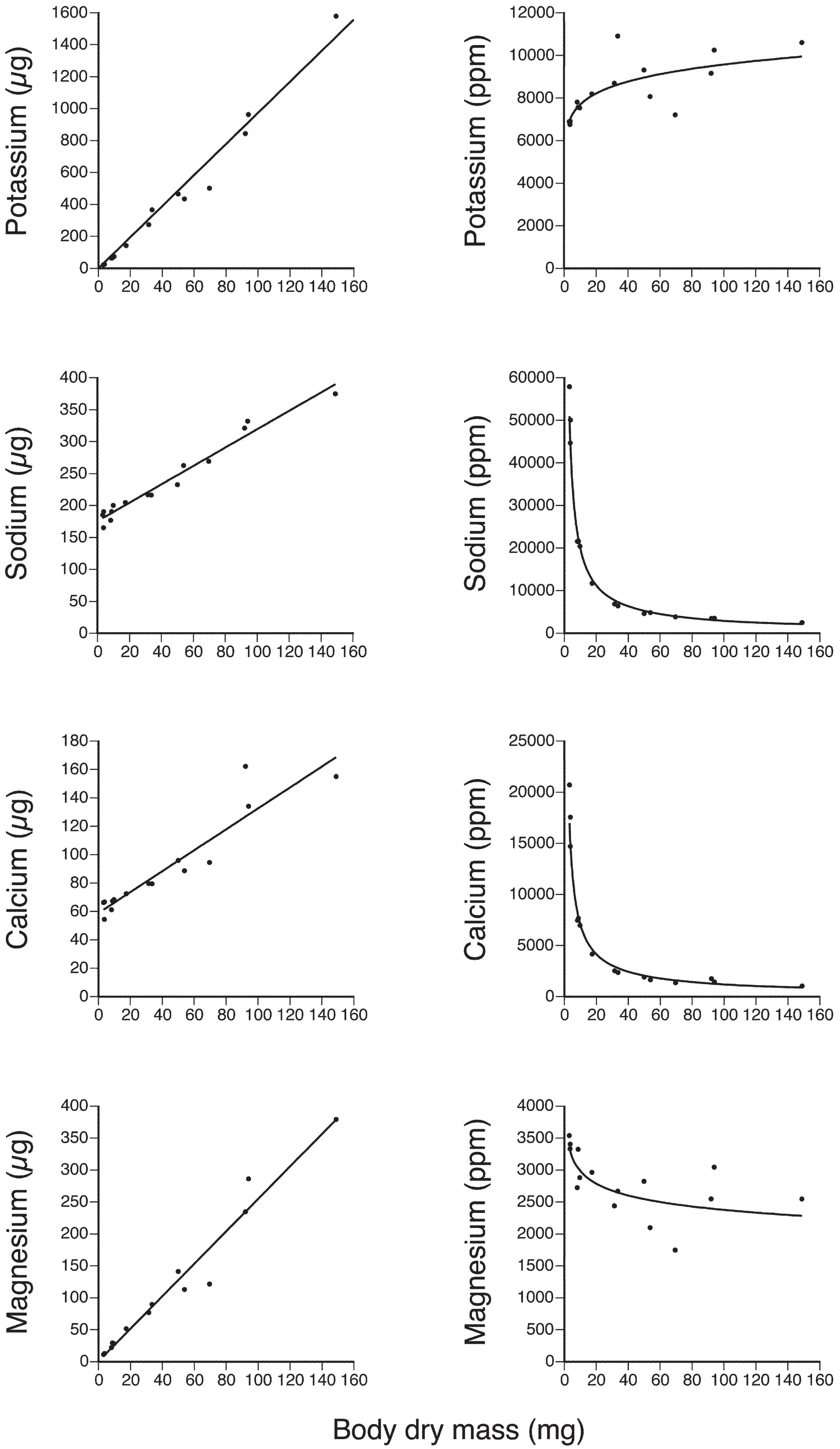

Fig. 4. Total amounts and concentrations of potassium (K), sodium (Na), calcium (Ca) and Magnesium (Mg) plotted against body dry mass.

