

# Larval nutrition affects lipid storage and growth, but not protein or carbohydrate storage in newly eclosed adults of the grasshopper *Schistocerca americana*

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## Abstract

Nitrogen availability from dietary protein can have profound effects on the physiology and evolutionary ecology of insect herbivores. While many studies consider the effects of nutrition on consumption and gross body composition of protein and other important nutrients, few consider partitioning to storage for future use. I used chemically defined artificial diets to quantitatively manipulate the amount of dietary carbohydrates and proteins available to growing larvae of the grasshopper *Schistocerca americana* to determine how larval nutrient availability affects growth and all three classes of stored nutrients (proteins, lipids, and carbohydrates) carried over from larval feeding into adulthood. Larvae on poor diets increased consumption, but could not compensate for diet quality, eclosing small and containing no significant nutrient stores at adulthood. Individuals fed intermediate to high nutrient content diets as larvae were significantly larger and contained a significantly greater proportion of lipid stores at adult eclosion, but not protein or carbohydrate stores than individuals fed low nutrient content diets. This suggests that larval derived lipid stores may be more important to adult fitness than carbohydrate or protein stores. This result is contrary to previous studies performed on the role of larval nutrition and allocation to protein stores, and this difference is likely due to variation in the relative availability of protein in adult diets across species.

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**Keywords:** Diet manipulation; Body size; Storage protein; Lipid; Protein partitioning

## 1. Introduction

Numerous ecological and nutritional studies suggest that the nitrogen from dietary protein is a limiting resource for herbivorous insects. Specifically, relative protein availability has been shown to affect important individual-level fitness-associated traits such as body size, growth rate, and fecundity; and at higher levels of organization has been linked to population dynamics, life histories, and even biological diversification (McNeill and Southwood, 1978; Slansky and Feeny,

1979; Mattson, 1980; Slansky and Scriber, 1985; Simpson et al., 1995; Joern and Behmer, 1997; Fagan et al., 2002). Therefore understanding protein partitioning and the physiology of protein utilization in herbivorous insects is critical to understanding the ecology, evolution, and physiology of insect herbivores; and to developing strategies to control economically important herbivorous insect pests.

Much work has focused on the effects of diet on total body protein content in phytophagous insects, and fine scale analyses of dietary protein and carbohydrate intake and output in feces have been used to understand patterns of nutrient uptake and processing (Slansky and Scriber, 1985; Zanotto et al., 1993, 1997; Simpson et al., 1995; Telang et al., 2004). However, relatively little attention has been paid to how protein is partitioned

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within an organism (Anderson et al., 2005). The effects of nutrient availability on allocation of resources to growth, reproduction, and lipid storage have been studied in a wide range of organisms. However, allocation to stored protein is often ignored with storage typically subsumed within estimates for protein growth (Slansky and Scriber, 1985; Zanotto et al., 1993; Raubenheimer and Simpson, 1994; O'Brien et al., 2002; Simpson et al., 2002). This is unfortunate because stored protein reserves can be both ecologically and physiologically important. Stored reserves allow insects to decouple times of resource-need from times of availability; facilitating the synchronization of life cycle events in response to environmental variation. Therefore, understanding protein economy in herbivorous insects necessitates understanding allocation to protein storage in the context of both dietary nutrient availability and allocation to other parameters such as lipid storage and somatic growth.

Hexameric storage proteins are the primary nitrogenous reserve in most insects (Telfer and Kunkel, 1991; Burmester, 2001, 2002; Goldstrom et al., 2003). Insects can accumulate significant storage protein reserves (e.g., up to 17% of the dry weight of *Heliothis virescens* pupae), and these storage proteins are known to play important roles in molting and reproduction (Telfer and Kunkel, 1991; Pan and Telfer, 1996, 2001; Seo et al., 1998; Burmester, 2001, 2002; Telang et al., 2002). However, studies of the effects of nutrition on the expression and accumulation of these proteins have mostly compared starved and well-fed individuals (Tojo et al., 1981; Riddiford and Hice, 1985; Tojo et al., 1985; Kumaran et al., 1987; Webb and Riddiford, 1988). Only three other studies have quantitatively manipulated larval nutrition and measured effects on storage protein content, and the life histories of the insects used in these studies has varied widely. First, Telang et al. (2002) used chemically defined artificial diets to manipulate the ratio of carbohydrate and protein available to larvae of the moth *H. virescens*, showing that larvae reared on foods with a low protein to carbohydrate ratio gained less mass, grew more slowly, and contained significantly less storage protein reserves than individuals fed balanced or high protein diets. Second, by limiting food availability, Hatle et al. (2001) showed that nutritionally restricted adult *Romalea microptera* grasshoppers accumulated storage proteins more slowly and had delayed reproduction compared to well-fed animals. Third, Telang and Wells (2004) manipulated larval food availability in the autogenous mosquito, *Ochlerotatus atropalpus*, and showed that well-fed female larvae produced larger, more fecund adults with greater lipid, glycogen, and storage protein reserves. Before we can understand how protein resources are partitioned in herbivorous insects, additional studies of the effects of protein availability on protein storage are needed within this group.

