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METABOLIC RESERVES IN *PERIPLANETA AMERICANA* (DICTYOPTERA: BLATTIDAE)

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Abstract Dietary carbohydrates, lipids, and proteins constitute the nutrient reserves for the American cockroach, *Periplaneta americana*. Adults collected from an urban population were evaluated by their body weight and for their storage and utilization of metabolic reserves. Mean dry body weight of females collected in December was significantly ($P<0.05$) higher than that of females in February, May, June, July, September, and October. Mean dry body weight of males collected in December was significantly ($P<0.05$) higher than that of February and August males. Mean content of total lipids in November females was 419.9 ± 38.7 mg/g dry weight, and for February females was 197.1 ± 42.8 mg/g dry weight. Glycogen contents in females and males significantly ($P<0.05$) increased in January and February. Highest glycogen was in February females at 217.9 ± 29.9 mg/g dry weight, and lowest was in August females at 86.1 ± 32.1 mg/g dry weight. Mean glycogen content in November females was 109.0 ± 31.4 mg/g dry weight, and the highest was in February males at 235.8 ± 31.1 mg/g dry weight. The lowest glycogen content was in November males at 81.0 ± 33.2 mg/g dry weight. Mean content of total protein in November females was 498.8 ± 31.2 mg/g dry weigh, and for February females it was 399.7 ± 33.6 mg/g dry weight. Mean total protein in November males was 483.7 ± 28.4 mg/g dry weight, and for February males it was 392.7 ± 38.8 mg/g dry weight. Reduced winter foraging corresponds to little or no food intake, and utilization of metabolic reserves results in a significant decrease in lipid and protein storage. Glycogen in over-wintering *P. americana* is used in cold hardening. Carbohydrates serve both a nutritional and a cold hardiness function in overwintering.
Key Words Nutrient reserves, over-wintering, lipids, proteins, carbohydrates

INTRODUCTION

In temperate zones, insects and other arthropods adjust to the seasonal limitations, such as scarcity of food and cold temperatures. Species survive adverse conditions by specific physiological and behavioral adaptations, including decreased foraging, increasing food reserves, and reducing their metabolic rate. Food reserves are generally stored in the form of proteins, lipids, and carbohydrates. Proteins play an important role in growth and development, and carbohydrates and lipids are major sources of energy during times of starvation (Chapman, 1971). Food reserves are important during the non-feeding period for insects that over-winter as adults or immatures. The stores in the fat body may initially constitute 30 percent of the body weight, but are depleted to less than ten percent by the end of the winter (Clements, 1963).

There is considerable published information on the foraging habits, and the physiological and biochemical aspects of metabolic reserves of *Periplaneta americana* (L.). Previous studies report a seasonal foraging pattern for this species in temperate regions (Haines and Palmer, 1955; Bao and Robinson, 1988). Seasonal foraging is characterized by limited movement or dispersal during cold months (Haines and Palmer, 1955; Bao and Robinson, 1988), and reduced activity during this time represented a physiologically distinct over-wintering period. Bignell (1982) and Downer (1982) provided comprehensive reviews on nutrition, nutrient reserves, and metabolism of this species. However, these data are based almost exclusively on laboratory studies. Little is known about nutrient reserves and utilization by *P. americana* during over-wintering in the field. Nor have any studies been conducted on a seasonal scale based on analysis of individuals from a field population.

More information on how pest species of cockroaches store and utilize metabolic reserves may provide insights into cockroach control strategies, particularly with regard to baiting. The American cockroach is a pest in temperate and tropical regions around the world (Robinson, 2005). Gel baits and plastic bait stations are common control strategies for large and small cockroach species. The objectives of the research

reported here were to characterize and analyze the types of metabolic reserves in *P. americana*, and test the hypothesis that cockroaches utilize these reserves during the over-wintering period.

MATERIALS AND METHODS

Sample Collection and Preparation

Adult American cockroaches were trapped twice monthly from a field population at Lincoln Terrace apartments, a large apartment complex in Roanoke, VA USA. The yearly mean temperature of basements was 23.9 ± 0.1°C, and mean relative humidity was 74.2 ± 11.9%; the temperature averaged 8.9 ± 0.3°C higher than the mean yearly ambient temperature.