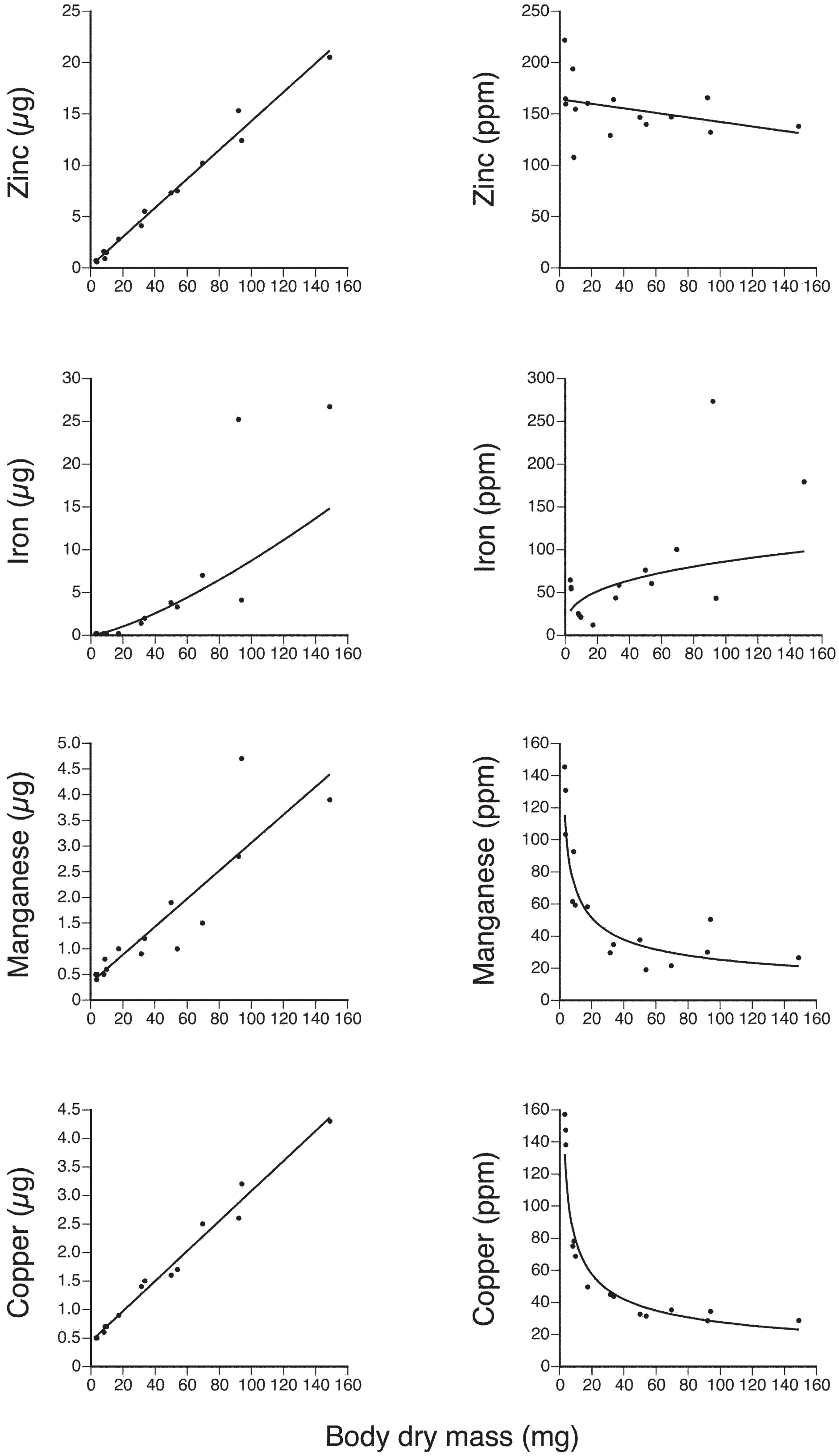

Fig. 5. Total amounts and concentrations of zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) plotted against body dry mass.

out development. For instance, sodium, which is critical for nerve cell function, and calcium, which is involved in muscle contraction, showed huge drops in concentration through the first three instars, but then leveled off in the last three instars. The very high initial levels indicate that females allocate significant quantities of these two ions to eggs, and that these two micronutrients might be limiting for reproducing female grasshoppers. High allocation of sodium and calcium to eggs may be important, given the degree to which the concentrations of these ions vary between different plant species, and given the high growth rates grasshoppers undergo during the first three instars (a 10-fold increase over a period of about 12 days). Tissue concentrations of potassium (used in nerve cells) and magnesium changed slightly over the course of development, but were small relative to those observed for sodium and calcium. Among the trace metals, which are all found in the hemolymph (Chapman 1998), the concentrations of manganese and copper dropped sharply as body size increased. These two trace metals serve important roles in catalytic reactions involving oxygen, so a large allocation of these two metals to eggs may be important during the first few instars, where growth occurs rapidly.

In summary, this paper provides a detailed picture of elemental relationship with increasing body mass in grasshopper nymphs, and clearly shows it is a dynamic process. It provides a baseline dataset from which we can begin to explore in more detail the manner in which elemental composition changes as grasshoppers transition from somatic to reproductive growth. It also allows us to begin to explore how foods with different nutritional compositions might influence the homeostatic control of elemental composition. It is our aim to begin to study, using the experimental design of the "Geometric Framework" (Raubenheimer & Simpson 2004), how such food imbalances influence the stoichiometric balance of a range of insect herbivores, including generalist and specialist grasshoppers.

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