I manipulated larval nutrition to determine the effects of nutrient limitation on growth and all three major nutrient storage parameters, protein, lipid, and carbohydrates, at the last larval-adult molt in the grasshopper *Schistocerca americana* (Downer and Matthews, 1976; Steele, 1985; Burmester, 2001). The last larval-adult molt represents an important physiological and life history transition in insects. Resources accumulated during larval feeding that are not used for maintenance (e.g., basal metabolism) or molting can be allocated toward two major life history parameters: (1) growth and the resultant adult body size, or (2) storage for later use in adulthood. Adult body size is a correlate of fitness in many organisms and in insects large body sizes have been associated with greater survival and fecundity (Roff, 1992; Stearns, 1992; Nylin and Gotthard, 1998; but see Leather, 1988). However, some insects are known to carry significant nutrient reserves over from larval life into adulthood (Boggs, 1981; Wheeler, 1996; Raubenheimer and Simpson, 1997; Telang et al., 2002). These resources can be used for a variety of adult functions including: dispersal, post-molting somatic growth (e.g., mass gain in internal tissues), reproduction, and maintenance. Protein stores carried over from larval life may play important roles in adults, particularly in nitrogen-limited consumers such as herbivorous insects. A recent study of storage protein accumulation and utilization in *S. americana* suggested that these proteins play roles both in molting and reproduction and that larvally derived protein stores are carried over into adulthood (Hahn and Wheeler, 2003).

The goal of the current study was to determine whether allocation to growth and all three major categories of stored nutrients at adult eclosion in *S. americana* would respond to larval nutrient availability or whether allocation to some categories of storage would be fixed and others flexible. Therefore, I manipulated nutrient availability in growing *S. americana* larvae by restricting them to chemically defined artificial diets that varied in their carbohydrate and protein content. If larvally derived protein stores play an important role in adults, I expected that protein stores at adult eclosion would increase with dietary protein availability. Because compensatory feeding behavior may allow animals eating dilute diets to approach their optimal nutrient intake by increasing consumption, I quantified both total food intake and intake of the primary macronutrients, proteins and carbohydrates, across diet treatments (Slansky and Scriber, 1985; Simpson et al., 1995). In addition, nutrient intake and processing can also be time-limited. Therefore, I quantified development time and growth rate as two measures of performance across diet treatments (Slansky and Scriber, 1985; Simpson et al., 1995; Abrams et al., 1996; Nylin and Gotthard, 1998; Flanagan et al., 2000).

## 2. Methods

### 2.1. Insects and rearing

Insects were obtained from a *S. americana* Drury colony maintained at The University of Arizona. This colony was founded from individuals collected near Lake Georgetown, Travis Co., Texas in October 2001. Insects were kept at 28 °C and 20% relative humidity and provided with 100 W light-bulbs for radiant heat on a 14L:10D cycle. First and second instar nymphs were reared in sibling groups in 30 × 30 × 30 cm cages and provided with the standard rearing diet of Romaine Lettuce and wheat germ ad libitum. Upon passing into the third instar, 72 female nymphs were separated and reared individually until adult eclosion in 3 L plastic bottles containing one of six diet treatments. Ninety percent of adult body mass is accumulated in instars 3–6 (Hahn, unpublished), therefore this was an appropriate time-period within larval development to manipulate nutrient availability. Each 3 L bottle contained a 2 × 10 cm strip of 0.5 cm mesh hardware cloth for perching, one 10 ml plastic Petri dish with a slit cut in the top containing deionized water, and one 10 ml plastic Petri dish containing artificial diet. Nymphs were provided only artificial diet and water from the onset of the third instar until eclosion. Fresh water was provided every day and fresh diet was provided every second day. Within 12 h of eclosion, food was removed and adults were frozen at –70 °C 12–24 h later to allow the cuticle time to harden.

### 2.2. Artificial diet treatments

Artificial diets were based on those for *Locusta migratoria* by Simpson et al. (1988) and have been successfully used in *S. americana* before (Behmer and Elias, 1999). Six artificial diet treatments differing in protein and carbohydrate content were used to manipulate larval resource availability. Diet treatments were as follows: 14%, 28%, 42%, 56%, 70%, or 84% of the dry mass of the diet was a 1:1 ratio of proteins and carbohydrates. The protein source was a 3:1:1 mixture of casein, albumin, and peptone; while the carbohydrate source was a 1:1 mixture of sucrose and dextrin. All diets contained 5% micro-nutrients (vitamins, salts, cholesterol, and linolenic and linoleic acids), and the remainder was a non-nutritive bulking agent (alpha-cellulose). Diets were presented as dried homogenized powders.