Glass jars (940 ml) were baited with distiller's grain in small containers capped with metal screen. Cockroaches were collected from the traps within 24 h of placement. Samples were pooled on a monthly basis, then were weighed and stored at -20°C before analysis. Each cockroach sample was kept in a glass tube during the 4-5 day lyophilization in a freeze dryer. Lyophilized cockroaches were stored in a desiccator for 24 h or until consecutive weights were stabilized and then recorded. Two cockroaches, each with their head, wings, and legs below tibia removed, were pooled and ground together for analysis. Pooled samples were taken from the same sex and month of collection. Each ground sample was stored in a 30 ml glass bottle kept in a desiccator in a dark and cool location. Males and female specimens were analyzed separately. Eight samples were replicated for each sex and each month. Two sub-samples were taken from each of the ground and pooled samples and extracted for analyses of lipids, sugar, glycogen, and nitrogen.

Lipid Quantification

The Folsch and Sloane-Stanley (1956) method was modified to extract and quantify lipids. Samples of 15-20 mg were weighed into a 1.5 ml microcentrifuge tube; 750 µl chloroform : methanol (1:1 v/v) was added, followed by 30 sec vortex and 5 min centrifuge at 10,000 RPM. The supernatant was transferred into a microcentrifuge tube and the procedure repeated; the re-extracted supernatant was collected. Tubes with supernatant were placed under N_2 stream on a heated sand bath at 30°C for 3-4 h until dry. Tubes were kept in a desiccator for 6 -12 h to stabilize weights, then re-weighed, with the difference between the initial and final weights equal to the total lipid content for each sample. An estimate of total body lipids was calculated by extrapolating from the samples. The mean percentage recovery was determined by a series of spike-over analyses with a known amount of corn oil (10 -15 mg) added to one of the paired samples during each evaluation. The overall recovery was 99.2 ± 5.7%.

Carbohydrates Quantification

A modified Van Handel's (1965) method of separating glycogen from free sugars was used to extract and quantify carbohydrates. Carbohydrates were quantified as glucose equivalents through use of the anthrone reagent (Trevelyan and Harrison, 1952). **Free sugars**. Samples of 5-10 mg each were weighed in 1.5 ml microcentrifuge tubes. Samples were treated with 50 µl of saturated Na_2SO_4, then extracted with 1.0 ml of 66% ethanol saturated with Na_2SO_4, vortexed and centrifuged at 10,000 RPM for 5 min. The supernatant was transferred into a glass tube and the pellet was re-suspended and re-extracted with 1.0 ml of 66% ethanol saturated with Na_2SO_4. The second supernatant was transferred into and mixed with the first in the tube. The supernatant was then diluted 2-fold with Na_2SO_4-saturated 66% ethanol. One ml of this dilution was added to a capped test tube containing 5.0 ml of anthrone reagent [0.01g anthrone in 5.0 ml of diluted H_2SO_4 (5.0 ml conc. H_2SO_4:2.0 ml d.H_2O)] in an ice water bath. Solutions were vortexed and immersed in boiling water for 10 min, tubes were then placed an in ice water bath to halt the reaction. Quantification of total free sugars per sample was achieved by comparison against glucose standards (5, 10, 25, 50, 75, and 100 µg/ml) in 66% ethanol by a spectrophotometer (Perkin Elmer Lambda 3B Dual Beam). Absorbance was read at 620 nm against a blank of ethanol saturated with Na_2SO_4. An estimate of total body free sugars was calculated by extrapolating from the samples. **Glycogen**. The pellet that remained from free sugar extraction was dried under N_2 stream and then re-suspended in 1 ml of d.H_2O, and vortexed for 15 sec. The solution was diluted 2-fold with d.H_2O and 1 ml of the dilution was used for the anthrone reaction as described above. The glycogen content per sample was quantified through comparison against glycogen standards in

d.H_2O. Absorbance was read at 620 nm against a blank of d.H_2O. An estimate of total body glycogen was made by extrapolating from the samples. The mean percentage recovery was determined through a series of spike-over analyses with four paired samples per test. 50 µg of glucose or 50 µg of glycogen was added to samples. Mean recovery of 94.6 ± 3.7% for free sugars and 92.2 ± 3.1% for glycogen were attained.