### 2.3. Food consumption and development time

Uneaten food and frass were removed every second day, and a known mass of fresh food was supplied. Uneaten food was collected over the course of the

experiment, dried to constant weight in an oven at 60 °C, and weighed. The total amount of food consumed by larvae over the experimental period was the total food provided minus the total uneaten. Macronutrient consumption was calculated as the total amount of food consumed multiplied by the proportion of the diet composed of protein and carbohydrate.

The number of days elapsed between molting into the third instar and adult eclosion was used as development time. There was no significant difference in the masses of 3rd instar larvae at the start of the experiment; therefore, growth rate was calculated as  $(\ln [\text{Wet Weight at Eclosion (g)} - \text{Lipid Mass (g)} - \text{Storage Protein Mass (g)} - \text{Glycogen Mass (g)} - \text{Trehalose Mass (g)}] - \ln \text{Wet Weight at onset of the 3rd instar}) / \text{development time from the 3rd instar until eclosion in days}$ .

### 2.4. Somatic growth

Two estimates of somatic growth were taken: storage-free dry weight and hind femur length. Individuals were freeze-dried to constant mass and weighed. Storage free dry weight was calculated as the total dry weight of the individual minus the four stored materials (i.e., storage free dry weight = dry weight – [total storage protein + total glycogen + total trehalose + total lipid]). After weighing, the length of the left hind femur was measured to the nearest 0.05 mm using a mechanical caliper. Molted exuviae were considered part of the maintenance budget of growing hoppers and are not considered in analyses of growth.

### 2.5. Nutrient storage

Dried adult grasshoppers were homogenized in liquid nitrogen and sub-samples of this homogenate were used for biochemical analysis of storage compounds. Glycogen, trehalose, and lipid content were assessed using the method of Van Handel (1965). This method separates glycogen, trehalose, and lipids. Lipids were further processed to remove phospholipids by running the lipid-containing extract through a column containing 0.2 g of 100-mesh silicic acid which was subsequently washed eight times with 1 ml of pure chloroform each time to elute neutral lipids. Lipids were spectrophotometrically assayed against a range of known standards using the vanillin–phosphoric acid method. Trehalose and glycogen were similarly assayed using the anthrone–sulfuric acid method.

Storage protein content was quantified using a sub-sample of the grasshopper homogenate according to Hahn and Wheeler (2003). Newly eclosed adults contain a single hexameric storage protein identified as *S. americana* persistent storage protein (saPSP). Sub-units of saPSP were clearly identifiable as a single band representing the most abundant soluble protein appearing

on 6–15% SDS–PAGE gradient gels (Hahn and Wheeler, 2003). To quantify saPSP subunits, gels were scanned at 633 nm using a laser densitometer (LKB Ultrascan XL). Standard curves were generated using known quantities of bovine serum albumin (BSA) from 0.2–4.0  $\mu\text{g}$ . Internal standards of 1.0 and 3.0  $\mu\text{g}$  BSA were included on each gel to correct for gel to gel variation.

## 2.6. Statistical analyses

One-way ANOVAs were used to assess whether larval diet had effects on body size, consumption, and development time. Pearsons' correlations were used to determine any relationships between either measure of body size, storage free dry weight or hind femur length, and storage independent of diet. There were positive correlations between both measures of body size and several storage parameters. To determine whether allocation to nutrient storage at adult eclosion was fixed or flexible ANCOVA was used to determine if larval diet had an effect on storage when body size was held constant as a covariate. Analyses did not differ whether storage free dry mass or hind femur length was used as the body size covariate. Therefore, only analyses using storage free dry mass as a covariate are presented. Where necessary, traits were transformed to meet the assumptions of normality and homogeneity of variances. Using the natural logarithm of most variables sufficed, except for hind femur length, where the reciprocal transformation was used. All analyses were performed using the JMP IN statistical package (SAS Institute, 1996).

## 3. Results

### 3.1. Consumption

Total consumption was notably higher in the lowest nutrient concentration diet than any other diet (Fig. 1a, ANOVA,  $F_{5,71} = 79.68$ ,  $p < 0.001$ ). Among the other diets, intake decreased steadily with nutrient concentration. The difference between the lowest concentration diet and all other diets was less pronounced when total macronutrient intake was considered (total proteins+carbohydrates). Total macronutrient intake was lowest on the lowest nutrient concentration diet, and increased as nutrient concentration increased (Fig. 1b, ANOVA,  $F_{5,71} = 3.84$ ,  $p = 0.004$ ).

### 3.2. Development time and growth rate

Total development time was notably longer in individuals fed the lowest nutrient content diet, and not significantly different among individuals fed all

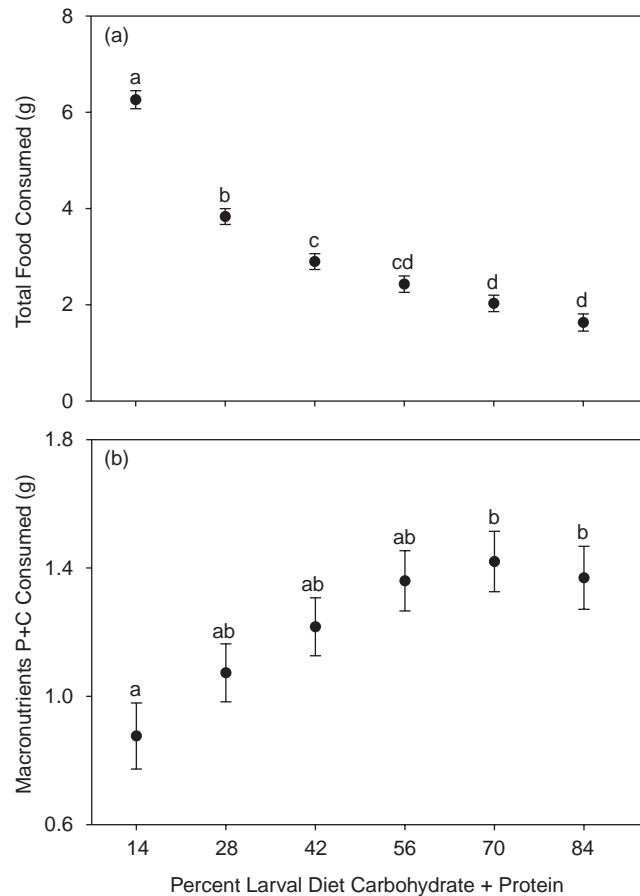


Fig. 1. Effects of larval diet on feeding. (a) Mean quantity of artificial diet consumed by individuals in each treatment group. (b) Mean quantity of macronutrients (proteins+carbohydrates) consumed by individuals in each treatment group. Horizontal bars represent one standard error. The same letter denotes groups that are not significantly different after a Tukey's HSD correction for multiple comparisons ( $\alpha = 0.05$ ).

other diets (Fig. 2a, ANOVA,  $F_{5,68} = 6.99$ ,  $p < 0.001$ ). Similarly, growth rate was lowest among individuals fed the lowest nutrient content diet, followed by the second lowest nutrient content diet, but there were no significant differences among individuals fed diets with greater than 28% nutrient content (Fig 2b, ANOVA,  $F_{5,68} = 6.65$ ,  $p < 0.001$ ).

### 3.3. Somatic growth

Larval diet had significant effects on both storage-free dry mass and hind femur length (Figs. 3a and b, ANOVAs, ln storage-free dry mass,  $F_{5,41} = 6.64$ ,  $p < 0.001$ , and reciprocal hind femur length,  $F_{5,61} = 3.12$ ,  $p = 0.015$ ). Intermediate nutrient content diets supported greater growth than the lowest nutrient content diet for both measures. Although there was no statistically significant difference in body size among the