Total nitrogen and Protein Quantification

Samples of approximately 0.25 mg each were weighed into 15 ml test tube. Each sample was treated with 1.0 ml of digestion mixture (313 mg hydrous $CuSO_4$, 129 mg selenious acid [H_2SeO_3], and 138 ml 5N H_2SO_4 per liter in d.H_2O) and heated at 150°C for approximately 8 to 12 h. Samples were then distilled at 300°C for approximately 12 h. The tubes were removed from heat and 3.0 ml of d.H_2O, 3.0 ml of 3.3N NaOH, and 2.0 ml of Nessler's reagent [7.0 g potassium mercuric iodide (K_2HgI_4) and 1.75 g gum ghatti refluxed in one liter of d.H_2O for 9 h] was added. Solutions were then vortexed and set for 15 min before comparing against a series of nitrogen standards (5, 10, 25, 50, 75, and 100 µg/ml) and a digestion mixture blank. Absorbance was read at 490 nm in a spectrophotometer. An estimate of total body nitrogen contents was calculated by extrapolating from the samples. The mean percentage recovery was determined through a series of spike-over analyses with four paired samples per test. A 10 µg of nitrogen standard was added into samples. The mean recovery of nitrogen was 97.9 ± 6.7%. Crude protein contents were converted by multiplying nitrogen content per sample with a fraction of 6.25 (Hatch and Brayton, 1985).

Data Analysis

Each data set was tested for normality (Proc Univariate normal procedures, SAS Institute 1989) prior to significance tests. Differences in the mean contents of lipids, carbohydrates (glycogen and total free sugars), and proteins among months were tested with analysis of variance (ANOVA) and means were separated with Tukey's studentized range (HSD) test at P=0.05 level (SAS Institute 1989).

RESULTS

Dry Body Weight

Monthly mean dry body weight of female and male *P. americana* are shown in Figure 1. Mean dry body weight of females was significantly (P<0.0001) higher than that of the males. The highest dry body weight was recorded in December in both sexes. Either female or male mean dry body weight in December was significantly (P<0.0001) heavier than that in February. Mean dry body weight of December females was significantly (P<0.05) higher than the mean dry body weight of females recorded in February, May, June, July, September, and October. Mean dry body weight of the December males was significantly (P<0.05) higher than that of the February and August males. Mean dry body weight decreased constantly from December to February in both sexes. The differences in mean dry body weight between December and February was 162.9 mg for female, and 80.1 mg for male. This was equivalent to 30.7% and 22.5% of dry body weight loss in females and males during the over-wintering period, respectively.

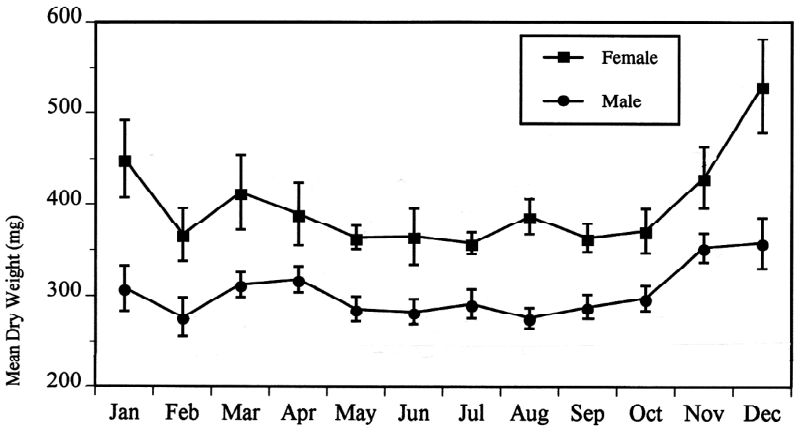

Figure 1. Monthly mean dry weight (mg) of *P. americana* adults collected from building basements, Roanoke, VA. Vertical bars denote ± SEM of the mean dry weights.

Lipids

Monthly mean contents of total lipids are presented in Figure 2. Females had significantly ($P<0.05$) higher total lipid contents per unit dry weight than males on a yearly basis. Total lipid content per unit dry weight in both females and males showed seasonal fluctuation. Mean lipid contents significantly ($P<0.05$) decreased in December to February, increased and fluctuated in March to May, significantly ($P<0.05$) increased in June to August, then fluctuated in the fall and peaked in November. Mean content of total lipids in November females was 419.9 ± 38.7 mg/g dry weight, and accounted for approximately 42% of the dry weight. In contrast, mean content of total lipids of February females was 197.1 ± 42.8 mg/g dry weight, and accounted for only about 20% of the dry weight. This represented a 53% loss in lipid reserves before and after the over-wintering period in females. Similarly, mean content of total lipids in November males was 386.2 ± 41.7 mg/g dry weight, and accounted for approximately 39% of the dry weight. Contrasting to high lipid level in November, mean content of total lipids of February males was 204.4 ± 23.2 mg/g dry weight, and accounted for only about 20% of the dry weight. This represented a 47% loss in lipid reserves before and after the over-wintering period in males.