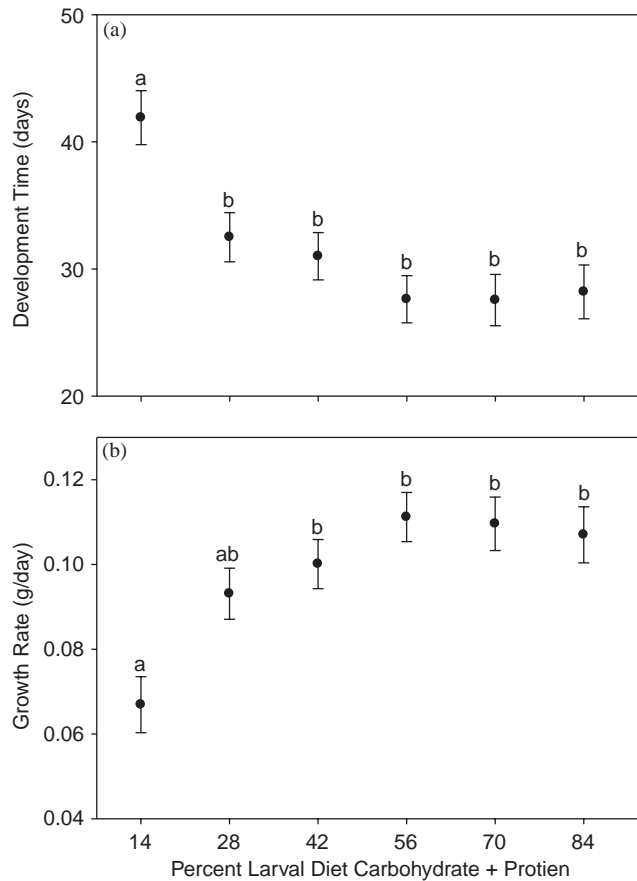


Fig. 2. Effects of larval diet on development time and growth rates. (a) Plot of mean development time in days by larval diet treatment. (b) Plot of mean growth rate by larval diet. Horizontal bars represent one standard error. The same letter denotes groups that are not significantly different after a Tukey's HSD correction for multiple comparisons ( $\alpha = 0.05$ ).

individuals fed diets ranging from 28% to 82% total macronutrients, individuals fed the 56% diet grew the largest and hereafter this diet is termed the best growth diet a designation of convenience for comparison with other treatments.

### 3.4. Nutrient storage

Irrespective of larval diet, larger individuals contained greater lipid, trehalose, and protein stores for both measures of body size, but there was no significant correlation between either measure of body size and glycogen stores (Table 1). Therefore, lipid, trehalose, and protein storage were analyzed using ANCOVA with storage free dry mass as the covariate. Larval nutrition had a large significant effect on lipid stores carried over into adulthood. Individuals fed low nutrient content diets accumulated significantly less lipid stores than individuals on higher nutrient content diets (Fig. 4a, Table 2). There was no effect of larval

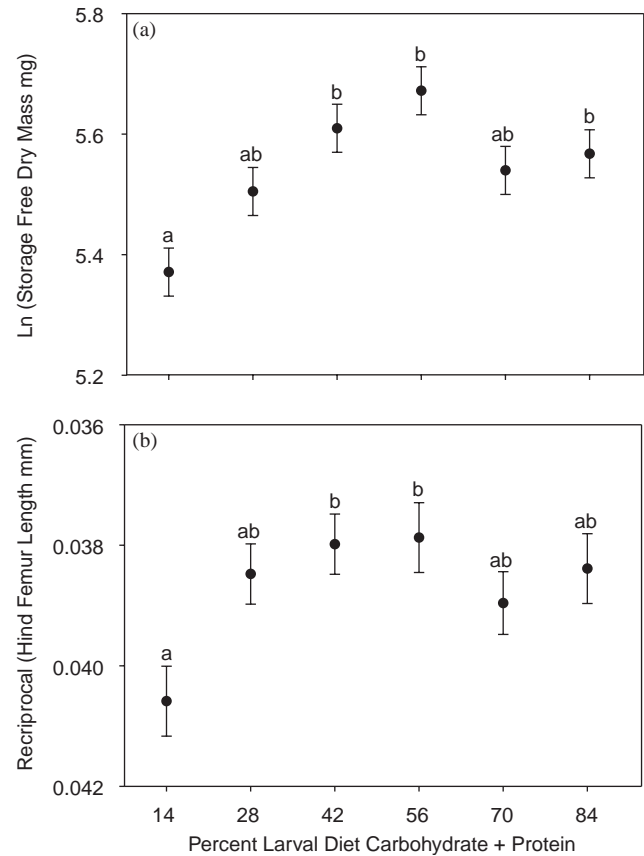


Fig. 3. Effects of larval diet on somatic body size. (a) Plot of the natural log of storage free dry mass by larval diet treatment. (b) Plot of the reciprocal of hind femur length by larval diet treatment. Horizontal bars represent one standard error. The same letter denotes groups that are not significantly different after a Tukey's HSD correction for multiple comparisons ( $\alpha = 0.05$ ).

diet on trehalose or protein stores carried over into adulthood when storage free dry mass was held constant (Figs. 4b and d, Table 2). In addition, there was no effect of diet on glycogen stores (Fig. 4c, ANOVA,  $F_{5,47} = 1.07$ ,  $p = 0.390$ ). Therefore, there was diet-induced flexibility in allocation to lipid storage at adult eclosion, but not glycogen, trehalose, or protein storage.

Nutritionally induced flexibility in lipid but not protein or carbohydrate storage was evident when the proportions of mass at adult eclosion accounted for by somatic growth (storage-free dry mass) and the four stored nutrients was compared (Fig. 5). The proportions of body composition accounted for by protein and carbohydrate stores were not different across the larval diet treatments. In contrast, lipid storage was proportionally much greater on the intermediate 56% P+C diet than the lowest nutrient content diets (14% and 28% P+C) (ANOVA,  $\ln \% \text{ mass lipid}$ ,  $F_{5,49} = 27.45$ ,  $p < 0.001$ ).

Table 1

Pearson's correlations between two measures of somatic growth and nutrient storage parameters amongst individuals across all diet treatments combined

Somatic growth parameter	Nutrient Storage Parameter							
	Lipid		Trehalose		Glycogen		Storage Protein	
	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
Storage free dry mass	0.859	<0.001	0.436	0.004	−0.165	0.296	0.488	<0.001
Hind femur length	0.730	<0.001	0.283	0.054	−0.237	0.109	0.457	<0.001

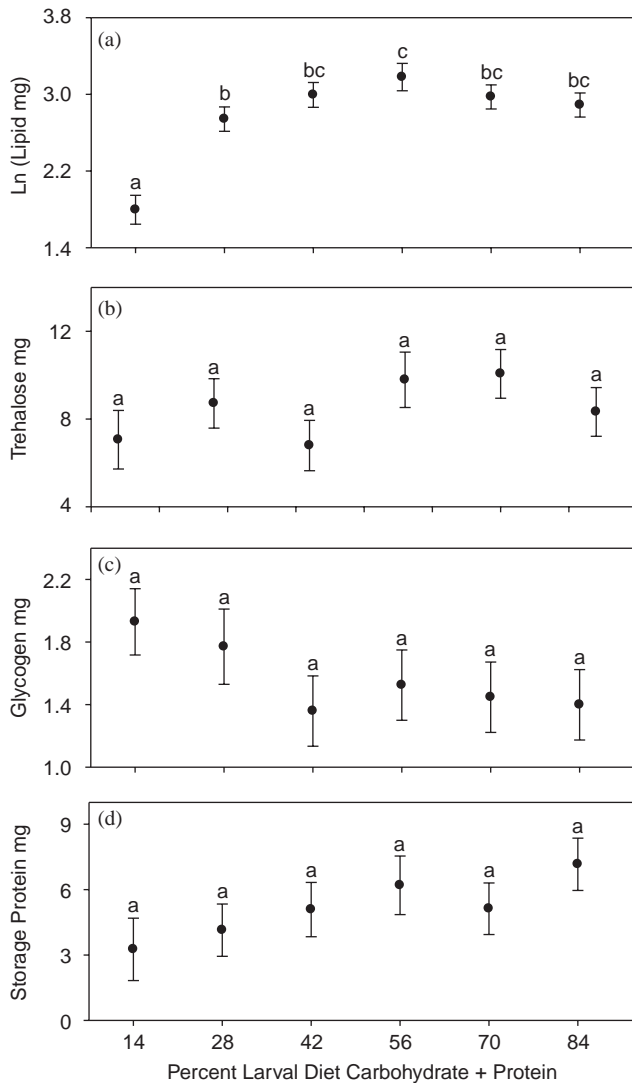


Fig. 4. Effects of larval diet on stored nutrient content. (a) Plot of the body size adjusted mean lipid content from the ANCOVA in Table 2 by larval diet treatment. (b) Plot of the body size adjusted mean trehalose content from the ANCOVA in Table 2 by larval diet treatment. (c) Plot of glycogen content by larval diet treatment. (d) Plot of the body size adjusted mean of storage protein content from the ANCOVA in Table 2 by larval diet treatment. Horizontal bars represent one standard error. The same letter denotes groups that are not significantly different after a Tukey's HSD correction for multiple comparisons ( $\alpha = 0.05$ ).

## 4. Discussion

### 4.1. Compensatory feeding and development time

As expected, compensatory feeding behavior was observed in response to larval diet concentration, where individuals that were fed low nutrient diets consumed more than individuals fed high nutrient diets. However, individuals fed low nutrient diets consumed less total proteins and carbohydrates than individuals fed higher nutrient diets. Compensatory feeding may have decreased differences in nutrient gains between diets that were close in content, but individuals were not successful in compensating across the wide range of nutrient concentrations in this study. In addition to compensatory feeding, insects are also capable of using numerous post-ingestive mechanisms to alter assimilation efficiency which could ameliorate some differences in diet quality (Slansky and Scriber, 1985; Simpson et al., 1995). However, differences in growth and storage parameters suggest that net nutrient input available for allocation differed between individuals on low and high nutrient diets. Not only did individuals on the lowest nutrient content diet consume less macronutrients they also took longer to do so and may have been affected by increased maintenance costs associated with longer instar durations. Growth rates are dependent on development time, and some authors have suggested that growth rates control the timing of larval–adult transitions in both insects and anurans (Lepis and Travis, 1994; Bradshaw and Johnson, 1995; Nylin and Gotthard, 1998; Flanagin et al., 2000). The only major difference in growth rates between diet groups occurred on the lowest nutrient content diet. This finding reinforces that compensatory feeding was effective for making up small differences in diet nutrient content, but the lowest nutrient content diet was outside the range of compensatory ability.