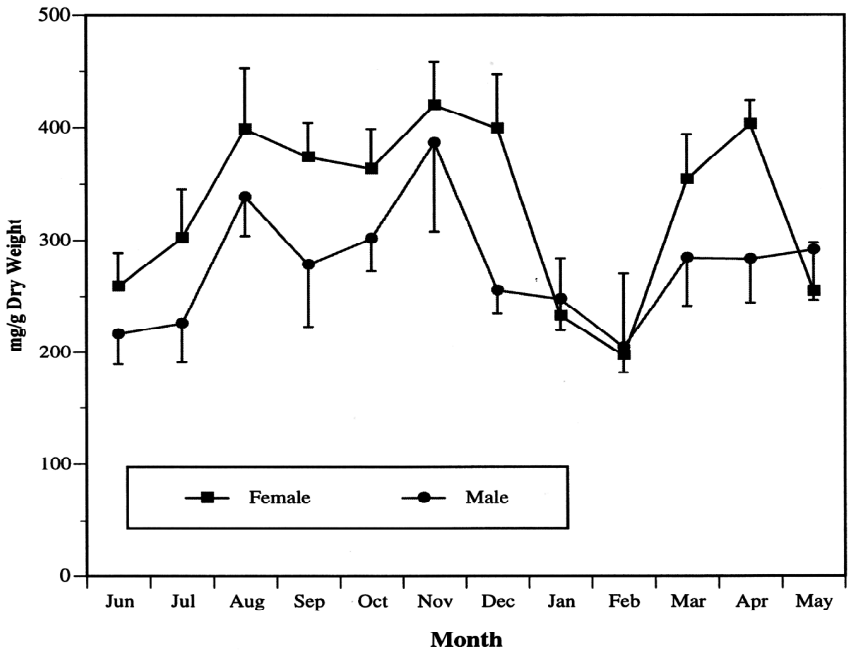

Figure 2. Monthly mean lipid contents of *P. americana* adults collected from building basements, Roanoke, VA. Vertical bars represent ± SEM of mean lipids.

Carbohydrates

Monthly mean contents of glycogen and total free sugars are shown in Figure 3 A and B, respectively. In contrast to the total lipid reserves of this species, glycogen contents per unit dry weight in both females and males significantly ($P<0.05$) increased in January and February. Though glycogen contents showed a seasonal fluctuation, there were no significant ($P<0.05$) differences among monthly reserves, except in January and February. Highest mean content of glycogen was recorded in February females at 217.9 ± 29.9 mg/g dry weight, and accounted for approximately 22% of the dry weight. Lowest mean content of glycogen was recorded in August females (86.1 ± 32.1 mg/g dry weight), and accounted for less than 9% of the dry weight. Mean glycogen content in November females was 109.0 ± 31.4 mg/g dry weight, and accounted for 11% of the dry weight. This represented a 100% increase in glycogen reserves before and after over-wintering in females. Similarly, highest mean content of glycogen was recorded in February males (235.8 ± 31.1 mg/g dry weight), and accounted for approximately 24% of the dry weight. The lowest glycogen content was recorded in November males (81.0 ± 33.2 mg/g dry weight), and accounted for only about 8% of the dry weight. This represented a 191% increase in glycogen reserves before and after the over-wintering period in males.

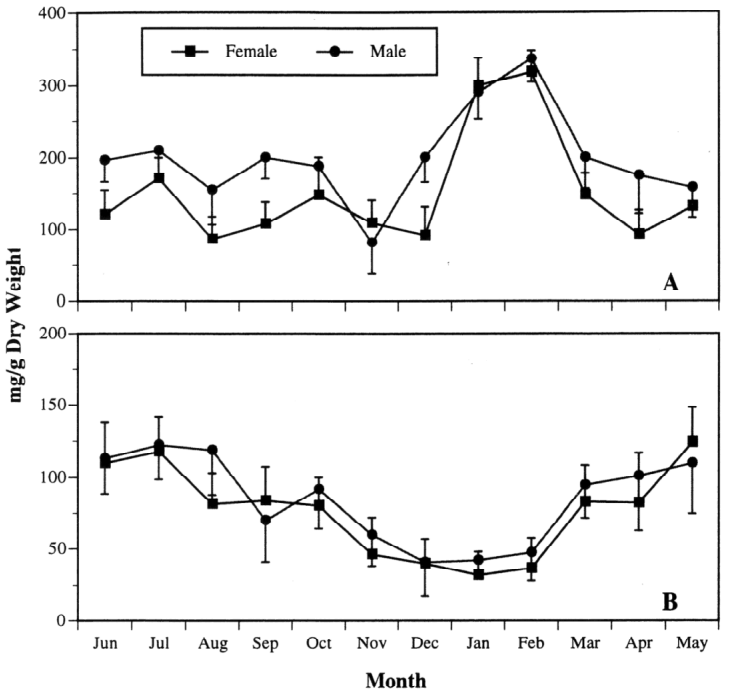

Figure 3. Monthly mean carbohydrate contents of *P. americana* adults collected from apartment building basements, Roanoke, VA. Vertical bars represent + SEM of mean carbohydrates. A = mean glycogen content; B = mean free sugars content.

Total free sugars per unit dry weight were not significantly (P=0.2316) different between the sexes, but were among months (P=0.006). Mean content of total sugar in November females was 45.9 ± 26.1 mg/g dry weight, and accounted for only approximately 5% of the dry weight. The mean content of sugar of February females was 41.4 ± 21.2 mg/g dry weight, and accounted for only about 4% of the dry weight. This represented a 10% loss in sugar reserves before and after the over-wintering period in females. Similarly, the mean content of total sugar in November males was 59.65 ± 21.8 mg/g dry weight, and accounted for approximately 6% of the dry weight. The mean content of total sugar of February males was 47.2 ± 19.2 mg/g dry weight, and accounted for less than 5% of the dry weight. This represented a 21% loss in sugar reserves before and after the over-wintering period in males. Sugar content per unit dry weight in both females and males showed a seasonal fluctuation. Total sugar contents were low in winter and accounted for about 4% of the dry weight in male or female, when foraging activity was minimal. Total sugar contents were relatively high in spring and summer, and accounted for up to about 14% of the unit dry weight in both sexes, when foraging was active.

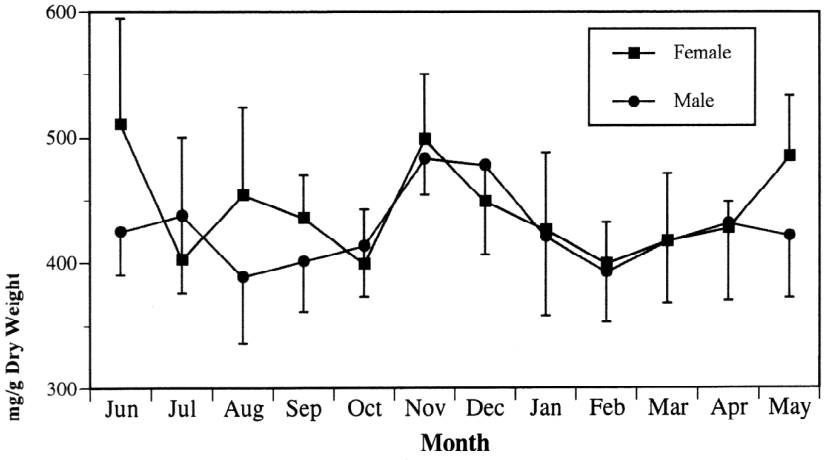

Figure 4. Monthly mean protein contents of basement *P. americana* adults collected from apartment building basements, Roanoke, VA. Vertical bars represent ± SEM of mean proteins.

Proteins

Monthly means of total protein are shown in Figure 4. *P. americana* adults showed high levels of protein in their metabolic reserves, ranging from 39% to 50% of their dry weight. Protein content per unit dry weight in both females and males showed seasonal fluctuation. Although not statistically significant due to large variation, mean protein content decreased in December to February, increased in March to May, (significant in females, $P<0.05$), fluctuated in June to August, and did not significantly ($P<0.05$) differ from the winter content, then peaked in November. Mean content of total protein in November females was 498.8 ± 31.2 mg/g dry weight, and accounted for approximately 50% of the dry weight. In contrast, mean content of total protein of February females was 399.7 ± 33.6 mg/g dry weight, and accounted for about 40% of the dry weight. This represented nearly a 20% loss in protein reserves before and after the over-wintering period in females. Similarly, the mean content of total protein in November males was 483.7 ± 28.4 mg/g dry weight, and accounted for approximately 48% of the dry weight. In contrast to high protein levels in November, the mean content of total protein of February males was 392.7 ± 38.8 mg/g dry weight, and accounted for about 39% of the dry weight. This represented a 19% loss in protein reserves before and after the over-wintering period in males.