### 4.2. Somatic growth and nutrient storage

Larval nutrient availability had significant effects on growth and lipid storage, but not protein or carbohydrate

Table 2  
ANCOVA tables for the effects of larval diet and body size on lipid, trehalose and protein storage

Trait	Source	df	F	p
Ln (lipid Content)	Whole model	6	35.98	<0.001
	Larval diet	5	8.39	<0.001
	Ln (Storage-free dry mass)	1	29.78	<0.001
	Error	35		
	Total	41		
Trehalose Content	Whole model	6	2.72	0.028
	Larval diet	5	1.33	0.275
	Ln (Storage-free dry mass)	1	2.84	0.101
	Error	35		
	Total	41		
Storage Protein Content	Whole model	6	2.49	0.041
	Larval diet	5	0.56	0.730
	Ln (Storage-free dry mass)	1	3.61	0.066
	Error	35		
	Total	41		

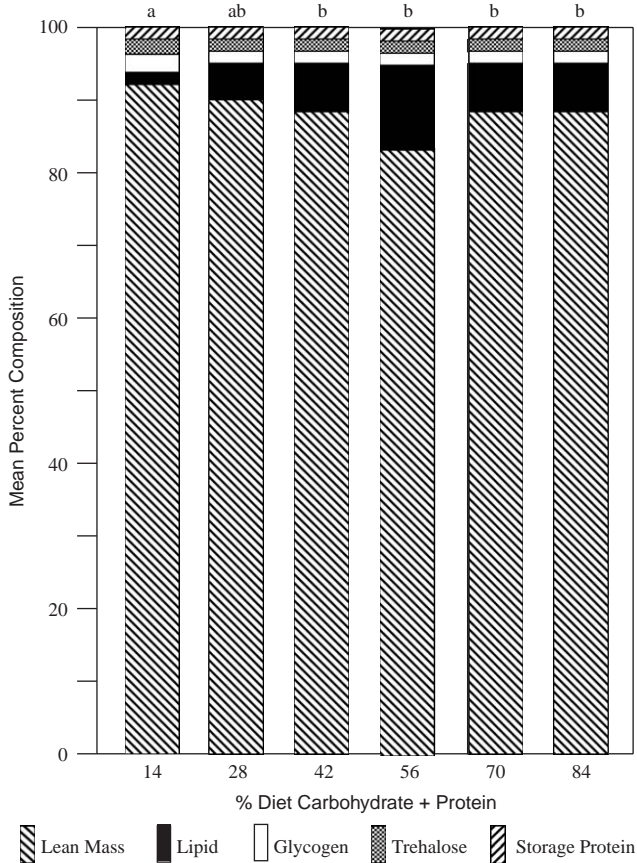


Fig. 5. Breakdown of the proportion of total mass accounted for by allocation to somatic growth (storage-free dry mass) and storage (lipid, glycogen, trehalose, and storage protein). The same letter denotes groups that are not significantly different in allocation to lipid after a Tukey's HSD correction for multiple comparisons ( $\alpha = 0.05$ ).

storage at adult eclosion in *S. americana*. An intermediate diet supported the greatest growth (56% P+C), but the largest difference in growth occurred between the lowest nutrient content diet and all others. Individuals fed the

lowest nutrient content diet had both significantly lower storage free mass and shorter hind femurs than individuals fed the best growth diet. Across diets, larger individuals contained more lipid, trehalose, and protein stores. However, when somatic growth was held constant there was a significant effect of larval diet only on lipid content. The pattern was particularly apparent when allocation to each storage parameter was expressed as a proportion of dry mass; lipid content varied from a mean of 1.77% in the low content diet to 10.24% in the best growth diet whereas responses to diet were much smaller for storage protein (1.05–1.95%), glycogen (0.84–0.44%), and trehalose (2.34–3.20%).

Across all diets, lipid was stored in equal or greater amounts than carbohydrates or proteins in *S. americana*. Some phytophagous insects, such as the moths *Plutella xylostella* and *H. virescens*, carry substantial protein reserves into adulthood from larval feeding (Wheeler et al., 2000; Telang et al., 2002). However, these moths do not feed on protein as adults and must carry over enough stored essential amino acids from larval life to produce eggs. Similarly, the autogenous mosquito, *O. atropalpus* also carries substantial protein reserves from larval life into adulthood to support production of a first batch of eggs without a blood meal; although adults will feed on carbohydrate and protein-rich resources to support the production of subsequent clutches (Telang and Wells, 2004). Although *S. americana* individuals are polyphagous and encounter significant variation in diet quality during their lifetimes, they can obtain both carbohydrates and proteins through adult feeding and adults undergo a significant period of feeding and somatic growth before reproduction, providing the opportunity to accumulate anabolic protein precursors necessary for growth and reproduction (Hahn and Wheeler, 2003). Comparisons of protein storage in these four species illustrates the influence of

life history, particularly the adult feeding strategy, on whether an insect carries significant protein reserves over from larval life into adulthood.