DISCUSSION

Dry Body Weight

Because *P. americana* adults are long-lived (Griffiths and Tauber, 1942), samples taken from random trap catches did not discriminate differences in age and physiological state. Therefore, a wide range of variations in body weight and size was recorded. Although the mean dry body weight of males and females did not show seasonal fluctuations as would be expected similar to their seasonal foraging pattern, differences in mean dry body weight were significant ($P<0.05$) from December to February in both sexes. Mean dry body weight showed a reversed relationship with respect to ambient and basement temperature changes in late fall and early winter. Increased dry body weight corresponds to increased lipid, glycogen, and protein reserves during this period. With the increased storage of metabolic reserves, mean dry body weight reaches a peak in December.

Nutrient Reserves

In insects that have a winter diapause, nutrient reserves usually increase before over-wintering and decrease after over-wintering (Lee, 1955). This pattern of nutrient utilization would be expected in over-wintering American cockroaches. Reduced winter foraging activity of this species in the basement environment corresponds to little or no food intake. Individuals must utilize metabolic reserves to maintain their minimum activities. Metabolism of reserves results in a significant ($P<0.05$) decrease in lipid and protein storage (Fig. 2, Fig. 4), and is reflected by the significantly ($P<0.05$) reduced dry body weight (Fig. 1) during this inactive period. Mean dry body weight from December to February was reduced by 30.7% in females and 22.5% in males. Results obtained in this study strongly support the hypothesis that American cockroaches utilize their metabolic reserves during the over-wintering period, and lipids and proteins are the two principal classes of metabolic reserves which are responsible for energy supplies in over-wintering adults.

Lipids and proteins. Lipids, carbohydrates, and proteins are metabolic reserves for almost all organisms. Among these, lipids are frequently the predominant nutrient reserve, accounting for 12-60% of the dry weight of over-wintering or diapausing insects (Lee, 1955). Lipids are considered the most economical form of energy storage and source because they yield a greater amount of metabolic water and calories per unit weight than either carbohydrates or proteins (Chippendale, 1978). However, carbohydrates and proteins are also important metabolic reserves to over-wintering insects (Steele, 1981; Behrens, 1985). The major components and storage forms of metabolic reserves in *P. americana* are lipids and proteins, which can contribute up to 91% of the dry body weight in November. Changes in lipid and protein storage over time result in seasonal fluctuation of dry body weight. During the over-wintering period, lipids are the principal metabolic reserve responsible for energy supply, and accounted for a 22.3% dry weight loss in females and 18.2% in males. Proteins are also an important energy reserve; they were responsible for a 19.9% dry weight

loss in females and 18.8% in males. Sugars are also used for energy during over-wintering, and accounted for a 4.5% dry weight loss in females, but 12.4% for males.

The protein concentrations per unit dry weight fluctuated in adults during spring and summer, and were not significantly ($P>0.05$) different from the protein reserve levels during the over-wintering period. Mullins and Cochran (1975) reported that female American cockroaches invest a considerable amount of urate nitrogen into each ootheca during the reproductive period. Males also contribute nitrogen reserves to offspring by means of transferring spermatophores (Mullins and Keil, 1980). The high reproductive rate of *P. americana* in spring and summer may be the basis of this protein deficit. Protein deficit may lead to increased foraging activity, and cannibalism in some individuals.

Utilization and gradual depletion of metabolic reserves, particularly lipids and proteins, occurs during over-wintering and results in nutrient deficit in post-over-wintering individuals in the population. Increased foraging activity of the American cockroach in spring is apparently a behavioral response to their metabolic reserve deficit. This deficit may be the physiological driving force for increased foraging when temperature increases in spring. This foraging behavior can be of particular importance in developing baiting strategies for cockroach control.

Carbohydrates. A 10-15% increase in glycogen reserve per unit dry weight of over-wintering *P. americana* adults corroborates well with the findings of Singh and Das (1980). Glycogen reserve was significantly increased by up to 30% higher in fat body and muscle tissues of nymphs and adults that were cold-acclimated at 15°C than the control group at 35°C. Steele (1981) reported that glycogen is not greatly affected by respiratory rates or metabolic demands through winter, and its concentrations show a strong inverse relationship with glycerol level. Glycogen in over-wintering *P. americana* may be used primarily in cold hardening rather than in energy production. In this regard, carbohydrates serve both a nutritional and a cold hardiness function in over-wintering *P. americana*.

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