Why do then females on the best growth diet carry significant lipid reserves over from larval feeding if they could readily obtain them from adult feeding? Although the function of larvally derived lipid stores in adult *S. americana* is unknown, carrying over significant lipid, but not protein or carbohydrate reserves, suggests an important function for larvally derived lipid reserves. Because of its high-energy content, lipid is the primary stored nutrient in insects and most other animals (Downer and Matthews, 1976). The grasshoppers *S. gregaria* and *L. migratoria* both accumulate significant lipid reserves in adulthood for use in dispersal, and both have been shown to accumulate some of these lipid reserves during larval feeding (Zanatto et al., 1993; Pener et al., 1997; Raubenheimer and Simpson, 1997; Simpson et al., 2002). In addition, *L. migratoria* larvae forced to eat diets with an excessively high carbohydrate-to-protein ratio accumulate significant fat reserves and survive starvation in early adulthood better than leaner individuals fed balanced or protein-biased diets (Raubenheimer and Simpson, 1997). After eclosion, the diet of adult grasshoppers can also have significant effects on survival and reproduction and larvally derived lipid reserves may interact with adult diet to affect survival, dispersal, or reproduction (Joern and Behmer, 1997; Moehrlein and Juliano, 1998). Because the nutritional quality of host plants in the field can vary widely, a polyphagous grasshopper like *S. americana* may experience significant variation in diet quality during its lifetime (McNeill and Southwood, 1978; Mattson, 1980; Bernays and Bright, 2001). Therefore, accumulating greater lipid stores during periods of excess may represent a bet hedging strategy for ameliorating the effects of unpredictability in future food quality (Boggs, 1981; Philippi and Seger, 1989; Rogers et al., 1993; Hopper, 1999).

It is noteworthy that some individuals fed the best growth diet in this study achieved similar dry masses to those fed the standard laboratory rearing diet of Romaine Lettuce and wheat germ in a previous study (Hahn and Wheeler, 2003). However, even in the best growth group in this experiment the overall variance in dry masses was greater, mean dry mass was 17% lower, and both storage protein and lipid contents were roughly 50% lower than individuals fed the standard rearing diet (Hahn and Wheeler, 2003; Hahn, unpublished data). Even though the artificial diet supported development in *S. americana*, it is clear that even the best growth diet was less suitable than the standard plant-based rearing diet. Interestingly, the impact of this unsuitability was greater on both lipid and protein storage than dry mass, suggesting that caution should be exercised when using artificial diets to measure nutritional effects on nutrient storage.

## 5. Conclusions

In contrast to other known insect examples, I have shown that manipulating larval diet had significant effects on growth and lipid storage, but not protein or carbohydrate storage in newly eclosed adult *S. americana* females. Both the autogenous mosquito *O. atropalpus* and the moth *H. virescens* must carry substantial protein reserves over from larval feeding to support adult reproduction which occurs without significant protein feeding in these species; whereas adults of *S. americana* undergo a significant period of feeding and somatic growth prior to reproduction. Therefore, the adult life history with respect to feeding likely has the greatest impact on the quantity of larvally derived protein reserves carried over into adulthood. Another difference between these three species is that *H. virescens* and *O. atropalpus* are both holometabolous while *S. americana* is hemimetabolous. Because of the large anabolic requirements of holometabolous development and the non-feeding pupal stage, the demands for nutrient storage would likely be greater for larvae of holometabolous insects than hemimetabolous insects and holometabolous insects would be expected to contain proportionally greater nutrient reserves at the end of larval feeding. However, this may not directly translate into holometabolous insects carrying proportionally greater larvally derived nutrient reserves into adulthood. Like *S. americana*, adults of many holometabolous species require a significant period of adult feeding before reproduction can occur (Wheeler, 1996). Therefore, within any particular taxa the adult nutritional life history will likely play a greater role than developmental mode in influencing the relative quantity of larvally derived nutrient reserves carried into adulthood. These comparisons reinforce the point that careful consideration of life history is critical when interpreting nutrient budgets. Understanding the varied roles of protein availability in shaping the physiology and life histories of phytophagous insects and other consumers will require further study of the role of protein storage in nutrient budgets. Achieving this goal will necessitate both further surveys of the relationship between accumulation and management of protein reserves across species whose life histories differ with respect to nitrogen availability and utilization, and studying the underlying physiological mechanisms that regulate storage and utilization in these species.

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